VETERINARY VACCINES

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ABSTRACT

Vaccination of animals for the prevention of infectious diseases has been practised for a number
of years with little change in product composition. Recent advances in molecular biology,
pathogenesis and immunology have laid the groundwork for the development of a new
generation of veterinary vaccines based on pure subunits as well as live vectored bacteria and
viruses. Along with novel methods of antigen preparation, the use of new adjuvants and delivery
systems will permit targeting of the appropriate immune response as well as offering flexibility
in terms of vaccination protocols. These new technologies are also being applied to the
development of vaccines to enhance animal productivity and to control reproduction.

KEY WORDS

Vaccine, antigen, adjuvant, delivery, immunoneutralization, immunization, live vector.

INTRODUCTION

Vaccination of animals against infectious diseases has proven to be one of the most cost-
effective means of reducing animal suffering and economic losses due to bacterial and viral
infections. However, infectious diseases continue to be a problem despite the continued use of
conventional products for decades. The technology for vaccine development and production has
remained basically the same for the past 200 years, involving the use of either killed organisms
combined with an adjuvant or live organisms with reduced virulence. Advances in immunology
and pathogenesis over the past two decades, based largely on recombinant DNA technology,
monoclonal antibodies and molecular genetics, have formed the basis for a new generation of
veterinary products which are starting to enter the marketplace and should continue to do so
for the foreseeable future. Subunit vaccines composed only of protective antigens are now cost

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effective to produce using gene expression technology in a number of systems, including bacteria, fungi, insect cells, and destructive or non-destructive mammalian systems. In parallel with the identification and production of subunit antigens, new adjuvants and delivery systems are being developed which allow one to stimulate the appropriate immune response (antibody vs. cell-mediated) at the desired site. Since many viral and bacterial pathogens colonize mucosal surfaces, delivery of the antigen to this site is crucial for stimulation of the mucosal immune system. New carriers such as cholera toxin and microcarriers, or the use of cytokine::antigen chimeras, offer the ability to deliver subunits to mucosal surfaces. Live vectors are arguably a better choice for stimulating mucosal immunity, and rapid advances have been made in the development of live bacterial and viral vaccines based on rational attenuation methods. While live vaccines have been used for a number of years, the exact mechanism of attenuation was often unclear and the genetic lesion responsible for the reduction in virulence was not characterized. Therefore, the possibility of reversion to virulence remained a remote possibility. Rational attenuation relies on the construction of well characterized deletions in specific genes coding for essential physiological functions or virulence determinants. Much of the work on bacterial attenuation has focussed on *Salmonella typhimurium*, although a number of other organisms have now been similarly attenuated. Likewise, attenuated herpesviruses and adenoviruses have also been constructed. While these gene-deleted organisms can be used as monovalent vaccines, they hold greater promise for the delivery of heterologous antigens.

The area of vaccinology has historically focussed on the control of infectious diseases, but there has been considerable interest recently in the modulation of animal productivity, behaviour and reproduction by immunization against hormones or their receptors. A vaccine which decreases fertility has been developed using synthetic peptide technology and is now commercially available and this product should be followed by others produced by recombinant DNA technology.

The present review will attempt to summarize both the present vaccines as well as highlight the recent discoveries that will shape the vaccines of the future.

BACTERIAL VACCINES

Historical Trends and Current Practices

Vaccination of animals for the prevention of bacterial infections has been practised for a number of years using both killed and live products. For many diseases, this has proven effective in reducing clinical signs of disease, but there is room for improvement in both safety and efficacy for some of these products. Many vaccines have been composed of killed bacteria

combined with an adjuvant to increase the immune response, and while this type of product is quite inexpensive to produce, there are several inherent problems. These include antigenic competition between protective and nonprotective components, the omission of protective antigens due to growth conditions or downstream processing, and the presence of potentially harmful components of the bacteria. There are a large number of components on the surface of bacteria, and those which are protective will make up only a small fraction of those presented to the immune system following vaccination. This "antigenic competition" ultimately will result in a decreased response against the protective antigens and a corresponding decrease in efficacy of the product. This problem is further compounded when vaccines contain multiple serotypes of an organism or more than one bacterial species. For example, there are 12 serotypes of *Actinobacillus pleuuropneumoniae* and more than 30 of *Streptococcus suis*, and while each does not have to be included in a vaccine, several of each are needed for broad spectrum protection.

It is now known that many important virulence determinants and potentially protective antigens are produced only under culture conditions which mimic those found in the host. For example, outer membrane proteins involved in iron acquisition by Gram-negative bacteria are usually only produced in the absence of free iron (20, 21) and therefore are not produced under "normal" laboratory growth conditions. Likewise, the synthesis of virulence determinants such as toxins and fimbriae can be regulated in a similar fashion (19, 74). Iron-regulated membrane proteins have been shown to be protective antigens in a number of systems (117, 133) and therefore manipulation of growth conditions to increase their expression may improve the efficacy of whole cell preparations. Bacterin preparations are often composed only of cellular material and therefore also lack protective immunogens found in the supernatant. An excellent example of this type of virulence determinant would be the RTX toxins produced by bacteria such as *Pasteurella haemolytica* and *Actinobacillus pleuropneumoniae* (22, 78, 79), agents responsible for pneumonia in cattle and pigs, respectively. In both cases, these toxins have been shown to be protective immunogens and therefore could be considered essential vaccine components. Some new products have been enriched for these secreted toxins and have shown some efficacy under field conditions (5, 62). Finally, bacterin preparations may include components such as lipopolysaccharide which interfere with the immune response to other protective components (125), and their removal would increase vaccine efficacy. Despite these problems, there are numerous examples of bacterin-based vaccines which have proven to be effective including those for vibriosis (*Campylobacter fetus*), calf scours (*Escherichia coli*), ovine footrot (*Bacteroides nodosus*), Clostridial infection, and *E. coli* mastitis to name a few. Furthermore, the use of these and similar vaccines has helped to identify specific virulence

determinants and protective components which have served as a basis for further vaccine improvement. For example, vaccination with whole cell preparations of *E. coli* established the protective capacity of K99 fimbriae and subsequently led to the development of extract products enriched for this protective antigen (1-3). Likewise, recombinant vaccines for ovine footrot have been developed due to earlier studies conducted with whole cell preparations (29, 56) (see next section). Therefore, while vaccination with bacterins has not proven to be 100 percent effective, they have been useful first generation products and in some cases could probably not be improved upon in terms of efficacy. Bacterial extracts can be viewed as refinements to whole cell vaccines in which specific protective components are enriched. The problem of antigenic competition is somewhat reduced in extract vaccines, and downstream processing procedures have been shown to further increase the safety and efficacy of such products (128).

A limited number of live bacterial vaccines have been developed using conventional technologies, including *in vitro* passage, passage through non-host animals, and chemical mutagenesis. *Brucella abortus* strain 19 has been used extensively as a live vaccine, and 65-85 percent of immunized animals develop protective immunity (99, 131). Streptomycin-dependent mutants of *P. haemolytica* and *P. multocida* which cannot grow in the absence of the antibiotic have been developed as vaccines (10, 16, 65), but have not found widespread use. The genetic lesion responsible for this attenuation has not been reported. New live vaccines for which the attenuation mechanism and the exact structure of the mutation are known are now being developed and offer an attractive alternative to vaccination with conventional live or killed products (see below).

The Future

Subunit vaccines. True subunit vaccines containing one or more protective antigens are attractive since only those components required for the induction of a protective cell mediated and/or humoral immune response are present. This offers flexibility in terms of vaccine formulation, adjuvant choice, and mechanism of vaccine delivery. Numerous virulence determinants produced by veterinary bacterial pathogens have been identified and characterized over the past 15 years, including adhesins (40, 107), capsular polysaccharide (49, 134, 139), toxins (76, 88, 124), outer membrane proteins (20, 21, 46, 101), and others. However, the purification of these components by conventional biochemical techniques is generally not feasible for vaccine production due to the high downstream processing costs. Recombinant DNA technology and high level gene expression offers an attractive alternative for the cost effective production of bacterial subunits and although only a few vaccines are currently being produced

this way, we anticipate that several new products will follow in the near future. A number of bacterial expression systems are available which can result in the desired produce being up to 40 percent total cell protein. These include Gram-negative bacteria, such as *E. coli* (45) and *Pseudomonas aeruginosa* (31), and Gram-positive organisms such as *Staphylococcus aureus* (100) and *Bacillus subtilis* (54). High level gene expression in *E. coli* often results in the recombinant protein being in an insoluble form (inclusion bodies) which must be harvested and solubilized prior to use. This has proven effective for some bacterial antigens (e.g., *P. haemolytica* LktA; *A. pleuropneumoniae* TfbA, CytA, and OmlA) (4, 43, 50, 117), but others may require more extensive downstream processing, such as refolding of the protein, prior to formulation. There are also systems which result in the formulation of soluble products, and these will likely find use mainly for those antigens which require biological activity or assembly for induction of a protective immune response.

The Fimbriae are excellent examples of antigens which require assembly on the cell surface, and an effective ovine footrot vaccine containing recombinant *B. nodosus* fimbriae, has been produced using *P. aeruginosa* as a host (29, 56). These recombinant fimbriae were antigenically identical to those isolated from the original *B. nodosus* strain. When the fimbrial genes were cloned into an *E. coli* host, assembly of the fimbriae did not occur, and the unassembled subunit protein was not protective (30, 56). This illustrates the importance of choosing the appropriate expression system and host for the production of recombinant proteins. Another fimbrial-based vaccine for the prevention of *E. coli* scours in pigs has also been developed (47), and in this case the host for gene expression was an *E. coli* strain. The vaccine contained equal quantities of K99, K88ab, K88ac, and 987P fimbriae. Both the footrot and scours vaccines could have been produced from the natural host bacteria using conventional purification techniques. However, the recombinant bacteria produce up to ten times more fimbriae and are easier to grow at high culture densities. Also, more than one fimbrial type can be produced by one strain making production even more cost effective.

The Gram-positive expression systems are particularly useful for secretion of recombinant proteins to the growth medium which facilitates product recovery, since cell disruption is not required. One *S. aureus* system based on the protein A (*spa*) gene has been shown to direct secretion of the product to the growth medium in both *E. coli* and *S. aureus* (64, 100). Recombinant DNA technology also allows one to modify the gene of interest to produce chimeric molecules as well as antigens which have been modified to include affinity tags for purification, protease sites for cleavage, or lipid moieties which may increase their immunogenicity (42, 97, 113). The latter modification may find use in veterinary vaccines as

replacements for oil-based adjuvants are introduced since the adjuvant molecule is covalently linked to the antigen thus making formulation and delivery more efficient and cost effective.

Live bacterial vaccines. One of the most exciting areas in contemporary bacterial vaccinology is the emergence of live bacterial vaccines based on rational attenuation methods. Live vaccines have several potential advantages over killed products, including (a) the ability to be delivered by a number of routes with one immunization, (b) the presence of all relevant antigens, including those expressed *in vivo* since the organism will multiply in the host, (c) the ability to elicit both cell-mediated and humoral immunity at the desired site within the animal, (d) low cost, and (e) the ability to serve as vehicles for the delivery of heterologous antigens. While live vaccines have been used before, the exact nature of the lesion responsible for attenuation was unknown, and therefore reversion to virulence remained a possibility. Advances in molecular genetics has made it possible to construct well defined mutations, usually deletions, in specific genes which affect survival in the host. Since the establishment of an infection requires the expression of genes coding for virulence determinants as well as components needed for physiological functions of the cell, mutations in either group can potentially reduce virulence. Representative examples of attenuating mutations of each class are listed in Table 1.

Table 1. Summary of Attenuating Mutations

Gene	Function	Genus
aroA, C, D	Aromatic amino acid biosynthesis	Salmonella, Bordetella, Bacillus Yersinia, Shigella, Aeromonas
purA, E	Purine metabolism	Salmonella, Yersinia
ompR	Porin regulation	Salmonella
phoP, Q	Phosphatase	Salmonella
galE	Galactose epimerase	Salmonella, Shigella
cya, crp	Adenylate cyclase	Salmonella, Bordetella
carAB	Pyrimidine and arginine synthesis	Escherichia
lktA, cytA	Cytotoxin	Actinobacillus, Pasteurella
toxA	Alpha toxin	Staphylococcus
cap	Capsule biosynthesis	Bacillus

Mutations in virulence genes specifying capsule and toxin synthesis have been shown to attenuate *Bacillus anthraces* and *P. haemolytica* (60), respectively, although the latter may not be a good choice for a vaccine strain since the leukotoxin is a dominant protective antigen.

However, since non-toxic forms of the molecule are still protective (50), a live vaccine producing a genetically detoxified *lkt*A gene product should still be protective. Most of the work on live vaccine development has focused on Salmonella species attenuated by mutations in "housekeeping genes." Vaccination with *aro*A mutants has been shown to induce antibody, delayed-type hypersensitivity and cytotoxic T-lymphocyte responses in mice (37, 106, 120). In one vaccine trial, oral immunization of seven-day-old calves with an *aro*A *aro*D double mutant of *S. typhimurium* resulted in protection against experimental challenge seven weeks later (63), demonstrating the potential for oral vaccination of a large animal. Others have shown that animals vaccinated twice with killed cells develop an antibody response against the organism but are not protected against challenge (77, 116, 126). *S. typhimurium pur* mutants are also attenuated, but vaccination results in a less persistent infection than with *aro*A mutants (15). Therefore, the degree of attenuation can be tailored to the desired level by using different mutations. Whichever type of mutation or approach is used to attenuate bacteria, at least two lesions will probably need to be incorporated in order to reduce the risk of reversion to virulence mediated by *in vivo* genetic transfer from another organism. This has already been accomplished using several combinations of alleles in *S. typhimurium* (63). The identification of novel attenuating mutations has been made easier by the IVET technology described by Mahan *et al.* (81), in which genes that are expressed at higher levels *in vivo* are identified based on promoter fusions with the *lac*Z gene. Using this technique, the *car*AB genes were identified as being expressed at higher levels *in vivo* by *S. typhimurium* and thus are potential candidates for immunization. Allan *et al.* showed that *car*AB mutants of avian *E. coli* serotypes O1, O2, and O78 were avirulent in a young chick model yet still were effective oral vaccines for the prevention of colibacillosis in turkeys (72). We anticipate that several new types of attenuating mutations suitable for incorporation into vaccines will be described as the IVET system is applied to more species of bacteria.

The use of live vaccines will probably occur to some degree in most production and management systems. However, these vaccines are especially attractive for the poultry industry where mass immunization via feed or drinking water is the only feasible method of control. Both *E. coli* and Salmonella vaccine strains are under development, and oral vaccination with *S. typhimurium cya/crp* and *E. coli car*AB mutants have been shown to protect birds against challenge with the homologous strains (72, 96). The *S. typhimurium* vaccine also protected birds against challenge with *E. coli* O78 (96).

Live vectored delivery of recombinant antigens. Attenuated bacteria can also be used as hosts for heterologous gene expression in order to make multivalent vaccines. Several attenuated Salmonella strains producing recombinant antigens have been produced, including the *E. coli* enterotoxin (17), K88 pili (25), *C. tetani* toxin (34), and rotavirus VP7 (122), to name a few. High level expression of the foreign gene is not required as for recombinant subunit vaccines, and secretion vectors which contain signal sequences to direct the foreign antigen to the cell surface are generally used, although this is not necessary in all cases. If protective epitopes of a protein have been identified, oligonucleotides coding for these sequences can be inserted in genes coding for surface structures such as fimbriae or flagella for cell surface expression (71). Further, by using promoters which are active only in the host, foreign antigens which are toxic to the host can be synthesized only after vaccination and not during growth of the vaccine strain. In principle, any host can be used to deliver recombinant antigens, and the ultimate choice must be based on safety and the type of immunity desired. There has been recent interest in the development of *Mycobacterium bovis* BCG as a delivery vehicle for heterologous antigens (61, 84). *M. bovis* has been used extensively for vaccination and is capable of inducing strong humoral and cell-mediated immune responses and should be capable of doing the same for foreign antigens presented on the cell surface. However, this system will likely be used mainly for human vaccines unless a diagnostic test which can distinguish vaccinated from infected animals accompanies the product.

VIRAL VACCINES

The Past and Present

Immunization to control viral diseases has a history of over 200 years with the development of live-pox virus vaccines followed subsequently by killed rabies virus vaccines. Since these initial observations, various approaches to control viral disease by immunization have been developed. These include: (i) virulent viruses, (ii) simultaneous administration of virulent viruses plus antibody, (iii) modified live (attenuated virus) vaccines, (iv) killed vaccines and (v) more recently, genetically engineered deletion mutants and subunit vaccines. The best example of using virulent virus as a vaccine is with infections laryngotracheitis virus which was introduced at a foreign site. In this case a respiratory virus was administered on the cloaca of birds (35). As the virus replicated at the site of injection it induced systemic and mucosal immunity before it spread to the respiratory tract. Unfortunately, the social behaviour and management practices under which poultry are housed results in some transfer of the virus from the cloaca to the respiratory tract of birds. Thus, although this was practised in the past, it is not without its short

comings and therefore has been replaced with attenuated live vaccines administered by the eyedrop method, as a spray or in the drinking water (44). Similarly, Orf virus is often used to immunize lambs by administering live virulent virus by scarification to the flank or other unwooled areas. Since lambs attempt to scratch and nuzzle the vaccination site, there is some transfer to virus to the lips and oral cavity of the lambs, with the concomitant decreased feeding and loss of productivity (44). Depending on the pathogenesis of the specific virus it is also possible to administer the vaccine at a time that is least detrimental to the host. For example, adult sows generally do not exhibit significant clinical signs following infection with transmissible gastroenteritis virus, but young piglets do. Based on this observation, some producers exposed pregnant sows to virulent virus just prior to parturition (121). This boosted the maternal antibody levels which were then transferred to the piglets and protected them during their most susceptible period of life. Similar approaches have been used in poultry where laying hens were immunized with virulent infectious bursal disease virus. This virus has little impact on adult birds, but induces the production of antibody which are then transferred to the yolk and subsequently to chicks, protecting them during the critical first 6 weeks of life (35). An alternative approach, using virulent viruses, capitalizes on the antigenic relatedness of viruses. Heterotypic immunity can be introduced by immunizing pigs with virulence strains of bovine virus diarrhea (BVD) to protect pigs against Hog Cholera Virus (35). Unfortunately, this practice is not acceptable if pigs come in contact with cattle.

Although virulent viruses have been used to control infections in animals, most vaccines used now are conventionally produced attenuated live or killed whole-virus vaccines. The attenuated vaccines are produced by passage through tissue culture or unnatural hosts. As a result of these multiple passages (in some cases >300), mutants are selected which have reduced virulence for the natural host. Mutants have also been developed by chemical mutagenesis and selection for various criteria including inability to replicate at body temperatures (temperature-sensitive mutants). Although these modified live viruses replicate *in vivo* they induce subclinical infections and concomitant immunity. Since these modified live vaccines can replicate, their safety has recently been challenged. The attenuating mutations are produced at random therefore it is impossible to predict where a mutation will occur and furthermore under what circumstances they may revert to virulence. Some of these mutations are single point mutations which can revert back to virulence upon a single passage *in vivo* (89). Secondly, some attenuated vaccines have been shown to be immunosuppressive (50, 59, 118). This characteristic is of major concern in situations where animals may be immunized with multiple vaccines (a common practice). The consequences of immunosuppression may be a poor immune response to

concurrently administered vaccines, or increased susceptible to concurrent infections. The fact that live-viral vaccines need to be refrigerated prior to administration to ensure their viability, combined with the above concerns has increased the use of killed vaccines. This is especially the case in rural and less developed regions of the world. Unfortunately, to induce adequate long-lasting immunity, killed viral vaccines require high doses of antigen, strong adjuvants and repeated administration to maintain protective levels of immunity. Furthermore the method of inactivation may alter the crucial epitopes of the virus required for inducing protective immunity (28). Conventional-killed vaccines cannot be administered in the feed or water, thus making administration of these vaccines to poultry extremely difficult. Furthermore, killed vaccines generally do not induce mucosal immunity, the primary site of entry of most viruses. Since conventional-live attenuated and killed vaccines all have some disadvantages many individuals and companies have attempted to circumvent these short comings by using recombinant DNA technology to develop better and more efficacious live or subunit vaccines for poultry and livestock.

Vaccines of the Future

Advances in molecular biology and immunology combined with our understanding of the pathogenesis of many viruses has provided us with the tools to develop more efficacious and safer vaccines for use in controlling viral infections of animals. Although there is still some controversy regarding the use of genetic engineering to produce vaccines, regulatory agencies and companies are recognizing the merits of such an approach. Support for the recombinant DNA approach has come mainly from the observations that genetic engineering can produce very safe and effective commercial vaccines. In order to maintain this momentum, a continued interaction between a number of disciplines in biology as well as a consideration of the economics of vaccine production will need to be considered.

Subunit vaccines. Most viruses possess a variety of different proteins, some of which are important in inducing protective humoral and cellular immunity, where as others are of not relevance to protection and may indeed be detrimental by virtue of its ability to induce immunosuppression (127) or to enhance viral infection (98, 102, 104). Thus, for a subunit vaccine it is crucial to identify the one or two proteins or epitopes that are involved in inducing protective immunity. Once these are identified, the next step is to isolate and express the genes coding for these proteins in an appropriate expression system. Although one could chemically synthesize the individual epitopes, it is more economical to express a number of epitopes in

tandem in expression systems. Indeed it is possible to express a series of epitopes in one expression cassette to produce a "string of beads" vaccine to protect against a series of viral diseases (141).

A number of expression systems are being used to produce large quantities of viral proteins for subunit vaccines. These include: (1) prokaryotic systems, and (2) eukaryotic systems including yeast, mammalian cells and insect cells. Prokaryotic systems are very attractive for vaccine production since their yield is often extremely high and the cost of fermentation and downstream processing is low. In some cases, it has been possible to produce as much as 40% of the total cell protein as a specific desired product. Unfortunately, for many viral glycoproteins, prokaryotic systems are not suitable since they do not post-translationally modify the proteins appropriately. Furthermore, many conformational epitopes cannot be reconstructed following expression in prokaryotic systems. Thus, inappropriate modification and conformational changes can result in reduced immune responses. However, one should not take this as a generalization to indicate that no viral proteins can be produced in *E. coli* since feline leukaemia virus gp70 expressed in *E. coli* has been shown to induce excellent virus neutralizing antibodies and protection (83).

Due to the limitations of prokaryotic expression systems for complex viral proteins, considerable interest has been generated in development of eukaryotic expressions systems. To date a large number of different viral genes have been expressed in yeast, mammalian cells and insect cells using baculovirus. Since there are presently few subunit veterinary vaccines licensed for commercial use, the merits of expressing subunit proteins in different expression systems will be described without using direct examples of their application in the commercial arena. The advantages of yeast as an expression system include; extensive experience that is already available with expressing proteins in yeast and in fermentation at large scale. Indeed one of the first licensed recombinant DNA vaccines produced, for Hepatitis B, was and still is produced in yeast (137). Unfortunately, in some cases yeast may over-glycosylate proteins which may in turn influence immune responses to that specific protein. This may preclude its use for the production of some antigens.

A second expression system which has received extensive investigation includes the expression of viral proteins or glycoproteins using insect virus vectors grown in insect cells or larvae (66, 80). Using the polyhedron gene promoter of *Autographa californica* nucleopolyhedrous virus, a large number of viral proteins have been expressed and tested for their immunogenicity. In some cases the yield of protein produced in insect cell cultures has approached 200 mg•L^{-1}. The recent development of a synthetic medium for culturing insect

cells without serum should further improve the economics of vaccine production in insect cell cultures. In addition to reducing costs, the removal of serum from the culture should reduce the potential problem of extraneous agents being present in the vaccine. The biggest disadvantage of insect cell culture systems is that progress in insect culture fermentation is still in its infancy. Thus, unlike yeast or prokaryotic systems, large scale fermentation (1000 liters) has not yet been achieved routinely. Since the physiological state of insect culture systems appears to influence the final yield of protein, significant progress in culturing insect cells will be required before they are used routinely for commercial production.

The ultimate eukaryotic expression system for producing authentic viral glycoproteins are mammalian cells. The advantage of mammalian cell systems is that cloned eukaryotic genes are often expressed as fully functional processed proteins (8). Two different approaches have been used to produce proteins in mammalian cells. These include lytic systems using mammalian viruses which have been engineered to express a foreign gene, and a nonlytic cell system. Vaccinia virus was one of the first mammalian viruses used for expression of foreign antigens (95). A similar approach has then been adapted using other poxviruses (140). Other viruses, including herpes and adenoviruses have also proven to be extremely useful for expressing foreign protein in mammalian cells (109, 144). Unfortunately, the use of viruses as vehicles for production of protein has two major disadvantages. First, all of the virus must be removed during the purification process to ensure that no live-virus is present in the vaccine. Second, since the infection process results in the destruction of the culture, each batch of vaccine requires the scale-up of cells from a master seed all the way to the final fermenter. This is both expensive and time consuming. To overcome both of these impediments, novel mammalian expression system has been developed whereby genes are introduced into mammalian cells which then secrete the protein continuously. Unfortunately, in most of these cases the yields of proteins are relatively low. To overcome this impediment we have recently developed a mammalian cell expression system which produces large quantities of secreted proteins under the control of the heat shock promoter (70). The cells can be cultured on microcarriers in conventional mammalian cell fermenters, exposed to elevated temperature to activate the promoter which then drives the production of protein for an extended period of time. The cells can be recycled through numerous heat shock and secretion cycles to produce large quantities of proteins over extended periods of time. This system not only provides the opportunity to produce proteins which are toxic to host cells, by secretion of the protein, but the growth of these cells in serum free media provides for economical downstream processing steps. Although this system has only been used to produce single proteins, it should easily be adapted to produce

chimeric proteins similar to that which has been reported for paramyxoviruses (parainfluenza F and HN, and respiratory syncytial virus F and G proteins) and even combinations of proteins from different viruses such as F from RSV and HN from parainfluenza (11, 12, 57). Since production of secreted proteins results in a slightly modified protein it is imperative to test each protein for its ability to induce both cell mediated and humoral immune responses (41).

Gene deleted viral vaccines. Previously we described the random mutations which occur during attenuation of live-viral vaccines. Very few of these mutations have been localized and if the mutation is a point mutation, the probability of occurrence of a back mutation is high making these vaccines relatively risky. With the understanding of the role of different genes in virulence it is now possible to identify the location of a specific gene and delete it entirely from the virus. Indeed, the first licensed veterinary vaccines using a genetically engineered live-attenuated virus (pseudorabies) was produced by deleting the thymidine kinase gene (69). Based on this original success, a large number of other genes coding for nucleic acid metabolism (thymidine kinase, dUTPase) or for structural and nonstructural proteins have been deleted and tested for attenuation (68, 75). In each case it has been possible to identify nonessential genes which could be deleted from the virus and alter its virulence. This approach dramatically reduces the possibility of reversion to virulence since it is very difficult to reacquire the deleted gene. In addition to attenuating the virus by introducing deletions, such an approach also provides the opportunity to develop companion diagnostic tests to differentiate carrier animals from those who were vaccinated. This will be extremely valuable in countries where eradication of a specific disease is desired. Such approaches are extremely attractive for eradication of herpesviruses which induce latent infections. Presently, a companion diagnostic test is available for differentiating pseudorabies virus vaccinated and carrier animals, and similar approaches are being developed for bovine herpesviruses.

In addition to being able to genetically attenuate viruses by introducing appropriate deletions it is possible to introduce foreign genes into the deleted region of the genome. Thus, the attenuated viral strain would not only function as a vaccine for itself, but could act as a delivery vehicle for introducing antigens from other organisms. Vaccinia virus was one of the first viruses into which foreign viral genes were inserted (95), and it continues to be an extremely attractive vector for vaccine delivery since its genome is very large and it is possible to delete large quantities of the genome and still maintain a viable virus. Another attractive feature of vaccinia virus is that it is economical to culture in cells and it is also thermally stable. A number of countries are using vaccinia virus as a vector against infectious diseases of wildlife

species. For example, vaccinia virus containing rabies virus glycoprotein genes are becoming widely used for control of wildlife rabies (103). The thermal stability of the virus allows it to be incorporated into bait and seeded into rural areas where foxes, raccoons and skunks, potential carriers of the rabies virus, will eat the bait and be immunized against rabies virus (119). Using this approach the number of animals that can be immunized can be greatly increased, thereby reducing the epidemiological spread of the virus in the environment and to domestic livestock and humans. Introduction of genes for rinderpest into vaccinia virus is an attractive approach for controlling rinderpest in development countries (142).

To reduce the environmental concerns of distributing vaccinia virus and their potential to spread to other mammalian species poxviruses from other subgroups (avian) are actively being pursued as vaccine vectors (140). Attenuation is further being achieved by the introduction of cytokine genes into vaccinia virus (36).

Although vaccinia virus appears to be extremely attractive for foreign gene insertion, other viruses such as herpesviruses and adenoviruses are also being actively investigated as vectors for vaccine delivery (67, 82, 109, 144). Regions have been identified within the genome of numerous animal herpesviruses and recently in bovine adenoviruses which are nonessential for *in vitro* or *in vivo* replication. Using these vectors it has been possible to express foreign proteins and induce immunity to these proteins in the natural host. For example, recombinant adeno-rabies virus induces neutralizing antibody and protection against rabies virus challenge of a variety of species (108). The recent identification of deletable regions within the bovine adenovirus suggests that this could also be a very attractive vector for bovine vaccines (90).

DNA immunization. One of the most interesting and exciting areas of vaccinology has been the recent demonstration that direct injection of genes encoding protective antigens results in the expression of protein and development of immunity against that protein. Although this technology was only described recently ,progress has been extremely swift and numerous genes have been shown to function in various tissues and a wide variety of different species (18, 135). The recent observation that DNA can be introduced into eggs (*in ovo* immunization) and induce antibodies has significant implications for immunization of poultry (Paul Johnson, personal communication).

PRODUCTION ENHANCEMENT VACCINES

Until recently, most production parameters in livestock were controlled by diet, probiotics or hormone administration. These continue to be used to alter productivity of animals as evidenced

by the most recent licensing of bovine somatotrophin for enhancing milk production in dairy cattle. However, recent advances in molecular biology, immunology and endocrinology have added an alternative approach to modifying animal productivity. This alternative approach involves immunologically altering hormone levels by vaccination. Although this approach is in its infancy, preliminary evidence indicates that it is possible to regulate animal production and behaviour immunologically, including aggression, feed conversion, growth rates, carcass quality, reproductive efficiency (to either increase or decrease fertility) and other behavioural characteristics of animals (114, 115). The basis for these new vaccines is that many of the characteristics described above are regulated by a complex series of interactions between hormones and host-cell receptors. The interaction of a hormone with the appropriate host-cell receptor results in signal transduction with subsequent alteration of cellular function including the production of other hormones or regulatory molecules. Thus, if antibodies could be produced against a specific hormone or host-cell hormone receptor to block the interaction of the hormone with host cells, regulation of cellular function should be altered. In theory this appears to be straight forward until one considers that hormones are endogenous proteins, requiring the generation of an autoimmune response. The problem of inducing immunity to endogenous hormones is further complicated by the fact that many of the hormones are small. For example, the gonadotrophin releasing hormone (GnRH) is only 10 amino acids in size. To overcome the problem of size and low immunogenicity, peptide or steroid hormones have been conjugated to carrier molecules and combined with strong adjuvants (73, 110, 114). Although this has proven to be effective, alteration of the molecule often occurs during conjugation to carrier molecules, consequently inducing an inappropriate immune response. To overcome this limitation, fusion proteins are being produced to contain the carrier and the hormone as a single chimeric protein. Such an approach often results in immune responses to epitopes involved in interacting with the host-cell receptor. Furthermore, it should be possible to express a number of repeat hormone peptides in one molecule to further enhance the size and immunogenicity of the hormone (13). Such an approach has been used to enhance immune responses to individual epitopes as well as to whole hormones. Finally, multiple copies of the hormone or epitopes can be expressed in conjunction with an immune modulator (cytokine) to further enhance the immune response to the hormone. Examples of cytokine protein chimeric molecules are becoming more numerous, and in each case the results indicate that conjugating a cytokine to a protein enhances the immune response over the co-administration of cytokines and proteins (52). The fact that the immune response can be fine tuned to either develop a Th1 or Th2 response will further allow for more appropriate selection of cytokines to ensure that an

antibody response is generated. Thus, the economics of producing hormone-based vaccines as well as improving their efficacy in stimulating neutralizing antibody responses appears to be within reach. Presently, hormone based vaccines are primarily being investigated for altering reproduction and growth.

Reproduction Modulating Vaccines

The manipulation of reproduction has been of major interest in both veterinary and human medicine for the past 50 years. Activity is directed at both inhibiting reproductive function and improving fecundity. These efforts have resulted in the release of a number of commercial vaccines for use in livestock and experimental vaccines for controlling reproduction in humans. Increases in ovulation following immunization of animals with androstenedione linked to a carrier (85) has resulted in the development of a commercial vaccine Fecundin. Similarly, a reproduction enhancing vaccine based on the hormone inhibin, which promotes the release of follicle stimulating hormone (FSH) with subsequent increased ovulation, has also been developed (14, 38). In addition to active immunization, enhancement of reproductive performance can be obtained by passively immunizing animals. This has been demonstrated in cattle injected with a monoclonal antibody specific for pregnant mares serum gonadotrophin (23, 24). Following passive immunization an increase in ovulation was observed. These observations provide evidence that reproductive performance can be increased both by passive as well as active immunization.

Although enhancing fertility has certain benefits, interfering with the reproductive performance may even have more benefit for many producers. Presently, surgical castration and spaying are used to prevent uncontrolled breeding and to modify undesired behavioural patterns in many domestic animals and pets. Since surgical castration is irreversible the search for reversible methods of controlling reproduction continue, especially for use in race horses and in pets. In the case of race horses, stallions and fillies either have behaviour problems or do not perform at their full potential (26). Since the racing potential of these animals may not be known at an early age early, castration will limit the animals market value and access to desired blood lines after a successful racing career. Therefore, reversible immunocastration would be desirable. In cattle, early breeding can result in major calving and management problems. If one could delay onset of estrus and prevent ovulation this could have a significant impact on animal production. Vaxstrate, a GnRH vaccine has been licensed for exactly this purpose (123).

Growth Promoting Vaccines

The most recent and probably the most controversial development in productivity modifying approaches for livestock has been precipitated by recombinant bovine growth hormone, somatotrophin. Although it has clearly been shown that there is no detrimental effect of administering somatotrophin, public opposition to its employment in meat and dairy animals has been fierce. Therefore, alternative strategies are being pursued to endogenously alter somatotrophin, thus overcoming the perception of hormone adulterated milk and meat. The basis for this altered strategy is primarily based on the observation that enhancement of the activity of growth hormone, can be achieved by binding antibody to somatotrophin. This results in enhanced activity of the hormone (6, 7, 132). The actual mechanism for this is not fully known, but it is possible that antigen antibody complexes may increase the serum half-life of growth hormone or alternatively interaction of antibody with specific epitopes on growth hormone may increase the hormone's signal transduction capacity to make it more active. It is also possible to increase the level of growth hormone by neutralization of another hormone, somatostatin (138). Since somatostatin regulates the level of somatrotrophin release, a decrease in somatostatin activity by neutralization results in the increased release of somatotrophin from the pituitary gland. A vaccine containing somatostatin to enhance somatrotrophin levels plus epitopes of somatotrophin, which would induce antibodies to enhance the biological activity of somatotrophin, should have a significant impact on growth, lactation and more importantly, alter carcass quality by influencing fat synthesis. However, before this approach is embraced, it will be important to determine what other effects somatostatin immunity may have.

ADJUVANTS AND DELIVERY SYSTEMS

Killed vaccines and especially the newly developed subunit vaccines are frequently weak antigens and stimulate short-lived IgM antibody responses. Therefore, in order to reduce the amount of antigen and increase the kinetics, magnitude and duration of the immune response, either an adjuvant or carrier is required. The method of antigen delivery will also dramatically influence the quality of the immune response. For example, due to the common mucosal immune system, which is distinct from the systemic immune system, immunity at mucosal surfaces will require delivery of antigens to this site (9). This is best achieved with live vectors as described above. However, mucosal immunity can also be achieved by linking antigens to cholera toxin carriers, linking antigens to cytokines or incorporating them into microcarriers. Although adjuvants form a crucial component of all killed or subunit vaccines, the field of adjuvants and delivery systems will not be fully reviewed since some very recent reviews have addressed this area (58).

However, some of the salient futures of adjuvants and delivery of subunit vaccines will be described.

Adjuvants

The word adjuvant is used in many ways in biology and medicine. Most commonly, it is used to refer to compounds which act in a nonspecific manner to augment specific immunity to an antigen. Adjuvants are capable of stimulating an earlier, stronger or more prolonged response to an antigen and are useful when the quantity of antigen is limited. It has also been shown in some systems that the immune response to antigen, with respect to humoral or cellular immunity and antibody class and subclass, can be selected for by manipulating both the dose of antigen and the specific adjuvant used in vaccine formulations.

There are several properties which an adjuvant should ideally possess to be effective, safe and practical for use. These include; no toxicity, stable, easy to use, effective, readily available, cheap and lend itself to quality control. Although there are numerous compounds presently utilized as adjuvants, very few fit all of the desired criteria for clinical use. Furthermore, very few adjuvants will be licensed unless a fundamental understanding of the mechanisms by which adjuvants function are known. The existing dogma maintains that deposition of the antigen for a prolonged period of time at the injection site some how promotes the immune response. Deposition has depended largely on the use of nonmetabolizable mineral oils, or antigen absorbed to chemical compounds such as alum. It has been proposed that the deposition of antigen may act in two ways. Firstly, the resulting inflammatory response following injection recruits immune cells which are able to respond to the antigen; secondly, the depot allows the slow release of antigen for a prolonged period of time. Depots are primarily produced by nonmetabolizable mineral oils or antigens adsorbed to chemical compounds such as alum. Both mineral oil and alum adjuvants have been licensed even though they induce granulomas. To reduce granulomas, mineral oils are being replaced by vegetable oils or alternatively by producing oil and water emulsions rather than water and oil emulsions. Using this approach it is possible to reduce the concentration of oil to <1-2% of the vaccine.

A second important property of adjuvants is to activate macrophages or other antigen presenting cells resulting in the release of cytokines or immune modulators. Examples of adjuvants that can stimulate the release of cytokines include bacterial products, plant products, and lipophilic chemicals (DDA and Avridine). These cytokines then help expand the immune responsive cells and increase the number of reactive cells. A third property of adjuvants or carriers is to bind antigens to host cells via a specific receptors. The best example of this

approach is where antigens are linked to immunoglobulin molecules or directly engineered into the immunoglobulin leaving the Fc available for interacting with Fc receptors on antigen presenting cells (143). Finally, adjuvants can act by attracting cells to the site of the antigen to ensure its appropriate constellation of cells are present in the vicinity of the antigen and provide the appropriate stimulation required to drive the immune response. These parameters can be achieved by combining adjuvants and appropriate delivery systems. Furthermore, once the type of immune response required is known it is also possible to drive a Th1 and Th2 response to develop primarily in antibody or a cell-mediated immune response (94). The tremendous explosion and knowledge of immune regulation and interaction and roles of cytokines in immune responses is attracting a considerable amount of attention into the use of cytokines as immunological adjuvants (52, 53). These cytokines are either linked directly to the antigen or incorporated in antigen delivery systems. Since cytokines regulate cellular traffic, antigen presentation, maturation and differentiation of lymphocyte responses it has been postulated that they should provide an approach to both enhance and direct immune response (94). Thus, cytokines should provide us with very effective methods of enhancing immunity to vaccines whether they be whole proteins, epitopes or hormones.

Delivery

Regardless of the quality of the antigen and adjuvant, most subunit vaccines can be improved by appropriate delivery. This is especially important when one considers mucosal immunity. Two methods of delivery that have attracted the most attention for inducing mucosal immunity include biodegradable microspheres and live-vectored viral or bacterial systems (see above). In the case of biodegradable microspheres a number of polymers have been used to adsorb antigen to their surface or to incorporate antigens within the polymer (microspheres). In most instances incorporation of the antigen into microspheres not only protects the antigen from degradation and allows for slow or pulsatile release, but many of the polymers also act as adjuvants due to their particulate nature. These factors combined often lead to significantly increased immune responses over those observed with conventional delivery systems. Furthermore, it is possible to incorporate cytokines or immune modulators into the microspheres to further enhance immune responses. Based on the polymer and the ratio of different components, the antigen can be released at different times and over extended periods such that continuous antigen stimulation leads to the induction of memory and enhanced immune responses (27, 87, 93). Since microspheres can be developed to release antigen at different times, a single injection should result in antigen release at the time of immunization and at later times. Such an

approach would remove the need for booster immunizations. This is especially important in many animal diseases where management systems preclude multiple immunizations. Furthermore, a single immunization would be more economical for the producer since they would not need to handle animals as frequently. Although natural substances such as starch, gelatin and albumin have been used for micro-carrier development, the most popular compounds used today are synthetic bio-degradable products of poly (lactide co-glycolide) These polymers have been used extensively in surgery as resorbable sutures, surgical clips and for controlled release of drug implants. Their extensive use over 30 years provides confidence regarding their safety. Following injection they only produce a minimal inflammatory response (helpful for initiating the immune response) and then degrade by hydrolysis of ester linkages to produce lactic and glycolic acid, natural body constituents. Since the ratio of lactide to glycolide in the co-polymers influences the rate of hydrolysis it is possible to vary the time of antigen release post-administration. Variable release can also be achieved by employing different size microspheres. Microspheres of less than 10 μm usually release more rapidly than microspheres of greater than 50 μm in size. Thus a mixture of two different preparations, one to release antigen early and another one to release antigen late, provides one with a single vaccine that will act as a booster.

Although different size microspheres can be produced, reports clearly indicate that regardless of the route of immunization, microspheres of less than 10 μm are much more effective than larger microspheres. This enhanced immune response associated with smaller microspheres was correlated with phagocytosis and transportation of the particles to regional lymph nodes adjacent to the injection site as well as a more rapid release of antigen.

A second major advantage to microspheres is their potential as oral delivery vehicles. Numerous studies have clearly shown that microspheres can protect antigen if administered orally. Microspheres less than 10 μm in size are absorbed by M cells and are taken up by macrophages in the Peyer's patches (27, 87). Antigen is then transported to mesenteric lymph nodes and the spleen. As a result of oral delivery, a mucosal immune response can be developed against subunit antigens. Such an approach provides an alternative to live-vectors for the induction of mucosal immunity to many pathogens. Whether incorporation of antigens into the feed will be a feasible method of reducing immunization costs needs to be explored. If this was possible, this would dramatically reduce the cost of handling animals during immunization.

Liposomes are also excellent delivery systems for enhancing immune responses to subunit antigens (48). Since liposomes can be made to contain multiple antigens, immunomodulators and agents to target liposomes to specific cells, they have attracted considerable attention as

vaccine delivery systems. As with micro-carriers, the composition of liposomes can be varied to influence antigen release and uptake by antigen presenting cells. The major advantages of liposomes and ISCOMS (see below) is that natural antigen presentation can be simulated. This delivery system is ideal for those antigens that are more immunogenic when incorporated in membranes and others that need to be complexed, a process that only occurs in association with membranes. Furthermore, if antigens are exquisitely sensitive to the reagents required to make microspheres (solvents, heat, etc.), liposomes may provide an alternative for antigen delivery. As with microspheres, liposomes can vary in size ranging from small unilamillar to multi-lamillar vesicles containing several lipid bilayers separated by an aqueous layer. Furthermore, depending on the method of preparation, the ratio of phospholipids to cholesterol and specific amphiphiles, one can vary the charges, stability and fluidity of liposomes. Each of these variables will influence their delivery properties and whether the antigens are entrapped in the aqueous phase or incorporated into the lipid membrane.

Liposomes themselves have been demonstrated to have adjuvant properties. Early studies have indicated that viral antigens and histocompatible antigens can be incorporated into liposomes to induce T cell cytotoxic responses against histocompatible viral infected target cells (32). These studies were among the first to clearly demonstrate the importance of antigen presentation in development of an appropriate immune response. The adjuvant activity can be augmented by the simultaneous incorporation of other immune stimulants such as lipid A, MDP and cytokines within the liposome (55). Since antigen presentation by macrophages and splenic dendritic cells occurs very efficiently with antigens incorporated into liposomes, the ability of MDP to stimulate a wide range of macrophage functions may be at least partially responsible for the enhanced immune responses observed following presentation of antigen to macrophages via liposomes. Inclusion of MDP with liposomes can also change the IgG isotype pattern versus that induced by the same proteins incorporated alone into liposomes (105).

Immune stimulatory complexes, ISCOMS, have also been shown to be very potent antigen delivery systems (92), especially for delivering glycoproteins from enveloped viruses. Using this system, greatly enhanced immune responses have been demonstrated for numerous viruses and presently two vaccines (equine influenza and feline leukemia virus) are being marketed which contain individual components of these viruses incorporated into ISCOMS. The advantage of ISCOMS is that they not only induce significant humoral immunity, but also delayed type hypersensitivity and cytotoxic T cell immune responses following parenteral immunization of antigens incorporated into ISCOMS (129). Furthermore, they have been demonstrated to be effective delivery systems for inducing immunity following oral and vaginal administration (130).

Since a wide range of immune responses are elicited by proteins incorporated into ISCOMS, one must assume that both Class I and Class II responses are induced. This indicates that the antigen processing following delivery by ISCOMS occurs by both the endocytotic and cytoplasmic pathways. This is crucial for the development of desired immune responses to an antigen. ISCOMS containing one or more viral envelope proteins of over 20 different membrane viruses from eight different families have been prepared and tested *in vivo* and all were shown to be highly immunogenic in various animal species (91).

ISCOMS are open (cage-like structures) normally between 30-40 nm in diameter. Since they are composed of cholesterol and Quill A, a complex mixture of glycosides isolated from the bark of *Quillaja saponaria*, they themselves are not immunogenic and therefore can be repeatedly administered to individuals without any adverse side affects. Furthermore they do not induce carrier-induced suppression. A major limitation of ISCOMS is that soluble proteins do not usually possess exposed hydrophobic regions and therefore do not associate well with the ISCOM structure. This is becoming a major impediment to their use since a number of expression systems have been developed that secrete large quantities of protein into the media. In order for efficient secretion to occur, the proteins have been engineered in such way to remove the hydrophobic membrane anchor. Although this provides a mechanism of producing large quantities of recombinant protein, it precludes their incorporation into ISCOMS. To overcome this difficulty, attempts have been made to expose other hydrophobic regions within the protein by partial denaturation at low pH (86). If this treatment alters the immunogenicity of the protein this approach cannot be used. Recently, Reed overcame this impediment by covalently attaching palmitic acid to facilitate incorporation of soluble proteins into ISCOMS (112). Thus, it should now be possible to genetically engineer proteins in such a way as to produce large quantities of soluble proteins, purify them and covalently attach palmitic acid to them for incorporation into ISCOMS. An alternative approach would be to incorporate short hydrophobic tails, by genetic engineering methods, that would be of sufficient length to allow association with ISCOMS, but not interfere with secretion of the protein into the media during its synthesis. Such experiments are presently in progress to reduce the downstream processing required for enhancing immune responses to subunit vaccines.

Recent progress in infectious disease research has allowed the identification of relevant T cell and B cell epitopes involved in inducing protective immunity to a variety of different pathogens. However, due to the small nature of epitopes, immune responses to them is often very poor unless they are attached to larger carrier proteins. To overcome this deficiency a number of individuals have genetically engineered these epitopes into self-assembling virus-like

particles. These includes incorporation of epitopes into hepatitis B, tobacco mosaic virus, polio virus, etc., without disruption of the particle's ability to assemble and furthermore ensuring that the epitope is surface-exposed (33, 51, 136). Although this approach has been shown to induce antibody responses to epitopes from viruses, bacteria and parasites, there is a limitation to the size and the number of epitopes that can be incorporated into self-assembling particles without disrupting their ability to assemble. We have overcome this problem by capitalizing on the ability of rotavirus VP6 nucleocapsid protein not only to self-assemble into virus particles, but also to act as a platform for the attachment of other epitopes and proteins of varying sizes (110, 111). Antibody responses induced to peptide- and protein-VP6 conjugates in the presence or absence of different adjuvants demonstrated that immune responses to the conjugates was as effective in the absence of adjuvants as in the presence of complete Freunds' adjuvant (39). The anti-peptide responses receiving non-adjuvanted and complete Freunds' adjuvants preparations were similar in subclass and titre. Evidence that the VP6 particles may function differently from other protein carriers was that pre-existing immunity to the VP6 did not suppress serological response to conjugated peptides. Specifically, mice with pre-existing immunity to the VP6 carrier through natural exposure or through deliberate infection with rotavirus responded more rapidly to conjugated peptides than non-exposed mice. Thus, VP6 can be used as a carrier/adjuvant without the worry of carrier-induced suppression. Preliminary evidence indicates that uptake of VP6 by macrophages is extremely rapid and that these macrophage cultures are subsequently activated. This activation probably results in a more efficient processing and presentation of antigen due to the increase production of interleukins which influence lymphocyte subpopulations responding to the attached antigens. In addition to attaching epitopes via different linkage technologies it is also possible to incorporate cytokines into VP6, thereby further enhancing immune responses. This is an example of combination of a carrier with immune modulatory cytokines for enhancing immune responses to subunit antigens.

CONCLUSION

Recent progress in molecular biology, protein engineering and immunology have had a tremendous impact on our ability to identify the protective components of many pathogens, including those epitopes that stimulate T and B cell responses. Unfortunately, economical production of the subunit proteins is not sufficient to guarantee that a vaccine will be effective. In many cases the vaccine will only be commercialized if it can be incorporated into a delivery vehicle or adjuvant which gives rapid and long lasting immunity, preferably after a single injection. Although adjuvants have been used for decades, a lack of understanding of how they

work and which adjuvants will stimulate the most appropriate immune response remains an enigma. One common theme appears to emerging: for a compound to act as an adjuvant it must activate at least some cells of the immune system. The recent discovery of Th1 and Th2 cells and the possibility of shifting an immune response to either a humoral or cellular one has provided us with considerable appreciation of the integrate balance between different cytokines involved in generating a specific type of an immune response. Therefore, to be able to design the most effective vaccines in delivery systems, considerably more knowledge regarding the pathogenesis and host responses (humoral versus cellular) involved in clearing the specific pathogen in question is needed. This knowledge, combined with our appreciation of how different adjuvants stimulate the production of different cytokines, alter lymphocyte trafficking, induce antigen processing and trapping are all important areas of investigation that need to be pursued if we hope to capitalise on the recent advances of molecular biology for the production of subunit vaccines.

The understanding of virulence factors and our ability to identify the genes coding for these virulence factors provides us with a much more rational approach to attenuating viruses and bacteria, by deleting specific genes thereby decreasing the risk of back mutation and revert into virulence. This should ensure safer vaccines that can be delivered by the oral or mucosal routes, and stimulate immunity at mucosal sites and thereby blocking the establishment of an infection. Even more exciting is the possibility to use these genetically engineered vectors to carry multiple antigens from other pathogens, thereby providing the opportunity of immunizing animals against a variety of pathogens in a single immunization. This approach has many advantages over multiple vaccinations or combining multiple pathogens in one administration. One major impediment to rapid acceptance of these advances is the concerns by regulatory agencies, who are often extremely conservative when confronted with new technologies. It is hoped that the considerable success achieved with vaccinia-rabies glycoproteins recombinants will alleviate some of these fears. Although there are still a number of areas requiring considerable investigation, progress to date provides a level of confidence that the vaccines of the future will be more efficacious and safer than past products. Furthermore, the advances that have been made in the past decade provide us with a variety of opportunities to alter the vaccine architecture required to provide protection in a variety of species and management situations.

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