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Review

Carrier molecules for use in veterinary vaccines

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ABSTRACT

The practice of immunization of animals and humans has been carried out for centuries and is generally accepted as the most cost effective and sustainable method of infectious disease control. Over the past 20 years there have been significant changes in our ability to produce antigens by conventional extraction and purification, recombinant DNA and synthesis. However, many of these products need to be combined with carrier molecules to generate optimal immune responses. This review covers selected topics in the development of carrier technologies for use in the veterinary vaccine field, including glycoconjugate and peptide vaccines, microparticle and nanoparticle formulations, and finally virus-like particles.

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1. Introduction

Vaccination of animals as an infectious disease control method has been practiced for over a century with remarkable success. Prior to the past two decades, most veterinary vaccines were either killed products formulated with an oil-based adjuvant or live attenuated vaccines. The field of biotechnology and molecular immunology yielded rapid advancements starting in the 1980s, including the ability to produce subunit antigens in a cost effective fashion for the veterinary market. Since that time, there has been an explosion in the number of vaccines developed for use in production and companion animals as well as the types of killed products available; the latter includes conventional protein and carbohydrate subunits, recombinant proteins, peptides, and more recently, nucleic acid-based products. These antigens have a need for alternative adjuvants and carrier molecules capable of stimulating the appropriate type of immunity at the appropriate site in the body, and numerous technologies including protein conjugation partners, microparticles, nanoparticles and virus like particles have seen use in licensed veterinary and human vaccine products worldwide.

Vaccine targets are also changing, with non-infectious disease targets representing a considerable growth area. These include control of fertility, behaviour and production by immunization against

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Table 1
Overview of carrier technologies discussed in this review.

Carrier molecules	Types	Advantages	Disadvantages	Examples
Protein carrier	Proteins	Act as carrier molecules for oligo- or polysaccharides. Can act as antigen and elicit potent responses against carrier	Requires complicated coupling procedures. Immunogenicity may interfere with booster immunizations	Keyhole limpet hemcyanin (KLH), ovalbumin (OVA), hemolysins, AcrA of *C.jejuni*
	Toxins, toxoids	Common carrier molecules for oligo- and polysaccharides. Mostly toxoids, which are safe to use, immunogenic and very stable	Often very reactogenic, may cause adverse effects when frequently used	Tetanus toxoid, diphtheria toxoid, recombinant form CRM, *A. pleuropneumoniae* exotoxins; *Mannheimia* haemolytica leukotoxin
Microparticles	Poly-lactide-coglycolide (PLG)	Tested in a variety of species and with a variety of antigens, effective for both systemic and mucosal delivery. Have been used in combination with a variety of antigens including DNA-plasmids, proteins and carbohydrates	Require use of organic solvents. Not very stable, and often not effective for mucosal administration. No specific targeting of immune cells or lymphoid structures. Targeting can be facilitated through incorporation of specific ligands	Used for a variety of antigens including DNA-plasmids, proteins and carbohydrates
	Alginate	Organic solvents not required for assembly. Between 1 and 50 μm in size. Fairly stable. Can be used systemically and mucosally. Safe and cost effective	Assembly more complicated, requires special equipment, no targeting of specific immune cells	Use in combination with OVA, *B. abortus antigens, F. hepatica* antigens and others
	Polyphosphazenes	Biodegradable polymer, easy assembly, size can vary from 20 to 50 μm. No organic solvents required. Water soluble. Can be lyophilized. Side chains can be added to influence the type of immunity. Compatible with other adjuvants. Safe and cost effective	Water soluble, some formulations not very stable following mucosal administration	PCEP and PCPP, used with a variety of antigens including RSV, influenza, pertussis and *E. coli* antigens
	Liposomes	Carriers for proteins, peptides and nucleic acid. Liposomes may have adjuvant properties. Multiple layers that allow integration of other adjuvants. Safe to use	Not very cost effective. Large-scale production can be a limiting factor. Usually not very stable. Have been rarely used in animals	Rarely used in animals
Nanoparticles		Range in size from 1 to 1000 nm. Based on lipids, polymers, surfactants and carbohydrates, *i.e.* chitosan or alginate. Safe to use, have the advantage of increased uptake *via* mucosal surfaces. Improve antigen uptake. Ligands can be used to target specific immune cells	Assembly more complicated, incorporation of antigens or ligands sometimes complicated due to small size. Not suitable for all routes of administration	Have been used with enterotoxigenic *E. coli*, and DNA-plasmid encoding Newcastle virus antigens
VLPs		Can be used as vaccine itself or can act as carrier for genetically-fused or covalently linked antigens (chimeric VLPs). During assembly additional antigens can be incorporated. Easy assembly, cost effective and safe. Can be used to target specific immune cells	Assembly limited to specific proteins only, in the case of chimeric VLPs generation and assembly more complicated. Not very stable	Have been used with a variety of antigens including HBV, papillomavirus, rotavirus, influenza, *etc*

hormones or hormone receptors. In addition, protein misfolding targets such as prion diseases are of significant interest for the control of Bovine Spongiform Encephalopathy (BSE), Chronic Wasting Disease (CWD) and scrapie, largely as a means of mitigating the threat of transmission to humans or trade barriers. These types of products require the use of peptide immunogens in many cases and thus need to be conjugated to carrier molecules for optimal immune responses.

For the purposes of this review, a "carrier" is defined as a molecule which is either linked to a vaccine antigen by conjugation or encapsulation. Such carriers can have intrinsic adjuvant activity, but it is not a requirement. We will focus on five areas; glycoconjugate and peptide vaccines, microparticle and nanoparticle formulations, and finally virus like particles (summarized in Table 1). It is recognized that this is not a comprehensive review of carrier technologies in the veterinary field, but rather those which either have had or will have a significant impact on animal health and production.

2. Glycoconjugate and peptide vaccines

It is well established that carbohydrate-specific antibodies can provide protection against pathogenic bacteria expressing surface exposed capsule or lipopolysaccharide (LPS). This led to the early development of polysaccharide vaccines against specific serotypes of *Streptococcus pneumoniae* [1]. Indeed, polyvalent pneumococcal polysaccharide vaccines have been commercially available since the mid 1970s [2]. However, while polysaccharides elicit protective immune responses in healthy adult populations, they are poorly immunogenic in infants and the elderly which led to the development of protein-polysaccharide glycoconjugate vaccines [3]. Apart from their inherent poor immunogenicity, polysaccharides when used alone as vaccines typically elicit low affinity IgM antibodies independent of T-cell help and, as a result, fail to generate boostable memory B-cell responses. By contrast, covalent coupling of a polysaccharide antigen to a protein carrier yields a glycoconjugate that, when used to immunize mammals, elicits T-cell help for