Chapter 11
The Importance of Animal Models in the Development of Vaccines

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Abstract Efficient translation of basic vaccine research into clinical therapies greatly depends upon the availability of appropriate animal models. Testing novel vaccine candidates in animal models is a critical step in the development of modern vaccines. Animal models are being used to assess the quality and quantity of the immune response, to identify the optimal route of delivery and formulation, to determine protection from infection and disease transmission, and to evaluate the safety and toxicity of the vaccine formulation. Animal models help to make the translation from basic research to clinical application, and they often allow prediction of the vaccine potential, which helps in predicting the financial risks for vaccine manufacturers. Choosing an appropriate animal model has become increasingly important for the field, as each model has its own advantages and disadvantages. In this review, the criteria for selecting the right animal model, the advantages and disadvantages of various animal models, as well as the future needs for animal models are being discussed.

Keywords Animal model • Vaccine development • Vaccine delivery • Infectious disease

11.1 Introduction

Animal models are commonly used to assess a variety of immunological parameters including humoral and cell-mediated immunity, onset and duration of immunity, systemic versus mucosal immunity, protection against challenge infection and
A plethora of animal models exists, ranging from very small insects to very large livestock species, such as horses or cattle. Animal models are being used to investigate very specific immune mechanisms, such as the trafficking and interaction of effector cells, or they can be used to assess larger aspects of vaccine development, such as the induction of herd immunity or to model the spread of a certain disease within a naïve or vaccinated population. Animal models can range greatly, from transgenic and cloned animals to outbred species; from surgical models that facilitate access to certain immune compartments to “humanized” animals; from neonatal to aging animals; and from gnotobiotic to wild type animals. They can be used to model single infections versus co-infections, chronic diseases and autoimmune disorders, and they can be used to analyze herd immunity following vaccination, transmission amongst infected and non-infected animals, as well as studying transfer of passive immunity via the placenta, colostrum, and milk. Thus, choosing the appropriate animal model is critical for the development of modern, more effective vaccines. However, the use of animals for research also comes with an ethical responsibility to treat the animal in the best possible way, and to avoid suffering or unnecessary pain. Thus, the use of animals in research should be limited to circumstances for which no other model exists and should be monitored through ethics committees involving the public.

11.2 Animal Models for Vaccine Research

Testing vaccines in animal models is a critical step in vaccine development, and often the most critical decision point in the long process of developing and registering a vaccine. Hundreds of different models are available to assess various aspects of the immune response. A plethora of species, strains, and mutants are available for these studies and some of them are reviewed in this review.

Many countries promote replacement and reduction of animal experiments for research as much as possible (Wiles et al. 2006), however, as there is no other method currently available to test the induction of immune responses to vaccination the use of animals remains critical in the development of vaccines. However, choosing the most appropriate animal model is crucial for success of the projects and in the long run to save animals and research money. Most vaccines have been evaluated at one point in small rodents, most likely mice. Mice have the advantage of being readily available at a low cost, they are easy to handle, they have defined genetic backgrounds, and their immune functions are well characterized. Furthermore, an abundance of immunological reagents exists for mice allowing a very detailed analysis of the immune response to vaccines. Fewer reagents are available for other species, which limits the level of detail in the analysis. However, large animal species such as pigs, cows and sheep have the advantage of being physiologically and immunologically closer related to man and often are host to the same or closely related pathogens. (Elahi et al. 2007; Gerdts et al. 2001). Moreover, large animal species are predominantly outbred, which is important for the development of vaccines as
a normal distribution for vaccine responders and non-responders can be seen. The genome for most species has been sequenced and annotated (Bishop et al. 2011), or is in the final process of being annotated. A detailed overview of the potential advantages and disadvantages of various species for vaccine research is provided in Table 11.1.

Animal models can be grouped into models used to assess an immune response only, natural disease models, surrogate disease models and surgical or experimental models. These models vary greatly in their scope, their cost and their requirement for special infrastructure.

### 11.2.1 Models to Assess an Immune Response

Models to assess an immune response typically include mice and small rodents, and in most cases are based on the use of specific strains, or knockouts. For example, the linkages between innate and acquired immune response to vaccination can be assessed by using mice that are defective in innate signalling pathways, such as MyD88<sup>−/−</sup> or TRIF<sup>−/−</sup> mice. To assess the type of an immune response induced by a specific vaccine Balb/c mice versus C57 black are commonly used, since reagents are available to assess both cytokine secretion and specific antibody isotypes. However, numerous other strains are available to assess the immune response in mice. Other species commonly used include rabbits, rats and guinea pigs. The advantages of these models is the ability to rapidly assess the immune response to a certain antigen and are commonly used for large screen testing of adjuvants, vaccine formulations or for the assessment of the best route of immunization. Specific strains, knockouts, or even humanized animals are being used to assess certain qualities of the immune response including a shift towards T helper (Th) 1, Th2 or Th17 responses, induction of mucosal versus systemic immunity, onset and duration of immunity etc. The one key characteristic though is that these models can’t be used to assess protection against infection, and thus are somewhat limited for the development of vaccines.

### 11.2.2 Surrogate Models

These models are commonly used in preclinical vaccine development and refer to the use of species that only under experimental conditions can be infected with the pathogen of interest. These models are somewhat artificial as often higher infection doses, artificial routes of infection, or lack of clinical symptoms are being used. However, they offer the advantages of working with animals that can be easily housed and handled, are cost-effective or are well defined in terms of the immune system. Most often mice are being used, not only for developmental purposes but also from a regulatory point of view for registering a vaccine product, as it allows
| Species | Advantages | Disadvantages |
|---------|------------|---------------|
| Mouse  | Low cost ($20–120 per animal); easy to handle; can be housed in groups in cage systems/microisolators; immune system very well characterized; short breeding cycles; hemo-chorial placentation allowing transfer of some IgG subclasses; abundance of reagents and assays available; genetically well defined and matched; adaptive transfer of immune cells possible; plethora of well-defined transgenic strains available | Small size; access to mucosal surfaces and several immune compartments limited; manipulation of neonates very difficult; short neonatal period; limited routes for vaccine delivery; PRR-expression on dendritic cells differs from humans; short life-span |
| Rat    | Low cost ($60–120 per animal); easy to handle; can be housed in cage systems/microisolators; immune system very well characterized; relatively short breeding cycles; hemo-chorial placentation allowing transfer of some IgG subclasses; reagents available but limited; some MHC-matched strains available | Not as easy to handle as mice; less reagents available; small size; limited access to immune compartments and mucosal surfaces; need to be housed in smaller groups, sometimes individually |
| Cotton rat | Moderate cost ($80–120); can be housed in cages; excellent model for respiratory infections; reagents for common cytokines and biomarkers available; hemo-chorial placentation allowing transfer of some IgG subclasses; moderate life-span | Require individual housing; difficult to handle; small size, limited access to certain immune compartments; few reagents available |
| Woodchuck | Excellent model for hepatitis virus infections; hemo-chorial placentation; relatively long life span (up to 20 years in captivity) | Require individual housing; specific needs; difficult to handle; small size; limited access to certain immune compartments; few reagents available |
| Guinea pig | Moderate cost ($50–150); easy to handle; can be housed in cages; routinely used to produce polyclonal sera | Small size; limited access to immune compartments; few reagents available |
| Rabbit | Moderate cost ($50–150); easy to handle; can be housed in cages or groups; good serum donors; larger life span; hemo-chorial placentation | Require infrastructure to house larger numbers; fewer reagents available |
| Ferret | Moderate cost ($150–300); excellent model for respiratory viral infections including influenza; outbred species; access to mucosal compartments | Small size; difficult to handle; requires specific training and infrastructure |
| Pig | Moderate cost ($50–1,000); large size allows access to mucosal surfaces and various immune compartments; physiologically very similar to humans; epithelio-chorial placenta type; no transfer of antibodies; access to fetus and fetal tissues; genome has been sequenced and is currently being annotated; outbred species; MHC-matched lines available that facilitate adoptive transfer; “minipigs” that are smaller in size and easier to handle; widely accepted model for xenotransplantation; mucosal delivery of vaccines possible; large toolkit available | Requires facilities and training; anatomically reverse lymph nodes; grow very fast; host to endogenous retroviruses. |
| Species          | Advantages                                                                 | Disadvantages                                                                 |
|------------------|----------------------------------------------------------------------------|------------------------------------------------------------------------------|
| Sheep, goat      | Moderate cost ($100–300); easy to handle; large size allows access to mucosal surfaces and various immune compartments; epithelio-chorial placenta type; no transfer of antibodies; access to fetus and fetal tissues; long neonatal period; functional mucosal immune system at birth; mucosal delivery of vaccines easily possible; outbred species | Requires special facilities and training; seasonal breeders, lambs available max three times a year; only 1–2 lambs per ewe |
| Cattle           | Large size allows access to mucosal surfaces and various immune compartments; physiologically very similar to humans; epithelio-chorial placenta type; no transfer of antibodies; access to fetus and fetal tissues; mucosal immune system fully functional at birth; access to various immune compartments including colostrums and milk; long neonatal period; genome has been sequenced at annotated; reagents for the most common cytokines, isotypes and biomarkers available; mucosal delivery of vaccines; outbred species | Moderate cost ($500–2,000); requires special facilities and training; long breeding cycle, only 1–2 offspring per year |
| Horse            | Epithelio-chorial placenta type; long-life span; excellent model for the elderly; access to a variety of immune compartments including the mucosal surfaces; long neonatal period; mucosal immune system fully functional at birth; outbred species | High cost ($500–3,000); need for special facilities and training |
| Dog              | Moderate cost ($150–450); longer-life span; access to immune compartment and mucosal sites; endothelial-chorial placenta type; outbred species | Need for special facilities and training; not as easy to handle in larger groups; few reagents available; immune system not fully developed at birth |
| Cat              | Moderate cost ($100–250); longer-life span; access to immune compartment and mucosal sites; endothelial-chorial placenta type; outbred species | Need for special facilities and training; not as easy to handle in larger groups; few reagents available; immune system not fully developed at birth |
| Non-human primate | Very costly ($1,000–20,000); physiologically very similar to humans (depending on species); easy access to mucosal sites and many immune compartments; immune functions well defined; outbred species; hemo-chorial placenta type | Very costly ($1,000–20,000); need for special facilities and training; immune system develops post partum |

*MHC* major histocompatibility complex, *PRR* pattern recognition receptor
screening of large numbers of candidate vaccines in a rapid and efficient way and in most cases is more cost effective. In particular the ability to specifically knock out individual genes has helped in the understanding of very specific immune functions and the ability to adoptively transfer immune cells from one animal to another is another major advantage of using mice as surrogate model. More recently, the creation of “humanized” mice, which are generated by the transfer of human stem cells into fetal animals, has further enhanced the potential of surrogate models for vaccine development (Macchiarini et al. 2005; Shultz et al. 2007). However, the use of other species as surrogate models is becoming more and more popular. For example, cotton rats are widely accepted as an excellent model for respiratory viruses, and ferrets are being used to model Influenza virus infections. Guinea pigs and domestic pigs can be used for tuberculosis research, and pigs are being used for a number of pathogens including Enteromoeoba histolytica (Girard-Misguich et al. 2011), Chlamydia trichomatis and Hendra virus (Meurens et al. 2012). We recently developed a novel model for pertussis in newborn piglets (Elahi et al. 2005). This model resembles the disease in human much closer and allows the assessment of both vaccine induced immune responses as well as study of the interaction between the bacterium Bordetella pertussis and the host (Polewicz et al. 2011). Interestingly, pigs are natural host to B. bronchiseptica, and thus many of the results can be directly translated into the development of veterinary vaccines (Elahi et al. 2007). Thus, the use of surrogate models has many advantages over models that are being used to assess the immune response to vaccination only. Surrogate models can be used to understand the role of various aspects of the immune responses including innate and acquired immunity, mucosal versus systemic immunity as well as trafficking of effector cells from one immune compartment to another, but offer the major advantage that these findings can be correlated with protection against experimental challenge infection.

11.2.3 Natural Disease Models

These models are based on a specific pathogen and its natural host and have the advantage of resembling the interaction between host and pathogen within the appropriate biological context. Thus, natural models can be used to analyze various aspects of the immune response to immunization and infection including the role of virulence factors during invasion, penetration and toxicity, as well as the host’s immune response to the pathogen. Natural disease models include many large animal species, which has proven to be a very successful strategy for developing vaccines against both human and animal diseases (Table 11.1). For example, the use of large animal models has helped in the development of vaccines against several important infectious diseases including Herpes simplex virus (HSV) infections, Escherichia coli, Rota- and Coronavirus, Respiratory syncytial virus (RSV), Influenza and West Nile virus (WNV), to name a few (Baron and Coombes 2007; Hall and Khromykh 2004; Osterrieder et al. 2006; Potter et al. 2004; Rouse and Kaistha 2006). An important advantage of large animal models is the ability to use the natural route
of challenge and therefore obtain more relevant correlates of immune-mediated protection. In addition, using large animal models one can find high- and low-responders, which then can be further characterized using genome, proteome and kinome analysis (Jalal et al. 2009; Wilkie and Mallard 1999).

Vaccine efficacy also varies dramatically when immunizing the very young or the elderly (Lambert et al. 2005; Lang et al. 2011; Moxon and Siegrist 2011). Natural disease models including Parvovirus, E. coli and Rotavirus infections in pigs and calves have been used to establish the concept of maternal vaccination as an effective strategy to reduce the risk of infection in the neonate. These studies identified vaccine strategies to optimize the passive transfer of maternal immunity to the newborn and determined the duration of protection following passive transfer of maternal antibodies (Dobrescu and Huygelen 1976; Kohara et al. 1997; McNulty and Logan 1987; Mostl and Burki 1988). As a result, this concept has been introduced into human medicine and several vaccines are now available for immunization of pregnant mothers, and additional candidates are being considered by several countries in the world (Blanchard-Rohner and Siegrist 2011; Edwards 2003; Poehling et al. 2011).

Another major advantage of natural disease models is the ability to study co-infections between two or more pathogens. There is increasing evidence in the literature that co-infections substantially contribute to the establishment of disease, and in many cases are responsible for severe complications and even lethal disease outcomes. This is the case for many viral infections as these are typically followed by a secondary bacterial infection. However, it is also believed to be the case for two viral infections, such as Hepatitis B and C virus (Rodriguez-Inigo et al. 2005), or others. Several co-infection models are well established in large animals including models for respiratory infections in cattle such as combinations of Respiratory bovine coronaviruses (RBCV)/Pasteurella haemolytica (Storz et al. 2000), Bovine herpes virus 1 (BHV-1)/Mannheimia hemolytica model (Yates 1982), Bovine virus diarrhea virus (BVDV)/Mycoplasma bovis (Prystia et al. 2011) to name a few. Other examples include a Porcine reproductive and respiratory syndrome virus (PRRSV)/Streptococcus suis model in pigs (Xu et al. 2010). Thus, using natural disease models has the advantage of being able to study the effect of multifactorial or co-infections in the same host.

11.2.4 Surgical Models

They have been used to explore various aspects of vaccine formulation and delivery, including the route of administration, targeting to specific receptors and the induction of mucosal versus systemic immunity. Surgical models allow access to specific immune compartments such as the intestine, lymph nodes or skin tissues. For example, we developed an intestinal gut-loop model in large animals (Gerdt et al. 2001), that can be used to assess the potential of oral vaccines in vivo. Following the original concept of Thierry-Vella loops (Yardley et al. 1978), this model is based on the surgical creation of independent intestinal segments that can remain within the animal for
more than 6 months without altered blood or lymph support (Gerdts et al. 2001). After a certain period of time, the segments can be collected and the immune responses in each segment in Peyer’s patch, lamina propria and intestinal epithelium assessed (Meurens et al. 2009). The major advantage of this model is the fact that the loops are independent from each other and thus allow the assessment of multiple immune responses to different vaccine formulations within the same animal. This model is now available in a number of species including calves, sheep, pigs and even chicken (Aich et al. 2007). Other surgical models include cannulation of blood vessels or even lymphatics, which allows for the collection of large numbers of specific immune cells (Yen et al. 2006). For example, pseudoafferent lymph which is especially rich in dendritic cells can be collected after removal of the lymph nodes and subsequent stenosis of afferent and efferent lymphatics (Rothel et al. 1998). Other examples of surgical models include the insertion of catheters or pumps for vaccine release at very specific sites, slow release over time or even placement of a bolus to analyze a depot effect.

11.2.5 Experimental Models

Animal models are also being used to assess specific issue such as vaccine delivery, topical application or safety and toxicity of vaccine formulations, or individual components thereof. In most cases, this is required by regulatory authorities, which often require the use of at least two species to show safety, in most cases small rodents. However, large animal models have been recognized as useful models. For example, the physiology of the skin is very similar between humans and pigs, which make the pig a good model for studying intracutaneous or topical delivery of vaccines, as well as assessing the safety of novel vaccine formulations.

11.3 Choosing the Best Animal Model

The ethical use of animals in vaccine research requires that we only choose animals that resemble the disease as closely as possible or that will help to address very specific issues. This should be considered every time an animal experiment is planned. Three examples of considerations for choosing an appropriate animal model are provided below.

11.3.1 Induction of Both Mucosal and Systemic Immunity

The vast majority of pathogens enter via the mucosal surfaces. The induction of both systemic and mucosal immunity, therefore, is an important goal of future vaccines, and models are required to assess whether future vaccines effectively
induce mucosal immunity (Gerdt et al. 2006). Not every animal model is well suited for the assessment of mucosal immune responses, as the size of the animal itself and that of the oral and respiratory tract predetermines the accessibility of the mucosal tract, the volume of injection, and the actual route of immunization. For example, intranasal vaccination in mice is often associated with inhalation and ingestion of vaccine antigens, which makes it difficult to discriminate between intranasal, oral and intrapulmonary vaccination. In contrast, larger animal models can be used for the controlled delivery of vaccines to the nasal passages and provide easier access to the mucosal surfaces themselves and mucosal compartments in (Gerdt et al. 2006, 2007). For example, sufficient quantities of intraepithelial lymphocytes and lamina propria lymphocytes can be isolated from the mucosal surfaces of pigs, sheep and cattle, without having to compromise on the number of immune cells or having to pool cells from different compartments (Gerdt et al. 2001). Indeed, the nasal passages of sheep and cattle more closely resemble that of humans, and display similar patterns of development (Hein and Griebel 2003; Mutwiri et al. 2002). In these species the mucosal immune system develops well before birth, which stands in clear contrast to mice, in which the mucosal immune system only develops after birth. As mentioned above, intestinal models have been developed that allow controlled vaccine delivery to specific mucosal sites including the intestine and which can be used to evaluate mucosal vaccine delivery technologies and adjuvants (Gerdt et al. 2001; Mutwiri et al. 2005).

### 11.3.2 Immunization of Neonates

Neonates are amongst the most susceptible to infectious diseases and millions of infants and young children die every year due to infection with infectious pathogens. This is due to a number of factors including the challenges associated with a developing immune system, an inability to respond to glycoconjugate vaccines, limited access to vaccines, as well as the absence of vaccines for devastating diseases such as RSV and others (PrabhuDas et al. 2011). Vaccine research specifically for neonates, however, is currently hampered by the absence of good animal models to study the induction of immune responses and immune memory in the context of a neonatal immune system. For example, the neonatal period in mice is much shorter than in man, which makes the use of mice for developing neonatal vaccines highly problematic. A number of large animal models may be more representative of immune system ontogeny in humans (Elahi et al. 2007). For example, using a fetal lamb model we were able to show that oral immunization with a DNA vaccine was highly effective in fetuses and induced strong mucosal and systemic immune responses, as well as long-term memory in the developing immune system (Gerdt et al. 2000, 2002). Large animal models may be much more appropriate for evaluating vaccine immune responses in the neonate and addressing questions regarding possible interactions between vaccines and maternal antibodies (Polewicz et al. 2011). For example, novel vaccine formulations including adjuvants have to
| Delivery route  | Administration                          | Advantages                                      | Disadvantages                                      | Common animal models                                                                 |
|----------------|----------------------------------------|-------------------------------------------------|---------------------------------------------------|--------------------------------------------------------------------------------------|
| Oral           | Feed, water, intragastric tube, gut-loop model | Induction of mucosal immunity, easy to administer | Uptake via the mucosa remains difficult, requires special delivery systems | Most species including mice, rats, poultry, ferrets, pigs, sheep, cattle, dogs, and fish |
| Ocular         | Droplets, spray                         | Induction of mucosal immunity                   | Delivery difficult in most species                | Poultry, fish                                                                      |
| Intranasal     | Spray, droplets                         | Induction of mucosal immunity                   | Problematic in smaller animals; not practical in some live-stock species | Mice, rats, dogs, pigs, sheep, cattle, horses                                            |
| Intravaginal   | Suspension; emulsion                    | Mucosal immune response, local                  | Not practical in many species                     | Mice, guinea pigs, pigs, cattle                                                    |
| Topical        | Patches, crème                          | Systemic immune response                        | Not practical in many species                     | Mice, pigs, cattle                                                                 |
| Intradermal    | Patch, micro-needles, gene-gun, electroporation, laser | Systemic immune response; good uptake by APC    | Not always practical                              | Mice, pigs, cattle                                                                 |
| Intramuscular  | Syringe, needle, injector, gene-gun      | Systemic immune response                         | Local site reactions, may affect carcass quality  | Most species                                                                       |
| Intraperitoneal| Syringe, needle, injector                | Systemic immune response                         | Risk of injecting into intestine                  | Mice, fish                                                                        |

*APC* antigen presenting cell
be specifically tailored to the neonatal immune system, as recently demonstrated by combining three novel immune modulators into one adjuvant platform. This platform, consisting of host defense peptides, polyphosphazenes and CpG oligodeoxynucleotides, proved highly effective after a single immunization in both neonatal and adult mice when combined with pertussis (Gracia et al. 2011) and RSV antigens (Kovacs-Nolan et al. 2009). Other combination adjuvants are currently under development (Mutwiri et al. 2011).

11.3.3 Novel Routes of Delivery and Devices

An area of rapid development in vaccine research is the area of vaccine delivery. Both human and animal vaccines are moving away from needles, either because of the risk of broken needles in meat products or because of the low compliance rate in young children and infants. Interestingly, the recent pandemic has revealed that even in adults, the injection via needle is becoming less accepted by the public. Thus, novel strategies for vaccine delivery are required, using needle-free injectors, intradermal patches or topical applications. Appropriate animal models are required that firstly resemble the skin physiology in humans, secondly allow testing of injectors and that at the same time allow delivery of the vaccine under real conditions (Table 11.2). Both pigs and cows have been frequently used to assess such novel vaccine technologies, and allow intradermal application of even larger volumes of vaccine (van Drunen Littel-van den Hurk et al. 2006). Needle-free devices such as electroporation (van Drunen Littel-van den Hurk and Hannaman 2010) have been shown to be highly effective in cattle and pigs (van Drunen Littel-van den Hurk et al. 2008, 2010) and are currently developed for practical application. Other devices, such as needle-free injectors have been successfully tested in pigs.

11.4 Conclusions

Animal models are critical for the development of vaccines. They are required to determine the quality and quantity of an immune response to vaccination, they are required for assessing the safety and toxicity of vaccine formulations, they are used to determine the efficacy of the vaccine in providing protection against challenge infection, and they are often used to assess the potential of preventing disease transmission within a specific population. Thus, selecting the most appropriate animal model for the specific needs of the research project is critical, and rather than being driven by low cost and ease of handling, researchers should look for models that closely resemble the target species and thus produce results that could be quickly translated into real products. In the long term, large amounts of money, time and resources can be saved that way.
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