Animal Infection Models for Vaccine Development

Abstract
Animal health is considered one of the cornerstones of the "One Health" concept, which includes the interaction and fight against human, animal and wildlife pathogens able to cause disease. Moreover, reduction of antibiotic use is nowadays a compulsory future direction both in animals and humans. In these scenarios, the control of diseases through vaccination is considered of paramount importance. This review aims to discuss the need, design, use and benefit of utilising animal infection models to develop vaccine products able to reduce the impact not only of human diseases and zoonotic pathogens transmitted by animals, but also diseases causing significant economic problems in livestock.

Introduction

A vaccine is a biological preparation that improves adaptive immunity to a particular disease (WHO). The vaccine product contains a killed or attenuated disease-causing microorganism, its toxins, one or several of its proteins or its DNA or part of it (European Pharmacopoeia, 2013; Kutzler and Weiner, 2008). The previous stimulation of the immune system with these products allow subsequent recognition of the infecting pathogen as foreign, and destroying it. Vaccination is the administration of these products, and it is considered that it has saved more lives worldwide than many other therapeutic interventions combined (Babiuk and Gerdts, 2012). Therefore, vaccination has had, is currently having, and will continue to have, a major impact on the health of both humans and animals, including their inter-connection ("One Health" concept).

The webpage of the One Health Initiative defines the "One Health" concept as a worldwide strategy for expanding interdisciplinary collaborations and communications in all aspects of healthcare for humans, animals and the environment (http://www.onehealthinitiative.com/about.php). Such a scenario implies textually the advance of "healthcare for the 21st century and beyond by accelerating biomedical research discoveries, enhancing public health efficacy, expeditiously expanding the scientific knowledge base, and improving medical education and clinical care". All these concepts are very well represented by vaccinology, which is the science or methodology of vaccine development. Basic and applied research knowledge on diseases and pathogens generated all over the world has expanded incredibly during the last 100 years, and has been the cornerstone for vaccine-producing companies. In the meantime, the vaccine biomedical field has evolved towards a very high-tech and specialised industry, with increasing expenses, risk in the research and development process, and complex regulatory environment (Mahmoud, 2005). Moreover, vaccine development has become very stringent in terms of requirements for the qualitative and quantitative composition, the tests to be carried out, and substances and materials used in their production.

Efficacy and safety are two of the requirements to license a vaccine. There are a number of methods able to test those two aspects during the product development, but in most cases animal models are used. This review aims to discuss the need, design, use and benefit of utilising animal infection models to develop vaccine products able to prevent not only human diseases and zoonotic pathogens transmitted by animals, but also diseases causing significant economic problems in livestock.

Animal Disease Model Considerations

Translational medicine is often described as an effort to carry scientific knowledge "from bench to bedside", representing the process by which the laboratory research results are directly used to develop new ways to treat/prevent illness/cure patients (Cohrs *et al*., 2015). This terminology also applies to the vaccine world (translational vaccinology), and includes an interdisciplinary approach built on basic research advances (including the study of biological in vitro processes as well as animal models) used to develop new vaccine products. Therefore, the use of animal models is an intrinsic component of different stages of vaccine development. However, the use of animals for experimentation is highly regulated and must be driven by the lack of other substitutive methodologies. Importantly, vaccinology is based on the immune response of humans and animals as well as its measurement, which limits the scope of models to work with.

An in-depth knowledge on immune responses against a given pathogen within the target species is the basis for vaccine development, including safety and efficacy testing. Obviously, development of vaccines intended for humans has major limitations since some efficacy studies are not ethical or feasible. In fact, the Food and Drug Administration (FDA, USA) has recently published a document entitled "Product Development Under the Animal Rule Guidance for Industry" (FDA, 2015). The Animal Rule from FDA states that the agency "may grant marketing approval based on adequate and well-controlled animal efficacy studies when the results of those studies establish that the drug is reasonably likely to produce clinical benefit in humans". At this point, however, it must be emphasised that sometimes there is a lack of a fully predictive animal model, as well as well-defined markers of immune protection for human diseases (Girard and Plotkin, 2012). This scenario is summarised with the statement "mice lie and monkeys exaggerate" (attributed to Dr David B. Weiner, University of Pennsylvania, USA), which reflected the difficulties of finding a sound animal

model for human immunodeficiency virus infection, although it can potentially be applied to multiple diseases. Such a scenario would imply, in a number of cases, the need to test directly for efficacy in Phase IIb/III efficacy trials in human volunteers at risk for the particular condition (Girard and Plotkin, 2012). However, previous proof-of-concept stages and pre-clinical efficacy trials are usually done in animal models.

Figure 1. Snatch-farrowed, colostrum-deprived pigs being fed and protected against potential reverse zoonosis (Biocontention Unit with biosecurity level 3, CReSA, Spain).

Another key concept in the use of animals for medicine development, as well as experimentation in general, is the ethics associated with its practice. As early as the 50s, the removal of inhumanity from animal experimentation was discussed in public for the first time and the concept of the "three Rs" was coined (Russell and Burch, 1959). These guiding principles should be applied by any research planning to use animals in experiments (Balls, 1994). The first step is, however, to be sure there is no alternative to the use of animals (1st R: replacement). Second, the objective must be to reduce the number of animals used to a minimum, to obtain information from fewer animals or more information from the same number of animals (2nd R: reduction). Finally, the researcher must refine the way experiments are carried out, to make sure animals suffer as little as possible, including better housing and improvements to minimise pain and suffering and maximise animal welfare (3rd R: refinement).

Animal Disease Model Design

The current design of vaccines is rather different from that of just 10 years ago. The advent of cost-effective high-throughput technologies, coupled with systems biological methods of data analysis, has allowed researchers the discovery and characterisation of a huge variety of molecular components within cells, as well as characterising their interactions to model and understand the behaviour of the system as a whole (Hagan *et al.*, 2015). Systems vaccinology is a new field of research that applies "omics" technologies, in combination with bioinformatics, to study the molecular networks driving vaccine immunity as well as offering novel insights about the immune system (Pulendran, 2014).

Figure 2. Group of lambs allocated at the Biocontention Unit with biosecurity level 3 (CReSA, Spain).

In the context of vaccinology, these abovementioned tools permit emphasising the three Rs concept. However, bioinformatics predictions are not always sufficiently accurate, and testing immune responses and clinical protection in the most adequate animal model is compulsory for most of the vaccines.

Traditionally, the most used disease models involved mice. The use of mouse models has been key to understanding infection and disease and inferring data for other species, mainly human (Lowenthal, 2016). Mice are easy-handling, they have a fast generation time, they are easy to be genetically modified and there are plenty of mouse-specific reagents. However, for a number of diseases, clinical outcome experienced by mice has nothing to do with that of the target species (Lowenthal, 2016). Therefore, the mouse might not be the most appropriate model for a significant number of diseases, in spite of the advantages offered at a laboratory level. As an example, it has been demonstrated that genomic responses in mouse models poorly mimic ("mice lie") human inflammatory diseases (Seok *et al.*, 2013). It is true, however, that mice models have evolved over time, and the advent of humanised, knock-out and transgenic mice represent further steps for some human and animal disease models.

Similarly, non-human primates (NHP) are widely used for human disease models, but working with them is very tightly regulated and considerations of animal welfare and ethics are of higher priority (Julander, 2016). This implies that studies with NHP are usually very difficult and expensive, especially when using biocontainment laboratories with high level biosecurity (3 or 4). On the other hand, NHP tend to display more severe clinical and pathological outcomes ("monkeys exaggerate") than

humans for a number of diseases (Girard and Plotkin, 2012). For example, experimental infection of Rhesus macaques with yellow fever virus generally results in severe disease and a more rapid lethality as compared with the disease in man (Julander, 2016).

Figure 3. Group of calves allocated at the Biocontention Unit with biosecurity level 3 (CReSA, Spain).

Interestingly, certain animal disease models for humans fit with an apparently unexpected species. This is the case with human influenza viruses, for which the ferret model is considered the most suitable to reproduce clinical disease and pathology (Belser *et al*., 2011). As another example, gnotobiotic pigs constitute the best animal model susceptible to human rotavirus infection, which display diarrhoea, anorexia, dehydration, viremia and intestinal lesions mimicking those in young children (Levast *et al*., 2013). In addition, the best fitting model for studying virus-host interaction for hespes simplex virus 1 infection in humans is the pseudorabies virus infection in pigs (Meurens *et al*., 2012).

Large animals often represent superior models when it comes to relevance for humans (Levast *et al*., 2013; Seok *et al*., 2013). Among these, and besides NHP, the use of livestock species such as pig, sheep and cattle in animal models has been an increasing practice during the last 10-15 years. Those species generally offer, depending on the disease of interest, several characteristics as good animals modelling vaccine research: 1) resemblance to the human disease, 2) access to various immune compartments, 3) similar response to the vaccine as humans, and 4) availability of multiple readouts for vaccine efficacy and safety (Gerdts *et al*., 2015).

Livestock Species as Animal Models for Vaccine Testing

The use of swine, cattle, sheep and, to a lesser extent, horses, as experimental models has provided important advances in an increasing number of developmental immunology studies for studying several human diseases, such as influenza, tuberculosis, Crohn's disease, asthma and viral diarrhoea (Conti *et al*., 2014; Gerdts *et al*., 2015). Beyond the obvious advantages due to their body size (easier sampling of tissues or liquids as well as surgical intervention), it is considered that large animals and humans have been developed as outbred populations over time, so it can be expected that their immune systems may have been modelled by exposure to rather similar infectious agents (Conti *et al*., 2014).

Pig has been used as an important biomedical model for decades, including aspects of heart, skin, gut and respiratory physiology, reproductive function, xenotransplantation, tissue engineering and infectious diseases (Lunney, 2007). For the latter, swine probably represents one of the best animal models for vaccine development, since it has a highly similar immune system compared to humans. Specifically, it has been found that the swine immune system resembles that of humans for >80% of analysed parameters, while the percentage was <10% for mice (Dawson *et al*., 2011). Pig models (natural or experimental) for human infectious diseases are summarised in Table 1 (Levast *et al*., 2012; Meurens *et al*., 2012; Gerdts *et al*., 2015).

Table 1. Pig models for human infectious diseases.

Pathogen	Comments
Influenza virus	Various ages (Influenza C and Influenza A subtypes H1N1, H1N2, H2N1, H3N1, H3N2 and H2N3)
Rotavirus	Neonatal gnotobiotic pigs
Norovirus	Neonatal gnotobiotic pigs
Herpes simplex 1	Host-virus interaction
Nipah virus	Young pigs
Bordetella pertussis	Neonatal pigs
Escherichia coli	Various ages, including neonatal pigs
Salmonella spp.	Neonatal gnotobiotic pigs
Shigella spp.	Neonatal gnotobiotic pigs
Clostridium spp.	Neonatal gnotobiotic pigs
Brucella (suis)	Various ages
Staphylococcus aureus	Young pigs
Pseudomona aeruginosa	Various ages
Helicobacter pylori	Neonatal gnotobiotic pigs
Acanthamoeba keratitis	Adult Yucatan micropigs
Chlamydia trachomantis	Adult female (genital model)

Ruminants (bovine and ovine) have also been used as animal models for human diseases, but their range is probably more limited than that of the pig. Table 2 summarises the ruminant models (natural or experimental) available in the literature (Levast *et al*., 2012; Gerdts *et al*., 2015).

Table 2. Sheep and cattle models for human infectious diseases.

Pathogen	Sheep	Cattle
Parainfluenza virus	Neonatal	Various ages, including neonatal calves
Papilloma virus	-	Adult
Mycobacterium tuberculosis	-	Various ages, including neonatal calves
Escherichia coli	Various ages	Various ages, including neonatal calves
Schistosomiasis	--	Various ages
Brucellosis	Various ages	Various ages
Respiratory syncytial virus	Various ages, including neonatal lamb (models for human and bovine viruses)	Various ages, including neonatal calves (bovine virus only as a model)
Rotavirus	-	Neonatal gnotobiotic calves
Salmonella spp.	Various ages	-

Horse has been sporadically used as an animal model for humans, usually for preclinical trials for human vaccines. Due to age longevity of the horse, vaccine testing for those products intended for the elderly can be appropriate in some cases (Gerdts *et al*., 2015).

Those livestock species are excellent models to study some zoonotic diseases, since the vaccines are tested in their own animal host. Sometimes the animal host is able to display a clinical disease very similar to that of humans, but in a number of cases, probably as a result of host-pathogen co-evolution, hosts are just experiencing subclinical infections and act as reservoirs (Gerdts *et al*., 2015). Moreover, the relatively recent increase of contacts between humans and other species (mainly wild ones) in an ever-more borderless world has led to a very significant increase in the number of emerging infectious diseases (Jones *et al*., 2008). Livestock species may represent a bridge between these wild reservoirs and humans, and facilitate transmission of those agents, as well as modulating their virulence by passaging the pathogen through large domestic animal populations. One very recent example is that of Middle East Respiratory Syndrome Coronavirus (MERS-CoV), which caused significant epidemics with high mortality in people from the Middle East (several peaks during the last four years) and South Korea (2015) (WHO). Dromedary camels are considered the most significant animal reservoirs (Reusken *et al*., 2013), and so far it has been demonstrated that vaccination of these animals may decrease significantly the shedding of the virus (Haagmans *et al*., 2016) and, in consequence, its potential transmission to humans. Working experimentally with camels is rather difficult, especially under the biocontention conditions required by the virus, but other large animal species models have so far been developed, including alpacas (Crameri *et al*., 2016), llamas and pigs (Vergara-Alert, personal communication).

On the other hand, and although very useful, large animal models have a number of limitations as well, mainly associated to their size and weight (higher cost associated with housing and experimentation), existence of less immunological tools than mice, and less close to humans than monkeys (Gerdts *et al*., 2015).

Livestock in general is subjected to many non-zoonotic diseases that might be devastating from production, economic and social points of view (Perry and Grace, 2009). Losses are not just coming from the death of animals and consequent decrease of consumable meat or other products (milk, eggs, etc.), but also from slower growth of animals, poor feed conversion rate, poorer meat quality, increased veterinarian costs and farmer labour, and increased use of antibiotics, other medications and decontamination products. All in all, these have direct or indirect social impacts, since these consequences may affect human wellbeing, food availability, and food safety and quality. Therefore, the field of vaccine development in livestock has expanded substantially in the last 20 years, not only regarding technical capabilities but also on the number of pathogens to vaccinate against (Meeusen *et al*., 2007). Animal models for vaccine testing have been developed in target species for a majority of the most significant economic diseases. Moreover, there are still a number of significant livestock diseases for which no vaccines exist in the global market, or which have significant drawbacks, in spite of availability of animal models. Among these are African swine fever virus (Galindo-Cardiel *et al*., 2013), postweaning colibacilosis (Lin *et al*., 2013) and streptococcal meningitis (Segura *et al*., 2015) in pigs, Rift Valley fever in sheep (Busquets *et al*., 2014) and bovine contagious pleuropneumonia in cattle (Gull *et al*., 2013).

Discussion

Vaccinology has evolved considerably since the first vaccination practised by Edward Jenner in 1796 (Stern and Markel, 2005). Nowadays, vaccination is considered as one of the most effective public health tools available to mankind, and has helped in the eradication and control of multiple devastating human diseases. New drugs (including vaccines) serving unmet medical needs are one of the key value-drivers of research-based pharmaceutical companies. However, the efficiency of research and development (R&D), defined as the successful approval and launch of new medicines (output) in the rate of the monetary investments required for R&D (input), has been declining for decades (Schuhmacher *et al*., 2016). This latter statement is mainly applicable to pharmacological products, since the number of vaccines in the market has increased significantly in the last two decades. However, research-based pharmaceutical companies are completely aware of the key factors driving the rate of innovation, R&D cost and probability of success; such a scenario may potentially jeopardise the future development of vaccine products. This situation is evident in veterinary medicine, where the number of medicinal products (including vaccines) intended for minor species (sheep, goats, rabbits, game birds, etc.) is declining due to an also declining market. Moreover, a similar situation applies to human vaccines, since the achievement of a successful vaccine does not only depend on the identification of a public health need, existence of in vitro and in vivo models and a technology platform. The key to successful vaccination mainly depends on a complex set of highly skilled practical and administrative steps, governed by constantly-evolving regulatory frameworks, all of which are ultimately defined by the need for significant financial and technical resources. In consequence, the pharmaceutical industry is expressing concerns about unfavourable market conditions, making the status and future of vaccine development uncertain (Davis *et al*., 2010).

On the other hand, the ever-present threat from infectious disease has recently been demonstrated by outbreaks of Chikungunya, Ebola and Zika viruses in humans, becoming a “public health emergency of international concern”, according to the WHO. Therefore, and despite the constraints expressed above, research in R&D of novel vaccines is guaranteed, both for humans and animals. In turn, the development of new animal models for preclinical studies, always respecting the principle of the 3 Rs, will expand.

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