Available online at www.sciencedirect.com
SCIENCE DIRECT®
Vaccine 23 (2005) 2928–2942
Vaccine
www.elsevier.com/locate/vaccine

DNA immunization against respiratory syncytial virus (RSV) in infant rhesus monkeys

Kerrie Vaughan[a,*], Gary H. Rhodes[b], Laurel J. Gershwin[a]

[a] *Department of Pathology, Microbiology and Immunology, University of California, Davis, School of Veterinary Medicine, Davis, CA 95616, USA*
[b] *Department of Pathology, University of California, Davis, School of Medicine, Davis, CA 95616, USA*

Received 15 July 2004; received in revised form 19 October 2004; accepted 25 October 2004
Available online 23 December 2004

Abstract

A DNA vaccine was tested in infant *Rhesus macaques* to evaluate its safety, immunogenicity and protective efficacy. Monkeys were vaccinated and challenged with a clinical isolate of human RSV. Vaccinated animals developed humoral and cellular responses following inoculation with plasmid DNA encoding the fusion (F) and nucleoprotein (N), from closely related bovine RSV. Vaccinated monkeys had decreased RSV in their lungs post-infection, and there was a qualitative difference in histopathology observed between vaccinated and unvaccinated animals. The combined result of safety and immunogenicity in a neonatal primate model is encouraging, suggesting the feasibility of DNA vaccines against RSV in infants.
© 2004 Elsevier Ltd. All rights reserved.

Keywords: DNA immunization; RSV; Rhesus monkeys

1. Introduction

Respiratory syncytial virus (RSV) is major cause of infectious pulmonary disease in infants and children worldwide. Disease due to RSV infection occurs seasonally in both populations, however disease severity is maximal in infancy [1]. While the development of safe and efficacious vaccines for use in the neonatal population is a daunting task, it is requisite for successful RSV disease prophylaxis. Currently, there is no vaccine for use against RSV, nor is there any consensus regarding the most appropriate strategy to use in neonates. This is due in large part to several age-related issues associated with this target group. Immunological and developmental immaturity are the predominant issues for consideration in RSV vaccine research. However, an incomplete understanding of the combined influence of each of these early processes has hampered RSV vaccine progress for decades. Furthermore, the groups most susceptible to severe lower respiratory tract infection are pre-term infants and those with pre-existing conditions, such as asthma, congenital heart disease or bronchopulmonary dysplasia [2–4]. For these infants, lung function and development are significantly impaired, and immune responses to infectious microbes are compromised. In these susceptible populations, vaccine safety is of utmost importance. Therefore, the ability to safely and reliably induce protective and/or lasting immune responses in immunologically and developmentally healthy, albeit naïve, as well as those most vulnerable groups remains to be established.

Of parallel importance is the question of whether vaccine-specific immunity can be elicited and protective in the presence of maternally derived antibody. The presence of maternal antibody at the time of immunization has long been known to suppress or inhibit immune responses to a variety of conventional vaccines, including live-attenuated, inactivated and subunit formulations [5–8]. Not surprisingly, passively acquired (not maternally derived) antibodies have also been shown to decrease the immunogenicity of subunit, recombinant, as well as live-attenuated vaccines against RSV [9–11]. This phenomenon of passive antibody-mediated inhibition

* Corresponding author. Present Address: U.S. Navy Marine Mammal Program, Spawarsyscen, San Diego, CA, USA. Tel.: +1 619 767 4004; fax: +1 619 553 5068.
E-mail address: genevax@spawar.navy.mil (K. Vaughan).

0264-410X/$ – see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.vaccine.2004.10.046

of vaccine-specific immunity has therefore become a central challenge for neonatal vaccine design against RSV. Because moderate to high levels of RSV-specific maternal antibodies have been correlated with decreased disease severity [12,13], it is perhaps essential to develop an active immunization strategy that will complement pre-existing, passively acquired protection.

Thus far, no conventional candidate vaccine strategy has demonstrated the necessary combination of safety and immunogenicity for use in neonates against RSV infection. With rare exception, killed/inactivated and subunit vaccines have been safe. However, these formulations have historically lacked good immunogenicity. While live-attenuated and recombinant viral vaccines have been strongly immunogenic, they lack stability, and are therefore unsafe for use in this target population. Moreover, these vaccine formulations remain vulnerable to passive antibody-mediated inhibition due to pre-existing maternal antibody. Gene-based immunization provides a unique tool with which to potentially provide targeted immunogenicity with the greatest potential for safety. DNA vaccines elicit both humoral and cellular immunity [14], and therefore have greater potential to provide more complete protection against RSV infection. Moreover, studies have shown DNA vaccines to be safe and immunogenic in neonatal animal models [15–18]. We hypothesized that plasmid DNA encoding specific viral immunogens would induce balanced humoral and cellular immune responses, and that these responses so elicited, would be protective against RSV in infant rhesus monkeys. Furthermore, we hypothesized that vaccination with the closely related bovine RSV genes would immunize better in the presence of maternally derived anti-RSV antibodies. Bovine respiratory syncytial virus (BRSV) is a pneumovirus that produces a similar disease syndrome in young calves. Notable is that there is high degree of homology between BRSV and RSV immunogenic proteins (% homology provided below). To test this hypothesis, infant *Rhesus macaques* were inoculated with plasmid DNA encoding two key immunogens of bovine RSV. These animals were then challenged with a clinical isolate of human RSV. Our findings suggest that a DNA vaccine formulation can be safe, immunogenic and partially protective in infant monkeys injected as early as 1.5 months of age. These findings also suggest that, while moderate levels of pre-existing RSV-specific antibodies may affect humoral responses to DNA vaccines, they do not prevent the induction of specific IgG upon subsequent RSV exposure. Moreover, the presence of these pre-existing antibodies did not affect vaccine-induced cellular immunity, and therefore the ability of these monkeys to clear virus from their lungs. Whether these pre-existing antibodies were maternally derived or naturally acquired cannot be determined from this study. However, their presence is consistent with the natural scenario of RSV infection in human infants, and therefore is relevant to include in this investigation. Taken together, these data support the assertion that DNA vaccination may be an ideal vaccine strategy for use against RSV infection and/or disease in human neonates, and that the use of highly homologous bovine RSV genes may be useful in the presence of pre-existing RSV-specific antibodies.

2. Methods and materials

2.1. Animals and study design

Infant rhesus monkeys (*Macaca mulatta*) were provided by the California National Primate Research Center (CNPRC, Davis, CA). The CNPRC maintains a colony of captive breeding adults for research purposes. The infants used in this study were nursery-reared, and at the time of the initial inoculation, were 1.5 months of age. Age-matched infants were included as controls. Due to their young age, the infants were housed in pairs for the duration of this study. The animal studies described herein were approved by the UC Davis's Animal Use and Care Administrative Advisory Committee (AUCAAC).

2.2. Preparation of plasmid DNA

The full-length fusion and nucleoprotein genes of BRSV (provided by Ursula Buchholz, Federal Research Center for Virus Diseases of Animals, Insel Riems, Germany) were subcloned into the *Bgl*II and *Xho*I sites of the eukaryotic expression vector pND (provided by Gary H. Rhodes, University of California, Davis, Davis, CA). DNA sequence homology between the bovine and human virus F and N genes 81% and 90%, respectively (GeneBank sequence analysis using Clone Manager 5, Scientific and Education Software, Durham, NC). The pND-F and pND-N constructs were then propagated in *Escherichia coli*, and transformants were selected based on their growth in the presence of ampicillin. Restriction enzyme analysis and DNA sequencing were performed to confirm the correct orientation and identity of the inserted genes. Constructs were prepared for injection using alkaline/SDS lysis, followed by two rounds of CsCl–EtBr gradient ultracentrifugation. Each plasmid was then evaluated for protein expression in vitro by Western blot analysis of cell supernatants following liposome-mediated cell transfection and then in vivo, by performing RSV-specific ELISA on sera from inoculation of mice (data not shown).

2.3. Vaccination and RSV challenge protocol

Four of the six infants were vaccinated with plasmid DNA encoding the fusion (F) and nucleoprotein (N) of bovine RSV. Two of the six infants were unvaccinated controls. [A description of the preparation of each construct (pND-F and pND-N) is provided below.] Each vaccinated monkey received an injection of 200 μg of plasmid DNA in a total volume of 200 μl of sterile saline. All four vaccines received both pND-F and pND-N, injected separately into the quadriceps (200 μg of each plasmid in 200 μl injected into each mus-

Download English Version:

https://daneshyari.com/en/article/2410675

Download Persian Version:

https://daneshyari.com/article/2410675

Daneshyari.com