Impaired immune response to vaccination against infection with human respiratory syncytial virus at advanced age

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ABSTRACT

Elderly humans are prone to severe infection with human respiratory syncytial virus (HRSV). Aging of today’s human population warrants the development of protective vaccination strategies aimed specifically at the elderly. This may require special approaches due to deteriorating immune function. To design and test vaccination strategies tailored to the elderly population, we need to understand the host response to HRSV vaccination and infection at old age. Moreover, preclinical need of testing candidate vaccines requires translational models resembling susceptibility to the (unadapted) human pathogen.

Here, we explored effects of aging on immunity and protection induced by a model human HRSV vaccine candidate in a translational aging model in cotton rats (Sigmodon hispidus), and examined possibilities to optimize vaccination concepts for the elderly. We immunized young and aged cotton rats with a live-attenuated recombinant HRSV vaccine candidate, and analyzed the induced immune response and protection to challenge with HRSV.

In old cotton rats HRSV-infection persisted longer, and vaccination induced less protection against infection. Aged animals developed lower levels of vaccine-induced IgG, virus-neutralizing serum antibodies and IgA in lungs. Moreover, booster responses to HRSV-challenge virus were impaired in animals vaccinated at older age. However, increased dose and reduced attenuation of vaccine could improve protection even in old animals.

This study shows cotton rats provide a model for studying effects of aging on the immune response to the human respiratory pathogen HRSV, and possibilities to optimize vaccine concepts for the elderly.
IMPORTANCE

HRSV infection poses a risk for severe disease in the elderly. Aging of the population urges to increase efforts to prevent disease at old age, whereas HRSV vaccines are only in the developmental phase. Preclinical need of testing candidate human vaccines requires translational models resembling susceptibility to the natural human virus. Moreover, we need to gain insight in the waning immunity at old age, as this is a special concern in vaccine development. In this study we explored the effect of age on protection and immunity against an experimental HRSV vaccine in aged cotton rats (Sigmodon hispidus), a rodent species that provides a model representing natural susceptibility to human viruses. Older animals generate less antibodies upon vaccination, and require a higher vaccine dose for protection. Notably, during the early secondary immune response to subsequent HRSV infection older animals show less protection and a slower rise of the virus-neutralizing antibody titer.

Keywords: aging, RSV, vaccine, cotton rat, immunology.
INTRODUCTION

At advanced age, when over approximately 65 years, people become more susceptible to severe infectious diseases by respiratory pathogens. This increased medical threat is caused by impaired functioning of the immune system (immunosenescence). Immunosenescence is currently best exemplified by limited immunity induced by vaccines to prevent infection with pneumococcal bacteria and influenza virus in the elderly (1-4). It is now becoming recognized that human respiratory syncytial virus (HRSV) contributes to an important part of severe respiratory disease and mortality in the elderly (1-3, 5, 6). This probably has been underestimated since HRSV is regarded as a common cause of lower respiratory tract infections primarily in infants, and disease caused by HRSV is potentially attributed to influenza virus due to its symptoms matching influenza-like illness (7, 8). Some studies suggest that the extent of HRSV-associated hospitalization in the elderly population is close to that for influenza (3, 7, 9).

As the proportion of the aged population is rising, the need for preventing age-related diseases is also increasing. Vaccines that elicit protection against respiratory viruses would significantly contribute to maintaining health and, hence, quality of life at old age. Despite intense efforts to design an effective HRSV vaccine for a licensed vaccine is not available. Most of these efforts are aimed at protecting children. To design vaccination strategies tailored to the elderly population, we need to understand how the immune system of this age group responds to vaccination. To enable testing of human candidate vaccines in the elderly a model that resembles susceptibility to the natural human pathogen is required.

Hispid cotton rats (Sigmodon hispidus) provide a highly representative model for human infections since they are susceptible to a variety of human pathogens that do not require adaptation to infect and replicate (10-14). HRSV is a good example, as infection,
pathology and protection induced by candidate HRSV-vaccines in cotton rats have already been explored and reflect the human situation (11, 12, 15-17). In comparison with young cotton rats, aged cotton rats express prolonged cytokine response and higher degree of pathology in the lungs in response to a primary HRSV-infection (18, 19). However, age-related effects on immune response and anti-viral protection induced by HRSV-vaccination herein remain to be clarified. To this end, we explored the effect of aging on vaccine-induced immunity and protection against HRSV infection in cotton rats of various age groups. We vaccinated young-adult (2 months) and old cotton rats (8 to 9 months) with a live-attenuated HRSV vaccine candidate and explored induction of HRSV-specific antibodies in serum and lung mucosa, the primary entry site of HRSV, and protection to challenge with wild-type virus.
MATERIALS AND METHODS

Virus and vaccine preparation

The propagation of virus and preparation of vaccine has been described in detail elsewhere (17). In brief, the HRSV we used for infection and challenge experiments was wild-type HRSV-X (strain 98-25147-X). This clinically isolated HRSV serogroup A strain (17) also served as the basis for the recombinant HRSV (rHRSV) virus used as vaccine in this study. The recombinant HRSV was recovered from a plasmid encoding cDNA containing directed mutations in the intergenic regions of the HRSV-X genome as described elsewhere (17, 20). This resulted in infectious rHRSV particles that express all the viral proteins, but express an attenuated phenotype.

Immunization and challenge of cotton rats

Adult cotton rats (Sigmodon hispidus) of young (2 months), intermediate (6 months) and old (8 to 9 months) age were obtained from a specific pathogen free breeding colony (Charles River Laboratories, the Netherlands) and held at the animal facilities of Intravacc (Bilthoven). HRSV-infection was done i.n. with 100 µl containing 3x10^5 TCID_{50} wild-type HRSV (17). Cotton rats were immunized intranasally (i.n.) with 10 µl containing different doses of the rHRSV or with wild-type HRSV, and challenged i.n. with 100 µl containing 3x10^5 TCID_{50} wild-type HRSV 28 days later (17). Animal studies were approved by the Animal Ethical Committee of RIVM. Animal handling was carried out in accordance with Dutch national legislation.

Viral titration

HRSV titers were analyzed by 50% tissue culture infective dose (TCID_{50})(17). Lung titers were determined in homogenized right lungs. Nasal HRSV titers were determined from nasal
washes obtained by flushing the upper trachea with 2 ml PBS supplemented with 7.5% sucrose. HRSV titers were determined on Vero cells and expressed as log_{10}TCID_{50} per gram lung. The lowest detection limit was 2.1 log_{10}TCID_{50} per gram.

**Virus neutralization assay**

Neutralizing antibodies were analyzed in a fluorescence-based plaque reduction assay by testing two-fold serial dilutions of cotton rat serum starting at 1:10 (17, 21). Diluted serum was mixed with an equal volume of rHRSV containing an EGFP reporter gene, and added to Vero cell monolayers. After centrifugation for one hour at 700 x g, and incubation for one hour at 37°C, the inoculum was removed and cells were overlaid with 1% methyl cellulose and cultured for two days at 37°C. Plaques were detected in a fluorescence Elispot reader (Aid-diagnostika, Germany). Plaque reduction was calculated by regression analysis to provide a 60% plaque reduction titer.

**IgG and IgA analysis**

IgG was measured in blood serum and IgA was measured in homogenized lung tissue. RSV-specific IgA and IgG antibodies were detected by ELISA on polystyrene 96 wells micro titer plates coated with Triton-X100 inactivated HRSV-X by HRP-labeled goat-anti-mouse IgA (AbD Serotec, Oxford, UK), cross-reactive to cotton rat IgA, and chicken-anti cotton rat IgG (ICL, Portland, OR), respectively.

**Statistics**

Multiple comparisons were analyzed with ANOVA, including a Tukey’s Multiple Comparison test, to test for statistical significance of differences. Comparisons of two samples were analyzed with a t test. P<0.05 was considered significant.
RESULTS

Older cotton rats clear HRSV infection slowly
Cotton rats at the age of 2, 6 or 9 months were infected with wild-type HRSV-X (wtHRSV).
This clinically isolated HRSV serogroup A strain (17) served as the basis for the recombinant HRSV virus used as vaccine in this study. Naive animals were infected intranasally and sacrificed 4 to 10 days after inoculation to analyze virus titers in lung and nose. At 4 days after inoculation, the lungs (Figure 1A) and nasal washes (Figure 1B) of all animals showed high amounts of virus, but did not show different virus titers between the various age-groups. However, 6 days post-infection the young animals (2 months) showed a significant drop in virus titer in the lungs compared to animals of 6 months old (Figure 1C). This difference was more pronounced when compared to animals of 9 months old. In addition, virus titers in the nose of young-adult cotton rats were slightly reduced compared to the older age groups of 6- and 9 months of age (Figure 1D). At day 10 after challenge, virus could not be detected anymore in nose and lungs of old animals (9 months, data not shown), indicating that the virus was eventually cleared. Together, these data show that HRSV infection pertains for a longer period in older cotton rats.

Vaccination induces less protection to HRSV infection at older age in cotton rats
To assess if age would affect HRSV-vaccination efficacy in cotton rats, we vaccinated cotton rats at the age of 2 months (young) or 8 to 9 months (old) with \(10^3\) TCID\(_{50}\) of live-attenuated recombinant HRSV (rHRSV). Subsequently, we analyzed protection induced against challenge at 28 days post-immunization with wtHRSV (17). As measured five days post-challenge, virus was not detectable in the lungs of young animals (Figure 2A), whereas old immunized animals and mock-immunized controls showed detectable amounts of challenge-virus. These data indicate that in cotton rats vaccination efficacy is lower at old age.
Increasing the dose and reducing attenuation of virus improves vaccine efficacy in old cotton rats

Since increasing the dose of a vaccine can improve its efficacy (23) we tested whether immunization with rHRSV using a dose higher than $10^3$ TCID$_{50}$ would induce protection against challenge virus in old cotton rats. The 10-fold ($10^4$ TCID$_{50}$) and 100-fold ($10^5$ TCID$_{50}$) higher dose of immunizing rHRSV resulted in lower titers of challenge virus in a dose dependent fashion as detected in the lungs five days after challenge (Table 1). Most (4 out of 5) animals immunized with the highest dose ($10^5$ TCID$_{50}$) rHRSV were even free of detectable virus at this time point after challenge (Figure 2B, Table 1). These data indicate that increasing the vaccine dose can restore protection in old cotton rats.

The rHRSV used in this study is attenuated by mutations made in intergenic regions of the viral genome (17). Attenuation of virus may influence its capacity to induce protection against infection (24). Hence, we analyzed protection against challenge virus induced by the parental unattenuated wtHRSV at an immunizing dose at which the rHRSV was not protective ($10^4$ TCID$_{50}$) in old cotton rats. In contrast to the old cotton rats immunized with this dose of attenuated rHRSV, all animals that had been immunized with $10^4$ TCID$_{50}$ of the wtHRSV showed no detectable challenge virus by day four after challenge (Figure 2B), indicating stronger protective potency of non-attenuated virus in old cotton rats.

Old cotton rats generate lower titers of IgG and virus neutralizing antibody in response to primary immunization

The capacity to generate antibodies in response to vaccination and infection reduces at old age (25-29). To assess whether this age effect would be paralleled in the cotton rat model, we compared the production of antibodies in response to HRSV vaccine in young cotton rats to that of old cotton rats. Vaccination with rHRSV could protect young-adult cotton rats and...
partly old cotton rats. Therefore, we argued that rHRSV-vaccination would allow an
immunological window to analyze for differences between cotton rats at young age (2
months) and old age (8 to 9 months). We set up an experiment to longitudinally analyze the
response to immunization with rHRSV followed by a wtHRSV challenge given at day 28 post-
immunization. An increase in HRSV-specific serum IgG in response to rHRSV was found
starting two weeks after immunization. However, the antibody titer induced was
significantly lower in old animals (Figure 3).

To analyze the quality of antibodies at the day of challenge, we analyzed the virus
neutralizing (VN) titers in serum (Figure 4A). Although all animals generated detectable VN
in response to the $10^5$ TCID$_{50}$ dose rHRSV, the VN titer was significantly lower in old animals
compared to young animals that had received the same dose. A lower response of old
animals was also found after immunization with the $10^4$ TCID$_{50}$ dose. These data show that
old cotton rats generate less IgG and VN antibodies in serum in response to rHRSV vaccine.

Old vaccinated cotton rats exhibit reduced VN antibody response to challenge virus

At 5 days after challenge (day 33) a sharp IgG titer rise was observed in young and old
animals that had been immunized with rHRSV (Figure 3). This sharp rise is an early booster
effect induced by challenge virus in previously immunized animals, since this quick and steep
increase of antibody titer in immunized animals was only found upon challenge and not in
non-challenged cotton rats (data not shown). We questioned whether eliciting this early
recall response to challenge virus differed between the two age groups. To analyze this
booster effect, we measured the increase in antibody level from day of challenge (day 28
post-vaccination) to 5 days post-challenge. The absolute IgG titer was significantly lower in
old animals. The rise in IgG titer in response to challenge occurred in young and old animals,
but did not significantly differ. However, a sharp rise was also found for functionality of
serum antibodies as shown by the increased VN titer in both young and old animals (Figure
This challenge-induced boosting of VN antibody titer in immunized animals was significantly lower in old animals. These data suggest that aging reduces boosting of functionality rather than quantity of IgG produced during the early recall response in cotton rats.

Old cotton rats generate lower titers of mucosal IgA antibodies

Since HRSV infects the lungs via the mucosal tissue, we wondered if production of mucosal antibodies in lungs of old cotton rats is deteriorated. We therefore measured HRSV-specific IgA antibodies in lungs of animals sacrificed at 5 days post-challenge (Figure 5). All young and old animals that had been immunized with rHRSV, with either the higher ($10^5$ TCID$_{50}$) or the lower ($10^4$ TCID$_{50}$) dose, exhibited significant induction of IgA compared to non-immunized controls. However, old animals that had been immunized with the lower rHRSV dose showed significantly lower IgA titer in lungs compared to young animals that had received the same vaccine dose. This indicates that when a low vaccine dose is used, old cotton rats generate a lower mucosal antibody response than young-adult cotton rats do.
DISCUSSION

The elderly population is an important target group for vaccination since this group is vulnerable to severe lung disease caused by respiratory infections with pneumococcal bacteria, influenza virus and HRSV (1-3, 5, 30). Animal models allow controlled study of age-related effects on vaccine-induced immune protection against infection. Cotton rats are permissive for infection with human RSV, develop human-like pathology upon infection with human virus, and allow analysis of the immune response to HRSV. Towards improving and testing vaccine-induced protection at old age, we analyzed effects of aging on immunity and protection to virus-challenge in this representative model. We showed that aged hispid cotton rats mount decayed protection induced by immunization against HRSV infection.

Older naive cotton rats cleared primary lung infection with wtHRSV slower than did young animals. This confirms previous studies, which reported similar findings with prototype Long strain HRSV in older animals (18, 19). In old cotton rats immunization with rHRSV vaccine induced less protection to HRSV-challenge than in young-adult animals. Reduced vaccine efficacy in old cotton rats was also found upon immunization with another live-attenuated HRSV (GΔG HRSV (22), data not shown) or with formalin-inactivated HRSV (data not shown). These results indicate waning of the ability to mount a protective immune response to vaccination at advanced age. However, increasing the dose improved vaccine efficacy in aged animals. In addition, wtHRSV conferred protection upon immunization with a low dose at which the attenuated rHRSV did not. This indicates that vaccine-induced protection against infection in the elderly may be achieved by increasing the dose or by reducing the level of attenuation of vaccine.

Reduced clearance at old age was found in the lungs and in the upper airways, although virus pertained in the nose for a longer period than in the lungs both after primary infection and upon challenge in vaccinated animals. This is a general finding in cotton rats (15, 17) and has also been described in humans (31). Observed differences in viral load
between nose and lungs might partly be due to differences between sampling methods. However, cotton rat studies showed that virus neutralizing antibodies protect the lower airways more effectively than the upper respiratory tract (15). This suggests that slower clearance from the upper respiratory tract reflects protection against infection being more effective in the lungs than in the upper respiratory tract in humans and in cotton rats.

Antibodies are important players in protection induced by vaccination and natural infection. Earlier cotton rat studies showed that HRSV-specific antibodies confer protection, and human studies suggest that a basic level of antibodies is needed for protection against deep lung infection (15, 32-36). Generally, the level of induced virus-neutralizing antibodies tends to wane in elderly people. However, the relation between antibody levels induced by HRSV and age in humans is still subject to speculation. Observations on the effect of age on the immune response to vaccine may be blurred since social behavior and -environment, frailty and varying history of natural infections can influence the outcome of human studies (26, 29, 32, 37-40). To clarify the effect of aging on the capacity to mount antibody production in response to HRSV-vaccine, we analyzed the antibodies produced in response to vaccination with rHRSV that induced suboptimal protection in old animals but could fully protect young cotton rats. Old cotton rats showed significantly reduced vaccine-induced titers of IgG and neutralizing antibodies in serum at the time of challenge, four weeks after vaccination. Since antibodies contribute to protection, this finding suggests that immunosenesence of vaccine-induced immunity contributes to reduced clearance of virus. Moreover, old animals immunized with a higher dose of rHRSV showed more neutralizing antibodies and less replication of challenge-virus, suggesting more neutralizing antibodies induced by higher antigen dose attributes to better protection in immunosenescent animals. Also, immunization with non-attenuated wtHRSV induced a proper level of neutralizing antibodies (data not shown) and protection in old cotton rats. Together with a study by Price et al., reporting wtHRSV did not induce significantly less antibodies in older
cotton rats (18), this finding may explain why wtHRSV is a more potent inducer of protection than the attenuated rHRSV at old age.

The lower RSV-specific IgG level induced by immunization at older age in cotton rats reflects the general notion of immunosenescence found in humans. In contrast, clinical studies indicate that old humans express similar or even higher titers of RSV-specific IgG antibodies (29, 31, 39, 41). However, several of these studies indicate that the functionality of these antibodies is inferior compared to antibodies of younger people (29, 39). Of note, old humans have been exposed more often to RSV infection during their life span than young people. This may cause a different RSV-specific immune status that is not fully mimicked in animal models such as our cotton rat model and likely explains differences found between animal models and humans. Such differences in observations between models and clinical studies should be taken into account when interpreting models of immune responses at old age.

Naive cotton rats immunized in our study had not been exposed (as confirmed by serology) to HRSV-infection before vaccination. Antibodies analyzed prior to wtHRSV challenge in our study therefore reflect a primary response to vaccine. This does not completely reflect the natural situation since aged people are not naive. However, we measured also antibodies in rHRSV-immunized animals after subsequent challenge with wtHRSV. Since rHRSV is a live virus (17), rHRSV-immunization can be regarded as a mild HRSV infection and challenge with wtHRSV as a boosting re-infection. A booster effect in previously immunized individuals becomes apparent by a quick and steep rise of antibody titer during the recall immune response to infection within 5-7 days as shown in our cotton rat studies (unpublished data), lambs (42), young children (43) and aged humans (31). The early booster effect was significantly lower in old animals and this was most pronounced in the rise of the level of neutralizing antibodies, rather than the boost of IgG, indicating that aged individuals are less capable of mounting recall responses of functional antibodies to
HRSV. This may explain why aged people have lower antibody functionality rather than lower total IgG against HRSV in serum (18, 38, 39).

It is tempting to speculate that the rate at which production of functional antibodies rises early after infection may determine control of viral load during the first days of infection. Therefore, it may be interesting to further investigate the function of the quick increase in antibodies in a secondary immune response, the final maximum titer reached and the role of the slower initial rise thereof at old age. Besides antibodies, T-cells are important for an effective immune response required to clear virus. Since functionality and numbers of RSV-specific CD4+ and CD8+ T-cells drop with age (29, 44, 45), it would be useful to unravel how T-cell responses at old age are involved in induction of protective immunity by RSV vaccines, and how these responses can be improved to act in concert with antibodies to achieve protection at old age.

The lung mucosa is the primary entry site where protection against deep-lung infection with HRSV by antibodies should be exerted. Moreover, mucosal antibodies have been reported to more closely resemble HRSV-neutralizing function of the immune response than does serum IgG (35, 36). Since data on age-related effects on HRSV-induced mucosal IgA are relevant but scarce, we analyzed HRSV-specific IgA produced in the lungs of rHRSV-immunized animals upon challenge. Prominently, in response to rHRSV vaccine old cotton rats produced significantly less IgA in the lungs compared to young animals.

Collectively, our data show in cotton rats an age-dependent reduction of the ability to mount protective immunity to human RSV, both in a primary and secondary (booster) immune response. Moreover, these data indicate the cotton rat is a useful model to study human vaccines designed for the aged population as well as possibilities to promote vaccine efficacy in the elderly by increasing vaccine dose or by reducing the level of attenuation.
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The authors have no conflict of interest to disclose.


22. van Bleek GM, Osterhaus AD, de Swart RL. 2011. RSV 2010: Recent advances in research on respiratory syncytial virus and other pneumoviruses. Vaccine.


Table 1. Protection induced by the attenuated recombinant (r)HRSV-vaccine candidate against challenge with human wild-type (wt)HRSV (3x10⁵ TCID₅₀) in old cotton rats. As a positive control, one group was immunized with 10⁴ TCID₅₀ wtHRSV. Table shows for lungs and nose the % of animals that showed HRSV five days after challenge, and the mean titer per group detected in infected animals.

<table>
<thead>
<tr>
<th>n</th>
<th>age</th>
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<td>3</td>
<td>8 mo.</td>
<td>mock</td>
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<td>100% 4.9 (0.4)</td>
<td>100% 4.2 (0.6)</td>
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<tr>
<td>6</td>
<td>8 mo.</td>
<td>rHR HSV</td>
<td>10³</td>
<td>100% 3.8 (0.7)</td>
<td>100% 3.7 (1.5)</td>
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<tr>
<td>6</td>
<td>8 mo.</td>
<td>rHR HSV</td>
<td>10⁴</td>
<td>100% 2.9 (0.3)</td>
<td>100% 1.2 (1.5)</td>
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<tr>
<td>5</td>
<td>8 mo.</td>
<td>rHR HSV</td>
<td>10⁵</td>
<td>20% 3.6</td>
<td>40% 2.8 (0)</td>
</tr>
<tr>
<td>6</td>
<td>8 mo.</td>
<td>wtHR HSV</td>
<td>10⁴</td>
<td>0% -</td>
<td>16% 2.1</td>
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</table>

Animals vaccinated at age 2 months:
- mock: 100% of animals HRSV-titer in lungs
- rHR 10³: 0% of animals HRSV-titer in lungs
Figure 1. Clearing of HRSV upon infection in cotton rats at different ages. At the age of 2, 6 or 9 months cotton rats were infected intranasally with 3x10^5 TCID\textsubscript{50} HRSV. Virus titers were analyzed in lungs and nose at 4 days post-infection (A, B) and at 6 days post-infection (C, D). At ten days post-infection virus was not detectable anymore. *P<0.05 as analyzed with ANOVA.

Figure 2. Vaccine-induced protection to HRSV-challenge in cotton rats at different ages. At the age of 2 or 9 months cotton rats (n=5/6 per group) were immunized with the attenuated rHRSV (with doses indicated as TCID\textsubscript{50}) or mock control. One group of cotton rats of 9 months old was immunized with wild-type HRSV (HRSV 10^4 TCID\textsubscript{50}). Subsequently, all animals were challenged intranasally with 3x10^5 TCID\textsubscript{50} wild-type HRSV at 28 days post-immunization. Virus titers were analyzed in lungs five days post-challenge. Less protection induced by rHRSV in old cotton rats was found in two independent experiments of which one is shown. *indicates none of the challenged animals were free of HRSV at five days post-challenge.

Figure 3. rHRSV vaccine-induced serum IgG. Cotton rats at age 2 (white bars) or 9 (black bars) months (n=6/group) were immunized with rHRSV-vaccine dose 10^5 TCID\textsubscript{50}, and HRSV-specific IgG was analyzed in serum weekly. Animals were challenged with wild-type HRSV (3x10^5 TCID\textsubscript{50}) at day 28. This graph shows data of one experiment representing lower level of rHRSV-induced antibodies in old cotton rats found in
three independent experiments. *P<0.05 for difference between age groups 2
months and 9 months as analyzed with a t test.

Figure 4. rHRSV vaccine-induced HRSV-neutralizing antibodies before and after boost with
HRSV challenge. Cotton rats at age 2 or 9 months were immunized with rHRSV-
vaccine dose 10^5 TCID_{50} or 10^4 TCID_{50}, and HRSV-neutralizing function of serum
antibodies was analyzed at day 28, at the time animals were challenged by infection
with human 3x10^5 TCID_{50} wtHRSV (A). The net increase of antibodies was analyzed at
five days after challenge (B). This graph shows one of two independent experiments
on rHRSV-vaccine in young versus old cotton rats showing similar results. Values are
expressed as log_{2}. *P<0.05 as analyzed with ANOVA.

Figure 5. rHRSV-vaccine induced mucosal IgA antibodies. Cotton rats at age 2 or 9
months were immunized with rHRSV-vaccine dose 10^5 TCID_{50} or 10^4 TCID_{50}, and
challenged with HRSV (3x10^5 TCID_{50}) 28 days after vaccination. Mucosal HRSV-
specific IgA was analyzed in lungs 5 days post-challenge. This graph shows one of two
independent experiments on rHRSV in young versus old cotton rats showing similar
results. *P<0.05 as analyzed with ANOVA.
Figure 1

day 4 after HRSV infection

A

virus titer lung

RSV titer

age (months)

2 4 6 8

B

virus titer nose

RSV titer

age (months)

2 4 6 8

day 6 after HRSV infection

C

virus titer lung

RSV titer

age (months)

2 4 6 8

D

virus titer nose

RSV titer

age (months)

2 4 6 8
Figure 2 shows the percentage of animals protected in the lungs after challenge with HRSV. The graph compares the protection of animals vaccinated with different doses of HRSV and a mock control.

- **Mock Control**: 0% protection.
- **$10^3$ Vaccine**: Approximately 70% protection.
- **$10^4$ Vaccine**: Approximately 80% protection.
- **$10^5$ Vaccine**: Approximately 90% protection.
- **$10^4$ HRSV**: Approximately 95% protection.

The x-axis represents the percentage of animals free of HRSV upon challenge, ranging from 0% to 100%.
Figure 3

IgG in serum
OD (mean)

0 7 14 21 28 33
day after vaccination

* * *

Downloaded from http://jvi.asm.org/ on October 2, 2017 by guest
Figure 4

A. VN titer in serum

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<thead>
<tr>
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B. VN at time of challenge

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Net VNboost after RSV challenge

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<tr>
<td>10^4 vaccine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>no vaccine</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

VN increase induced by challenge

<table>
<thead>
<tr>
<th></th>
<th>2 months</th>
<th>9 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>10^5 vaccine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10^4 vaccine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>no vaccine</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 5

IgA in lungs (absorbance 450 nm)

2 months

9 months

10^5 vaccine

10^4 vaccine

no vaccine

0.0

0.5

1.0

1.5