

BRIEF COMMUNICATION

Age-Dependent Replication of Respiratory Syncytial Virus in the Cotton Rat

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Despite the documented disease burden of respiratory syncytial virus (RSV) in the elderly, little is known about the underlying risk factors or pathogenesis of RSV in a geriatric population. This report describes an age-dependent change of RSV clearance in the lung and nose of the cotton rat. Six days postinfection with RSV, lung and nose viral titers were significantly higher in all older age groups as compared with 4- to 6-week old cotton rats ($P < 0.05$). When comparing the 4- to 6-week old animals to the 15- to 16-month old animals 6 days postinfection, there was over an 800- and 100-fold increase in lung and nose viral titers, respectively. The cotton rat may prove to be a useful model in eliciting mechanisms of severe RSV disease in the elderly. *Exp Biol Med* 227:799–802, 2002

Key words: respiratory syncytial virus; elderly; cotton rat.

Respiratory syncytial virus (RSV) has been long been recognized to be the most common cause of lower respiratory tract infections in infants. In recent years it has also been identified as the cause of significant morbidity and mortality in the elderly. Studies have suggested that the extent of RSV-associated hospitalization in the elderly may be similar to that for influenza and *Streptococcus pneumoniae* infections (1–3). The risk factors for and pathogenesis of RSV disease in the elderly remain largely un-

known. It has recently been reported that older BALB/c mice have a reduced CD8⁺ and interferon- γ response to RSV infection (4). The cotton rat (*Sigmodon hispidus*), a widely used model of RSV infection, is more permissive for RSV replication than the BALB/c mouse throughout life. We describe an age-related change in the ability of cotton rats to clear the virus that may provide further clues to the mechanisms underlying RSV disease in the elderly.

Inbred cotton rats of both sexes were obtained from our breeding colony. Animals were housed in large polycarbonate cages and fed a diet of standard rodent chow and water. All experiments were approved by the corporate Animal Use and Care Committee, and were conducted in accordance with NIH animal care guidelines.

The prototype Long strain (group A) of RSV was obtained from the American Type Culture Collection. Virus stocks were prepared in HEP-2 cells and serially plaque-purified to reduce defective-interfering particles. A single pool of virus containing 10^7 plaque-forming units (pfu) per milliliter was used. Viral titers were determined by plaque assay on HEP-2 cell monolayers as previously described and were expressed as pfu/g tissue (5).

Cotton rats were grouped as follows: 4- to 6-week-old, 6- to 7-month-old, 9- to 10-month-old, 12- to 13-month-old, and 15- to 16-month-old. Under isoflurane anesthesia the animals were infected intranasally with a dose of 10^6 pfu of RSV per 100 g of body weight. The first experiments were designed to determine the effect of age on the clearance of RSV from nasal and pulmonary tissues. Four, 6, and 8 days after infection, animals in each of the age groups were sacrificed by carbon dioxide inhalation. Lungs and noses were removed and processed for viral titration as described (5). Because small numbers of older animals were available at any one time, the experiments were repeated until there were 8–21 animals per age group per time point. The sum-

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mary of such experiments is shown (Fig. 1). Virus titers are expressed as the geometric mean \pm standard error (SE) for each of the age groups. The geometric mean of the different cotton rat age groups was compared to the geometric mean of the 4- to 6-week-old cotton rats of their respective sacrifice days using the two-tailed Student's *t* test of summary data.

Peak lung and nose titers were observed for all age groups 4 days postinfection, ranging from 5.06–5.67 and 4.96–5.32 \log_{10} pfu/g in the lung and nose, respectively. There was a significant, although unimpressive, increase in the 12- to 13- and 15- to 16-month-old animals' lung titers when compared with the 4- to 6-week-old animals' lung titers ($P < 0.05$). There was no significant difference found in the viral titers in the nose when comparing the different age groups with one another.

On day 6, postinfection lung viral titers were significantly higher in each older age group as compared with the 4- to 6-week-old cotton rats ($P < 0.05$). Nineteen of 21 4- to 6-week-old cotton rats had cleared the virus below detection levels (2.0 \log_{10} pfu/g). The highest viral titers were observed in the 15- to 16-month-old cotton rats ($n = 8$) with a geometric mean of $4.98 \pm 0.31 \log_{10}$ pfu/g. This was over an 800-fold increase in lung viral titer compared with the 4- to 6-week-old cotton rats. The same trends that were ob-

served in the lungs on day 6 postinfection were also seen in the noses. RSV was almost completely cleared ($2.53 \pm 0.11 \log_{10}$ pfu/g) in the 4- to 6-week-old cotton rats ($n = 19$). As in the lungs, viral titers in the nose increased as a function of age, with the highest titer in 15- to 16-month-old animals ($n = 8$, $4.63 \pm 0.28 \log_{10}$ pfu/g). This was over 100-fold higher than the youngest animals.

On day 8 postinfection (data not shown), all but one of the 15- to 16-month-old animals had cleared the RSV from the lungs below detection levels, and the noses of the 15- to 16-month-old animals ($n = 8$) had very low levels of infectivity (2.63 ± 0.3 pfu/g, \log_{10}).

Because it seemed possible that poor nutrition of the cotton rats as a result of illness could explain the delayed viral clearance, groups of 10 each of 6-week and 14- to 15-month-old cotton rats, half infected with RSV, were weighed daily for 8 days. No consistent weight gain or loss was noted in either the infected or control animals, and weight variation from day to day in a single animal was less than 5%.

The second experiment was designed to determine the effect of age on pulmonary lesions. Animals studied histologically were sacrificed by carbon dioxide inhalation, after which the heart and lungs were removed together. The two left lobes were ligated around the bronchi and then removed

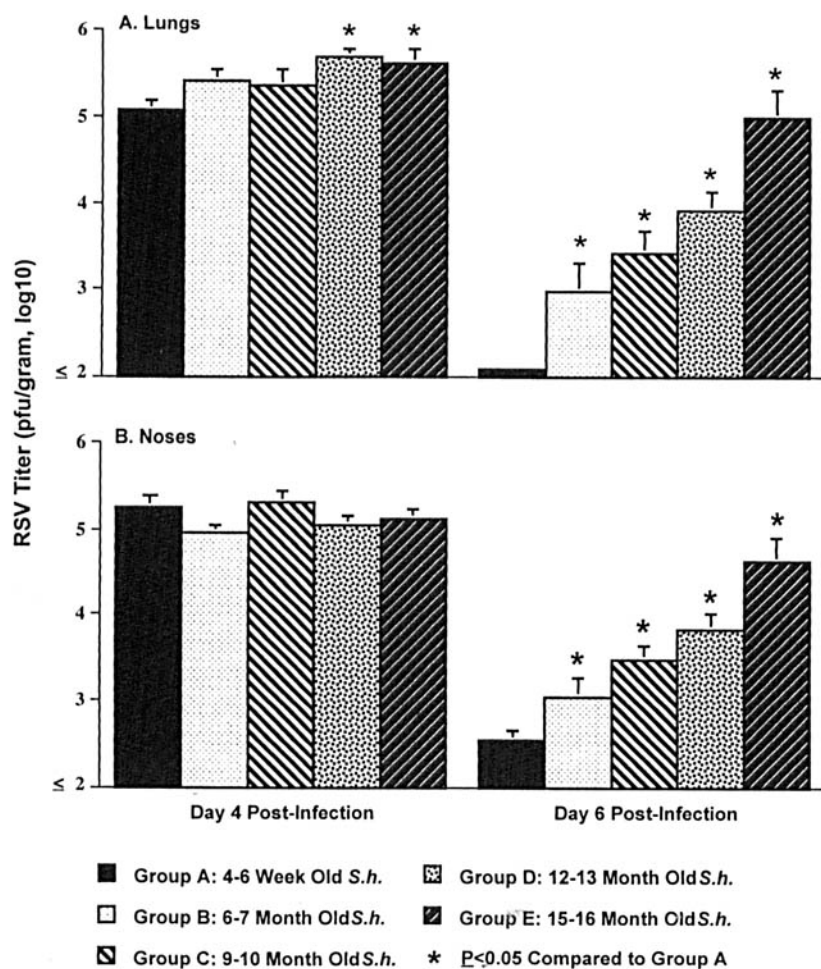


Figure 1. (A) Comparison of RSV lung titers 4 and 6 days after infection (8–21 animals per day per age group). *Significant at $P < 0.05$ compared with 4- to 6-week-old animals of the same time interval. Bars are SE. (B) Comparison of RSV nose titers 4 and 6 days after infection (8–19 animals per day per age group). *Significant at $P < 0.05$ compared with 4- to 6-week-old animals of the same time interval. Bars are SE.

for viral quantitation. The three right lobes were inflated through the trachea to their normal volume with 10% neutral-buffered formalin. The trachea was tied with a suture and the lungs immersed in formalin for several days. After paraffin embedding, coronal sections of the lungs were cut so as to show all three lobes on a single slide. Sections were cut at a thickness of 4 μm and stained with hematoxylin-eosin. Three pulmonary inflammatory changes were scored in each lung section: peribronchiolitis (inflammatory cells, primarily lymphocytes, surrounding a bronchiole), alveolitis (inflammatory cells within alveolar spaces), and interstitial pneumonitis (increased thickness of alveolar walls associated with inflammatory cells). Each lesion was scored separately for each histologic section. Before scoring, slides were examined to determine the range of lesions of each type. The maximum for each lesion was assigned a value of 100. The slides were randomized, read blindly, and scored for each lesion as a percentage of the maximum. The scores are relative and valid only for comparing the same lesion in different sections and not for comparing different lesions within a section.

Histopathologic changes in the lungs were similar in kind and intensity for all age groups for the three lesions evaluated. The age of the cotton rats did not correlate with lesion intensity, but the older animals had slightly delayed onset and recovery from lesions (data not shown). Only 1 of 36 statistical comparisons were significant at the $P < 0.05$ level. The lungs of young and older control and RSV-infected cotton rats are shown (Fig. 2). The larger alveoli in the older cotton rats are evident, but the degree of inflammatory change after infection is similar.

The third experiment was designed to determine the effect of age on RSV neutralizing antibody titers. Eight animals per age group were bled from the retro-orbital venous plexus 0, 4, 6, 8, 18, 28, and 56 days postinfection. The serum was stored at -20°C until assayed for neutralizing antibody. Serum samples were assayed individually using a plaque-reduction neutralization assay with a 60% endpoint (5). There was no age-dependent difference observed in the cotton rat in their neutralizing antibody response to RSV (data not shown).

In the young cotton rat model of RSV infection, viral replication is prompt and vigorous, and viral titers are appreciably higher than in the most sensitive mouse strain, BALB/c (4). After a brief eclipse, nasal and lung viral titers peak about 4 days postinfection in young adult animals, and lesions are generally maximal 6 days postinfection (5). RSV infection in cotton rats is semi-permissive, with the extent of viral replication proportional to the input (6). There are actually fewer lesions in primary infection as compared with secondary infection (7).

In the present study we have demonstrated that RSV titers are significantly higher in both the lung and nose in older cotton rats as compared with 4- to 6-week-old cotton rats at 6 days postinfection. In contrast, the viral titers in old BALB/c mice were slightly but not statistically significantly

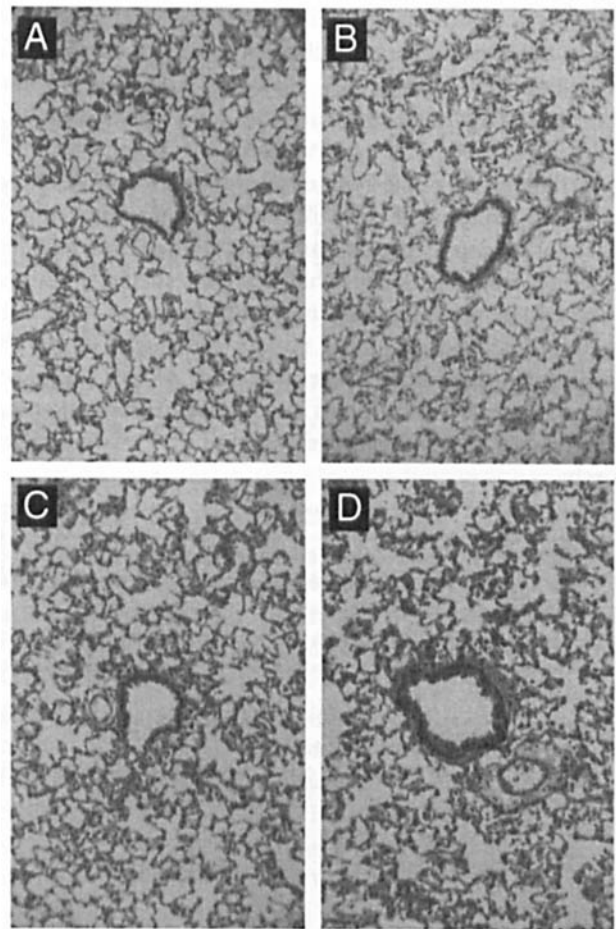


Figure 2. (A) Lung of 6-week-old cotton rat, uninfected. (B) Lung of 13-month-old cotton rat, uninfected. Note the larger alveoli. (C) Lung of 6-week-old cotton rat 6 days after infection with RSV. Note peribronchiolitis, alveolitis, and interstitial pneumonia. (D) Lung of 13-month-old cotton rat 6 days after infection with RSV. It is indistinguishable from the lung in (C) except for larger alveoli. Each photomicrograph magnification is $\times 71$, stained with hematoxylin-eosin, and a small bronchiole is in the center of each frame.

higher than in young mice (4). In the older cotton rats, the RSV infection is cleared or nearly cleared by 8 days postinfection. The degree of difference in viral titers at 6 days postinfection increases with increasing age of the host. Despite this, the extent of lesions found in these experiments is approximately the same in all ages of cotton rats tested.

Explanations for the findings in this study include an anatomical or functional change in the respiratory tract in older cotton rats, a reduction in innate immunity, or a reduction in adaptive immunity. In the absence of demonstrable intercurrent pulmonary disease, there is no reason to think that the older animals should be less vigorous in terminating a viral infection for anatomical reasons (8, 9). It seems possible that a reduction in the innate or adaptive cotton rat immune responsiveness could explain the findings; indeed, the older BALB/c mice were shown to have a diminished CD8^+ response (4). We are several years from having the reagents to do a thorough assessment of cotton rat cellular immunity to RSV, although we have an ongoing program in our laboratory to develop such reagents. It is

generally accepted that specific T cells terminate most acute viral infections, whereas antibodies are more important in prevention of infection (10). However, note that we have been successful in treating an acute RSV infection with a relatively large dose of passive antibody (11).

Although this may be a usable model for the study of RSV infections in the human elderly, it has some limitations. For example, it is impossible to duplicate the repeated RSV infections that occur in humans (3), especially because in the cotton rat pulmonary immunity lasts for at least 18 months, the majority of the cotton rat lifespan (12). The mechanisms that result in the difference in viral clearance that we describe in this report may provide information that could lead to prevention or treatment of RSV disease in the elderly.

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