Abstract: During the last two centuries a number of vaccines have been used for the control of infectious diseases in animals. Inefficiency of currently used conventional vaccines consist of either whole inactivated or live attenuated microorganism is frequently due to lack of appropriate adjuvants. The use of conventional adjuvant with antigen in preparation of vaccines, induced cell mediated immune responses, but not antibodies at high level. In recent years, to solve this problem, nano-vaccines have been used in animals.

In this kind of vaccine, nano-particles such as latex, polystyrene gold and silica covalently linked to antigen, elicited cell mediated immune responses as well as antibodies with minimal side-effects. The potential efficiency of these vaccines with a single dose has been evaluated in limiting of infectious disease in sheep and tumours in mice. This special issue of methods further addresses practical issues of production, affordability, reproducibility and stability of formulation, and also includes a discussion of the economic and regulatory challenges encountered in developing vaccines for veterinary use and for common Third World infectious diseases. In this article, nano-particles used in veterinary vaccine in discussed in detail.

Keywords: Nano-Vaccines, Nano-Particles, Adjuvant, Immune responses.

1 Introduction

Particulate vaccine delivery systems are well suited for veterinary and wildlife vaccine strategies. Indeed, they are often applicable to a large range of species as they do not rely on specific ways of activating the immune system but rather on basic characteristics of the mostly innate immune responses. However, in some cases adaptations may be required due to anatomical differences. The use of particulate adjuvant also allows for additional routes of immunisation, which are better suited to veterinary and wildlife species including oral delivery and long-distance ballistic intramuscular delivery. These systems protect the antigen from degradation and impact during penetration through the skin and muscle. In some cases, they allow for targeting of several antigens by linking them together. Particulate vaccine delivery systems allow for the induction of qualitatively different immune responses including CTL and other cell mediated immunity. These immune responses are often critical for immunity to key veterinary pathogens including most viral infections [1].

Inefficiency of currently used conventional vaccines is frequently due to lack of appropriate adjuvants. The new generation vaccines consisting of purified proteins and peptides (isolated from micro-organisms, produced by recombinant DNA technology or by direct chemical peptide synthesis, or expressed by relevant DNA constructs) are often weakly immunogenic. To be effective, these vaccines require immunostimulating compounds, adjuvants, which act nonspecifically to increase the immune response to a defined antigen. Search for harmless and effective adjuvants remains to be an urgent need in modern vaccinology [2].

Vaccines for large animals need to be cheap and easy to administer, with minimal side effects. Live or attenuated vaccines, administered by the subcutaneous (SC) or intramuscular (IM) route have been traditionally administered to livestock.
These are usually based on attenuated live or heat killed pathogens and incorporate natural “danger” signals, which promote immunity, but can also cause inflammation systemically and at the site of injection [3]. There are however many diseases for which whole organism vaccines have proven ineffective or unsafe to administer. Thus, newer vaccines with clearly-defined, quality-controlled antigens, such as plasmid DNA-encoded polypeptides, proteins produced in vitro by fermentation and synthetic peptides, have been developed, but these require concurrent administration of adjuvants to provide the activating “danger” signals to trigger an immune response. A current challenge is to develop vaccine formulations which promote protective immunity, but do not have adverse inflammatory side-effects [4].

Adjuvant development has a major role in vaccine technology [5]. Adjuvants are particularly important in eliciting responses to simple proteins delivered in solution. The lack of suitable adjuvants has often hampered the development of effective recombinant peptide and protein vaccines for many diseases. One way of achieving maximal immunogenicity with minimal reactogenicity is to use particulate antigens without adding or inducing inflammatory mediators. It has been demonstrated in mice that antigen covalently linked to inert nano-beads with a size range of 40–50nm are preferentially taken up by dendritic cells (DC) and induce high antibody titres, as well as cell mediated immune responses (including cytotoxic T cells), without significant side-effects. Thus, in mice, the nano-bead based adjuvant met the requirements as an ideal adjuvant for maximising immunogenicity while minimising reactogenicity [6].

2 Nanoparticles and Their Use in Vaccine Formulations

The ability for vaccines to illicit a particular type of immune response is important in conferring protection. Inert particles coated or conjugated to antigen are uniquely designed to provide a targeted immune response with little or no side-effects. Another important consideration is the ease of delivery since different routes of inoculation can also influence local reactions and the type of immune response induced. Micro- and nanoparticle are solid particles made from inert materials or biodegradable polymers. Nanoparticles are often in the range of 10–1000 nm, while micro-particles are larger at around 1–100 µm in diameter [7]. The use of these microparticles is thought to improve immunogenicity with minimal reactogenicity due to the absence of additional inflammatory mediators [8]. The more simplified nano-particle system utilises antigen covalently conjugated to solid core nano-bead of a defined size. It has been demonstrated in sheep that antigen covalently linked to inert nano-beads with a size range of 40–50nm induced antibodies and cell mediated immune responses [9]. The size of these nano-beads, which falls into the size-range of most viruses, is such that they appear to be taken-up preferentially by dendritic cells (DC) [10]. Interestingly, since both mouse and sheep DCs appear to take-up these particles it is possible that the mechanism of uptake of 40nm nano-beads would be conserved within a wide range of species.

2.1 Liposomes

Traditionally liposomes are composed of mainly phospholipids with an aqueous phase inside the particle madeup of a lipid bilayer. The antigen is either enclosed within the particle in the aqueous phase or intercalated into the lipid layer and therefore accessible from the outside. They have been shown to induce both humoral and CTL responses. Several variations on this theme have been given different names [11]. In many cases products such as monophosphoryl lipid A have been included into liposomes hereby confounding the effects of the liposome sensus strictu and immuno-stimulatory properties of its individual components. In addition, certain viral proteins incorporated into liposomes could have immunogenic, fusogenic, targeting and/or other intrinsic properties, which can make interpretation of results rather confusing. To complicate matters the size of the lipid vesicles can also profoundly influence the trafficking of antigen, the uptake by antigen presenting cells and ultimately the type of immune response induced. With the antigen entrapped into liposomes it is possible to deliver vaccines orally. In fish this has allowed the vaccination of carp against Aeromonas salmonicida infection. Liposomes have also been used for the delivery of DNA vaccines. In one study cationic liposomes were used to deliver a plasmid DNA expressing Bovine viral disease virus (BVDV) type 1 major glycoprotein E2, to cattle as a DNA vaccine [12]. In these experiments liposomes were ineffective at enhancing immune responses to the DNA vaccine, with animals vaccinated with the liposome preparation less...
protected then those vaccinated with DNA alone. Liposomes have also been used to deliver allergen extracts as immunotherapy for refractory canine atopic dermatitis [13].

2.2 ISCOM-Based Adjuvants

Immunostimulatory complexes (ISCOMs) were initially described by Morein et al. [14] more than 20 years ago, when viral antigens were incorporated into 40nm particles composed of cholesterol, phospholipid and saponins derived from Quillaia saponaria Molina trees. The use of viral antigens in these initial experiments is not surprising considering hydrophobic membrane associated antigens are much easier to formulate with ISCOMs compared to soluble proteins. In addition, ISCOMs were reported to induce strong CTL responses which are often necessary to effectively control viral infections. More recently, ISCOMATRIX has been developed which differs from ISCOMs in that the antigen is not incorporated into the 40nm particle but instead is mixed with generically prepared particulate adjuvant [15]. ISCOMATRIX induces both humoral and cell mediated immune responses including CTL. Since it is difficult to envisage a mechanism by which soluble (i.e. non-associated) antigen could enter the Class I pathway; one can speculate that the CTL responses induced by ISCOMATRIX occur when antigen is associated to the ISCOMATRIX particles, postmixing, through electrostatic or some other type of interaction. ISCOMATRIX has been shown to induce profound effects on the innate immune response at the level of the local lymph node, resulting in the induction of strong local cytokine responses which affected cell trafficking within the draining lymph node [16] and resulted in strong immune responses in sheep. Interestingly from a practical point of view, vaccination against one disease can have protective efficacy against opportunistic diseases. For example, an ISCOM-based Equine herpes virus 2 (EHV-2) vaccine has been shown to protect not only against infection with this virus; but also against Rhodococcus equi pneumonia for which EHV-2 is a predisposing factor. This type of “cross-protection” might increase in an unexpected way the economic viability of vaccinating animals. It is interesting to note that ISCOM-based H5N1 influenza vaccines were effective in chickens while the unadjuvanted vaccine was not [17]. This suggests that ISCOM-based adjuvants might also be effective in controlling this infection in humans, in case this strain would become highly transmissible between humans.

2.3 Virus-Like Particles

Currently, many vaccines use live (attenuated) or inactivated (killed) virus. Incomplete activation/attenuation or reversion of virulence of these vaccines poses some risk to both humans and animals. In the last 10 years development of an alternated vaccine strategies has involved the identification of viral proteins containing protective determinants. This has lead to the advance of recombinant systems such as Baculovirus, which enable the expression of large quantities of these proteins that mimic these viral proteins. These VLPs morphologically are similar to the empty capsids of viruses. As more information is ascertained with regard to the three-dimensional structure of these viruses it is now becoming possible to engineer vaccines with multiple viral epitopes in order to illicit protective immunity.

VLPs have been used successfully in a number of different species. Roy and colleagues utilising Bluetongue virus (BTV) found that vaccination of sheep with VLPs consisting of all four major proteins (VP2, VP5, VP3 and VP7) gave long lasting protection for 15 months postimmunization. In addition, vaccination with African horse sickness virus (AHSV) VP2 protein in the presence of adjuvant provided protection against challenge in horses [18, 19].

2.4 Ballistic Delivery of DNA

DNA vaccines have traditionally been delivered either intramuscularly or intradermally. While intramuscular injection is regularly practiced in veterinary species, intradermal injection using a needle is too time consuming to be of practical use in most species. Ballistic delivery of DNA coated gold particles therefore offers an attractive alternative. While there have been some reports that gene gun delivery of DNA vaccines induces immune responses in some species [20-23], it seems that this method of vaccine delivery will not be universally applicable. For example gene gun vaccination in sheep skin was ineffective at inducing immune responses. Gene gun immunisation into the ear of dogs seems ineffective at inducing neutralising antibodies to rabies, while injection through the same route did induce neutralising antibodies [24].
2.5 Long-Distance Ballistic Vaccination of Wildlife

The vaccination of large wildlife species presents additional dilemmas of having to approach each animal individually and delivering the vaccine with minimal disturbance to the animal and maximal safety to the operator. To achieve this goal ballistic vaccine delivery systems have been developed which can be shot into the muscle of large wildlife species (e.g. bison) from distances of about 20m using air-powered rifles. These biodegradable “Biobullets” made of photopolymerized PEG hydrogels can serve as biodegradable bullets that deliver volumes of about 90 µl [25], while protecting the carried load from the impact upon penetration into the muscle to a depth of 10 cm. Interestingly these Biobullets can be used to deliver particulate vaccines as demonstrated by the fact that 1 µm fluorescent beads could be effectively delivered intramuscularly using this system. Using this method it has been demonstrated that the bison can be effectively vaccinated against *Brucella abortus* from a safe distances of 20 m [26].

3 Conclusion

Numerous studies have shown that microparticle based technologies represent promising vaccine candidates. Nano-particles in the range of 40–50 nm have the potential to induce potent cell mediated (CD4 and CD8 T cells) as well as humoral immune responses. Recently it was shown that Lipofectin (a liposome) can induce higher IgG1 and IgG2a when combined with pDFll (DNA vaccine for Der f 11, a mite allergen). Nano- and microparticles containing entrapped or adsorbed antigens have been investigated as vaccine adjuvant alternatives to the currently used alum with an objective to develop better vaccines adjuvant and minimize the frequency of immunizations [27]. The discovery that inert nano-beads are able to induce strong immune responses in mice suggested that this novel class of adjuvants could induce immune responses without the side effects typically associated with local tissue damage.

Nano- and microparticle-based delivery systems have been used in veterinary species. These range from the Biobullets (1–2 cm-sized) shot at wildlife with an air-rifle, to the microparticle (0.1-10 µm-sized) used for DNA vaccination, to nano-beads/ISCOM (40 nm-sized) used as adjuvants in injected formulations. Several of these have been used commercially in veterinary species or in the wild. In all cases it seems that veterinary applications have used the intrinsic properties of these systems to solve practical issues associated with veterinary vaccines, including difficulty of access to the animal and need for particular type of immune response.

References