



Formulier

Projectvoorstel dierproeven

- Dit formulier gebruikt u om uw projectvoorstel van de dierproeven te schrijven
- Bij dit formulier hoort de bijlage Beschrijving dierproeven. Per type dierproef moet u deze bijlage toevoegen.
- Meer informatie vindt u in de 'Toelichting op de te gebruiken formulieren voor de aanvraag van een projectvergunning' op de website www.centralecommissiedierproeven.nl.
- Of neem telefonisch contact op. (0900-2800028).

1 Algemene gegevens

- 1.1 Vul uw deelnemernummer van de NVWA in.
- 1.2 Vul de naam van de instelling of organisatie in.
- 1.3 Vul de titel van het project in.

2 Categorie van het project

- 2.1 In welke categorie valt het project?
- U kunt meerdere mogelijkheden kiezen.*
- Fundamenteel onderzoek
- Translationeel of toegepast onderzoek
- Wettelijk vereist onderzoek of routinematige productie
- Onderzoek ter bescherming van het milieu in het belang van de gezondheid of het welzijn van mens of dier
- Onderzoek gericht op het behoud van de diersoort
- Hoger onderwijs of opleiding
- Forensisch onderzoek
- Instandhouding van kolonies van genetisch gemodificeerde dieren, niet gebruikt in andere dierproeven

3 Algemene projectbeschrijving

3.1 Achtergrond

Licht het project toe. Beschrijf de aanleiding, de achtergrond en de context. Besteed aandacht aan de bij vraag 2.1 aangekruiste categorieën.

Human respiratory syncytial virus (RSV) is the commonest cause of lower respiratory tract infection (LRTI) in children, worldwide, causing disease in an estimated 34 million children, >3 million hospitalisations, and 66,000–199,000 deaths in children under 5 years, each year (1). The majority of deaths occur in low- and middle-income countries (1, 2). Most children are infected during the

first year of life, and all have been infected by their second year. Although there is limited viral antigenic variation, the duration of immunity induced by RSV is short-lived and recurrent infections occur throughout life (3). The peak incidence of severe disease is in infants 2–7 months of age. Moreover, RSV infection in infancy is also associated with the subsequent development of chronic respiratory morbidity (e.g., asthma, wheezing) (4). Because RSV immunity is short-lived, repeated infections occur throughout life. Besides children, RSV also contributes to excess mortality in the elderly (5, 6) and in immunosuppressed individuals of any age (7). Few epidemiological data on RSV infections are available in adults, but it is estimated to cause up to 5% of community-acquired pneumonia, mainly in older adults and those with co-morbidities in whom a 9 to 12% case fatality rate is observed (8, 9). The economic impact of RSV disease in adults is estimated to be greater than that of influenza in relation to numbers of days lost from work (10, 11). There is no licensed RSV vaccine and only one effective anti-viral therapy (monoclonal antibody). The availability of an effective RSV vaccine would reduce the impact of RSV infections in the target groups and contribute to public health. An alternative strategy to protect neonates from RSV infection is vaccination of pregnant women. Therefore RSV vaccine candidates are being developed for three main target groups: infants and toddlers (6 mo to 5 yrs), pregnant women and the elderly.

The name Respiratory Syncytial Virus is derived from an effect mediated by the viral F protein, causing membrane fusion with virus infected and neighbouring cells forming so-called syncytia. RSV is a negative-sense, single-stranded RNA virus of the family Pneumoviridae. Two antigenic subgroups of RSV are known A and B, with subgroup A being responsible for severe clinical cases and subgroup B with asymptomatic cases. The RSV genome is approximately 15 k base-pairs, with 10 genes encoding a total of 11 proteins (12). Of these proteins the F protein (fusion), expressed on the virus surface, is pursued as a vaccine antigen. The F protein exists in two distinct conformations: pre- and post-fusion, but only the pre-fusion conformation is able to induce potent neutralising antibodies. The F protein amino acid sequence is relatively conserved and, unlike influenza, does not require frequent antigenic updating. The second surface-expressed protein is G, which is involved in initial virus attachment, it is highly variable and appears to be non-essential, as viruses without it only have an attenuated phenotype. Therefore, the G protein is not considered as a vaccine antigen. Besides the viral surface antigen F which aims at inducing neutralising antibodies, the conserved nucleo- (N) or matrix-proteins could be considered for cell-mediated immunity inducing vaccines.

The fact that neither natural (13) nor experimental human infection (3) induces robust immunity against reinfection, implies that a RSV vaccine is expected to prevent severe disease, rather than provide sterilising immunity. Passive immunisation with Palivizumab, a humanised mouse monoclonal antibody directed against the RSV pre-fusion F protein, is the only treatment option for severe RSV infection (14), as no RSV vaccines currently are available. Palivizumab has a relatively short half-life (about 20 days), and thus, monthly intramuscular injections are required during the RSV season to provide protection. It is also expensive, thus limiting its use to very high risk individuals (e.g., those born extremely prematurely with chronic lung disease of infancy or infants with major congenital cardiac disease) in high-income countries (15).

RSV vaccine development has been hampered by historical results obtained in the late 1960's with formalin-inactivated (FI) RSV vaccines, which increased the risk of severe RSV infection in children (16). This failure is likely due to absence of pre-fusion F protein in FI-RSV vaccines, with the remaining post-fusion F protein inducing only poorly neutralising Ab (17). Moreover, FI-RSV vaccines induce an undesired, potentially dangerous, Th2-biased response (18, 19). Recent advances in structural biology have led to the development of RSV-F protein antigens that are stabilised in the pre-fusion configuration (20, 21) and numerous RSV vaccine candidates are being clinically tested (22).

Cotton rats and mice are widely used as models of RSV infection and have provided insights into mechanisms of immunity to and the pathogenesis of RSV infections. Although cotton rats are semi-permissive for virus replication, they are about 100-fold more permissive than BALB/c mice per inoculum dose of virus, but they do not develop clinical signs of disease (23). Following IN inoculation, RSV replicates to high titres in the nose and lungs, but to lower titres in the trachea. Cotton rats are susceptible to infection throughout life, but virus replication is greater and persists for longer in the nasal passages of 3 day-old rats than in older animals (24, 25). Ferrets can also be used for the study of RSV infection, but only few reports have hitherto been published (26). Bovine RSV (BRSV) in young calves is an alternative model for epidemiology, pathogenesis and vaccine efficacy studies (27). A recent review

provides information on RSV infections in Non-Human Primates (NHPs) (27). grivets (*Ceropithecus aethiops*) (28), rhesus monkeys (*Macaca mulatta*) (29), bonnet macaques (*Macaca radiata*) and cynomolgus monkeys (*Macaca fascicularis*) (18, 30) are all semi-permissive for human RSV infection. Infant, young and adult macaques are all equally sensitive to RSV infection and mount local and systemic immune responses that protects from re-infection (30).

Of the different animal models used in RSV vaccine research, only NHPs have a unique close homology to humans in most components of their immune system (31-33). For instance, similar T and B-cell subsets have been described in NHPs (33). Moreover, the immunoglobulin gene germline repertoire is highly conserved between macaques and humans, which is important when induction of broadly neutralizing antibodies (34, 35). In addition, structure and function of Fc receptors, which are essential for the function of non-neutralizing antibodies, show many homologies between macaques and humans (36). Only very limited information is available on Fc receptors in ferrets or cotton rats and only few reagents for the in-depth analysis of immune responses are available (37). Finally, in NHPs the innate immune system, including molecular pathways and antigen presenting cell subsets, are much more homologous to humans than what is seen in mice (31). NHPs not only most closely reflect the human physiology, but also resemble humans in their clinical symptoms, limited pathology, pattern of viral replication and cytokine and chemokine responses following RSV infection (30). In conclusion, the strong immunological and physiological resemblances to humans make NHPs a unique model in pre-clinical safety, immunogenicity and efficacy evaluation of RSV vaccines.

3.2 Doel

3.2.1 Beschrijf het directe en het uiteindelijke doel van het project. Beschrijf de bijdrage van het behalen van het directe doel aan het uiteindelijke doel.

- Indien het directe doel bestaat uit verschillende subdoelstellingen, benoem deze dan hier.

The main objective of this proposal is to evaluate novel RSV vaccine candidates for safety, immunogenicity and protective efficacy following RS-virus infection in macaques. The capacity of new vaccine candidates to elicit durable neutralising immune responses will be evaluated under this project application. The ultimate objective of this project is to develop a RSV vaccine that induces durable protective responses against RSV infection. Vaccines that will be tested in NHP have to be in the final stages of preclinical development and require this final validation step in order to assure that there are no adverse effects that were missed in the preliminary studies in other species and that they are effective in an animal species that has an immune system closely related to humans.

The project has two objectives: 1. To evaluate novel RSV vaccine candidates for safety, immunogenicity and protective efficacy following RS-virus infection in macaques using the established RSV-macaque infection model and 2. Establishment of novel RSV infection models using a different challenge strain or route of infection.

3.2.2 Hoe wordt de haalbaarheid van het directe doel gewaarborgd?

At our institute we have been performing vaccine evaluation studies in NHP for over 20 years. Most vaccine candidates were directed against human immunodeficiency virus, hepatitis B virus, hepatitis C virus, and tuberculosis. Since 2012 we have been working on influenza virus infection in macaques and the evaluation of vaccines against influenza (38-43). We have the appropriate facilities and experience to work with pathogenic viruses, including influenza virus, at DM-3 and ML-3 biosafety conditions. In addition, we have the appropriate immunological assays for assessment of cellular, humoral and innate immune responses against influenza. Our long-standing experience with pathogenic viruses, including influenza, and with vaccine evaluation guarantees that the animal studies describe in this proposal will be adequately performed. RSV vaccine evaluation studies at BPRC will be performed in close collaboration with two institutes who have extensive experience in the RSV infection model.

3.2.3 Is voor de uitvoering van dit project andere wet- en regelgeving van toepassing die een invloed zou kunnen hebben op het welzijn van de dieren en/of de haalbaarheid van het directe doel?

Nee

Ja > Geef aan welke wet-en regelgeving van toepassing is en beschrijf de effecten daarvan op het welzijn van de dieren en de haalbaarheid van het project.

3.3 Belang

3.3.1 Beschrijf het wetenschappelijk en/of maatschappelijk belang van de hierboven beschreven doelen.

Annual RSV epidemics cause considerable morbidity and mortality world-wide and especially affect more vulnerable groups like young children, the elderly and people with underlying diseases. In addition, pregnant women are also considered as RSV vaccine recipients, where trans-placental IgG can confer protection in neonates. The economic impact of RSV disease in adults is estimated to be greater than that of influenza in relation to numbers of days lost from work (10, 11). Currently no RSV vaccines are available and a RSV vaccine that induces durable protection against RSV will contribute to a reduction of RSV morbidity and mortality in (young) children and risk-groups. Thus, a vaccine that confers durable protection against RSV would have great societal impact. Both novel vaccines, for instance in the form of DNA, mRNA or viral vectors, as well as new vaccine delivery methods require evaluation in appropriate animal models so that the level as well as the mechanism of protection can be adequately established, before these new vaccines can be evaluated in clinical studies.

3.3.2 Benoem de belanghebbenden in het project en beschrijf voor elk van de belanghebbenden wat hun belang is.

The stakeholders for an RSV vaccine are the aforementioned target groups (young children, pregnant women, people with underlying diseases and the elderly) for whom protection from RSV infection would increase their quality of life. The vaccination of risk-groups and the resulting decrease in RSV burden would also be of great societal benefit. For the animals as stakeholders, there are no direct benefits and they will experience moderate discomfort as a result of the experimental procedures.

3.4 Strategie

3.4.1 Geef een overzicht van de algemene opzet van het project. Besteed aandacht aan de eventuele fasering in de uitvoering en de samenhang. Vermeld eventuele mijlpalen, keuzemomenten en beslisriteria.

Annual RSV epidemics cause considerable morbidity and mortality world-wide and especially affect more vulnerable groups like young children, the elderly and people with underlying diseases. In addition, pregnant women are also considered as RSV vaccine recipients, where trans-placental IgG can confer protection in new-borns. The economic impact of RSV disease in adults is estimated to be greater than that of influenza in relation to numbers of days lost from work (10, 11). Currently no RSV vaccines are available and an RSV vaccine that induces durable protection against RSV will contribute to a reduction of RSV morbidity and mortality in (young) children and risk-groups. Thus, a vaccine that confers durable protection against RSV would have great societal impact. Both novel vaccines, for instance in the form of DNA, mRNA or viral vectors, as well as new vaccine delivery methods require evaluation in appropriate animal models so that the level as well as the mechanism of protection can be adequately established, before these new vaccines can be evaluated in clinical studies.

3.4 Research strategy

3.4.1 Provide an overview of the overall design of the project (strategy).

The criteria to consider vaccine candidates for evaluation are: a) the vaccine strategy must be novel, for instance with regards to choice of antigen, formulation, route of application, that have not been tested before in similar NHPs studies, b) demonstration that the vaccine or vaccine components are non-toxic, c) when specific host molecules are targeted then cross recognition of macaque homologues must have been demonstrated, d) the vaccine cannot be adequately tested in other than NHP animal models, for instance due to the mechanism of action or the type of immunological assessment needed, e) preferably immunogenicity of vaccine candidates should have been proven in other species, unless this is not possible because the specific vaccine modality used does not work in other species. This concerns only vaccines for which it is not possible to directly evaluate them in other species because interaction with specific host molecules is required that are only present in humans and in NHPs, but not in other species. In this case, we would like to add the additional requirement that for this type of vaccine a similar vaccine strategy that targets slightly different molecules but uses the same mode of action has been evaluated and found to be immunogenic in other species.

In order to evaluate the safety and immunogenicity of the vaccine concept, a vaccine evaluation experiment will be performed according to well established procedures, as described in appendix 1. Typically, one or a number of immunisations are given over a certain time period. Following immunisation induction of T-cell and antibody immune responses are measured, systemically in the blood as well as locally in the upper and lower respiratory tract. The strength of these responses as well as their duration are determined. Subsequently, the capacity of the vaccine to protect against infection is evaluated by experimental infection of the animals with RSV. For RSV infection we will use a macaque adapted RSV strain (18, 29), shown to be infectious in macaques.

Experimental infection will only be performed when the immunisation has induced immune responses against the virus that is to be used for experimental infection, such that protection against infection is to be expected. Whether protection is actually achieved depends on local interaction between cells of the immune system and local anti-viral antibodies with the virus and virus infected cells in the respiratory tract. This cannot be adequately modelled in an in vitro system and requires experimental infection of an animal. Ideally the vaccine should provide a robust level of protection and be able to reduce disease and virus replication in animals infected with a standard virus dose via delivery to the upper respiratory tract and lungs. However, since most people become infected via exposure to small droplets containing a limited amount of virus, a less stringent infection model; i.e. using a low dose of virus given via aerosol delivery, may sometimes be chosen. In the event evaluation of the capacity of a vaccine to protect against infection requires that a virus has to be used that has not been tested before in macaques, then this virus will first be tested in a small number of animals to determine if all animals become infected and what the amount of virus replication is (appendix 2). Virus infection is routinely performed by inoculating the animals via a number of routes or combinations thereof; i.e. intra-tracheal, oral or intranasal, using a standard virus dose. For refinement, we will investigate another mode of virus delivery, namely as an aerosol. The rationale for setting up this model of infection is that the virus is given in the form of small droplets that better reflect the natural mode of exposure. Once this method is established for RSV infection, it can be applied in subsequent vaccine evaluation studies, for instance in cases where less stringent criteria of protection against infection are needed (in case it is difficult to make a protective vaccine and it is necessary to establish relatively modest improvements in vaccine development that can only be measured when a low dose of virus is used).

3.4.2 Onderbouw de gekozen strategie.

The main objective can be divided in 2 sub- objectives:

Vaccine evaluation: Safety, immunogenicity and capacity to protect against infection will be evaluated using an established RSV infection model. The challenge virus to be used in the initial vaccine efficacy studies is a, well described, macaque-adapted human RSV isolate, shown to reproducibly infect macaques (18, 29).

Establishment of a new RSV infection model: In the event a new RSV strain is required for vaccine evaluation, the infection model will be optimised for dose and route of administration (e.g. i.t., oral, i.n. or aerosol delivery) in order to optimally assess vaccine efficacy. When aerosol administration for the macaque-adapted RSV challenge strain the challenge dose will have to be established.

In the event that the established macaque-adapted RSV challenge model will be used, this can be performed under sub-objective 1. If, however, a new challenge virus has to be used for vaccine evaluation, two steps are required: The infection model has to be established (sub-objective 2), before the vaccine can be evaluated. Therefore, two types of experiments are required to fulfil these objectives: 1. Vaccine evaluation experiments (described in appendix 1) and 2. Establishment of a new RSV challenge model (described in appendix 2).

Vaccine evaluation in macaques.

For this type of experiment animals will be immunised by a number of immunisations over a defined time period. During the study animals will be monitored for adverse effects of the vaccine, including monitoring of general behaviour and health. Blood, nasal washes and bronchoalveolar lavages will be collected to determine induction of systemic as well as local immune responses. Only if immune responses, suggesting protection against infection, are induced the efficacy will be tested by experimental infection with RSV. A group of control animals will be included for comparison. This control

group can be unvaccinated, or be previously infected (e.g. RSV). The choice of the control groups depends on the research question.

RSV infection in macaques.

In order to establish infectivity and potential pathogenicity of a virus that has not been tested previously in NHP, a small number of animals will be infected and monitored for clinical symptoms, fever, body weight and changes in blood parameters. Nasal and tracheal swabs and lung lavages will be taken to confirm that the animals were infected and to determine virus replication. To evaluate a new virus or a different infection route, the virus is inoculated via the desired route, using a standard virus dose. Proper application in vaccine evaluation requires that in these infection studies > 80% of the animals become infected and that the amount of virus produced in the respiratory tract over the infection period is clearly detectable and that the variation between the animals is sufficiently low to allow measurement of reduction in virus load in vaccinated animals with less than 10 animals per group. In case these parameters are not achieved, the experiment will be repeated with a 10-100 times higher dose. In case any of the animals reaches the humane endpoint within the first four days after infection then a 10-100 times lower virus dose will be evaluated. The same criteria will be applied to determine whether the aerosol infection model is sufficiently robust.

3.4.3 Benoem de type dierproeven. Vul per type dierproef een bijlage Beschrijving dierproeven in.

Volgnummer	Titel bijlage Beschrijving dierproef
1	RSV vaccine evaluation in macaques
2	RSV infection in macaques

Referenties

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