Infant baboons infected with respiratory syncytial virus develop clinical and pathological changes that parallel those of human infants

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Papin JF, Wolf RF, Kosanke SD, Jenkins JD, Moore SN, Anderson MP, Welliver RC, Sr. Infant baboons infected with respiratory syncytial virus develop clinical and pathological changes that parallel those of human infants. Am J Physiol Lung Cell Mol Physiol 304: L530–L539, 2013. First published February 15, 2013; doi:10.1152/ajplung.00173.2012.—Respiratory syncytial virus (RSV) infection of the lower respiratory tract is the leading cause of respiratory failure among infants in the United States of America and annually results in >300,000 deaths worldwide. Despite the importance of RSV, there is no licensed vaccine, and no specific form of therapy. This is largely due to the absence of an appropriate animal model for the evaluation of vaccines and therapeutic agents. We inoculated anesthetized infant (4 wk) baboons (Papio anubis) with a human strain of RSV intranasally or intratracheally. Baboons were monitored daily for clinical changes. Anesthetized baboons were intubated at various intervals, and bronchoalveolar lavage (BAL) was performed for viral culture and determination of leukocyte counts. Sham-infected baboons served as controls. Necropsies were performed on infected baboons on days 1, 3, 5, 8, or 13 after inoculation, with pathological analysis and immunohistochemical staining of lung tissues to detect RSV antigen. Infected baboons developed tachypnea and reduced oxygenation peaking from 4 to 8 days after infection and persisting for ≥14 days. Virus was recoverable in BAL fluid up to 8 days following infection. Necropsy revealed intense interstitial pneumonia, sloughing of the bronchiolar epithelium, and obstruction of the bronchiolar lumen with inflammatory cells and sloughed epithelial cells. RSV antigen was identified in bronchiolar and alveolar epithelium. We conclude that RSV-infected infant baboons develop clinical and pathological changes that parallel those observed in human infants with RSV infection. The infant baboon represents a much-needed model for studying the pathogenesis of RSV infection and evaluating antivirals and vaccines.

interstitial pneumonia; bronchiolitis; baboon model; animal models; respiratory syncytial virus vaccine

RESPIRATORY SYNCYTIAL VIRUS (RSV) is the most important respiratory pathogen of early life. RSV infection, in the form of interstitial pneumonia and bronchiolitis, annually results in >120,000 hospitalizations in the United States of America and >300,000 deaths worldwide (21, 24). RSV lower respiratory tract infection (LRTI) in infancy also may predispose to the development of childhood asthma (33). Despite the considerable impact of RSV infection, there is no approved vaccine and no effective specific form of therapy. The lack of an appropriate animal model has resulted in an incomplete understanding of the pathophysiology of the disease and an inability to evaluate the safety and efficacy of vaccines and pharmacological agents. An animal model developing clinical and pathological manifestations similar to those of human infants with RSV infection would provide a significant step toward overcoming these problems. To reflect human disease, an appropriate animal model should develop tachypnea, reduced oxygenation, and interstitial and peribronchial inflammation with obstruction of the bronchiolar lumen by invading leukocytes and exfoliated epithelial cells following infection. These manifestations should be most prominent in an infant of the species tested, since RSV infection in humans is most severe in infants.

Existing animal models of RSV infection fall short of these desired characteristics in various respects. In mouse models, RSV disease is mediated by aggressive lymphocyte responses, that is, removal of CD4 or CD8 lymphocytes before infection leads to milder illness despite permitting greater virus replication (14). This is in marked contrast to the situation in humans, where severe RSV bronchiolitis is characterized by an absence of CD8 cytotoxic T lymphocytes in the lung (37). This substantial difference in pathogenesis makes it unlikely that results from testing of vaccines and pharmacological agents in murine models will apply to RSV infection in humans. Lambs infected with RSV develop histological evidence of LRTI, but actual symptoms of LRTI (sustained tachypnea and hypoxia) are minimal or absent (20, 25, 31). Infection of cattle with bovine RSV strains may cause significant LRTI, but human RSV strains induce lesser changes (2). Moreover, in calves raised on farms, RSV-induced lung disease is characterized by intense neutrophilic inflammation, mucopurulent exudate, and growth of a variety of bacterial pathogens, suggesting the presence of bacterial superinfection (2, 35). Such secondary infection is extremely uncommon in human infants with RSV infection (28). When calves are raised in germ-free environments, bovine RSV challenge can result in pneumonia, but the disease seems to be lymphocyte-mediated, as it is in rodents (1). It is therefore uncertain whether findings in calves can be generalized to humans.

Numerous species of monkeys and apes have been experimentally infected with human RSV. Although RSV infection in chimpanzees causes profuse rhinorrhea, it has not resulted in LRTI (3). RSV infection in owl monkeys (27), cebus monkeys (9) (30), bonnet monkeys (26), cynomolgus macaques (8), and African Green monkeys (17) results in histological evidence of
mild interstitial pneumonia, but these animals have not developed tachypnea or hypoxia. None of these studies evaluated the effect of RSV on infant animals. The nature of inflammatory responses in infants may differ from those in older children and adults, which could affect the clinical response to RSV infection.

The olive baboon, Papio cynocephalus anubis, has often been used in biomedical research for transplantation, infectious disease, and vaccine studies. Here we report on the use of the infant olive baboon in the development of a novel model of RSV disease that closely mimics the findings observed in human infants.

METHODS

Baboons. Olive baboons obtained from the Oklahoma Baboon Research Resource at the University of Oklahoma Health Sciences Center (OUHSC) were infected at 4 wk of age. After infection, study baboons were monitored in a sequestered nursery. Animals surviving the study were returned to the baboon colony. Baboons were maintained and treated in accordance with guidelines and approved protocols of the Institutional Animal Care and Use Committees and the Institutional Biosafety Committees of OUHSC. Study protocols were submitted to and approved by the same committees.

Obtaining of clinical data and specimen collection. Baboons were monitored daily for respiratory rate, level of activity, and food intake. On study day 0 and at various intervals up to 21 days thereafter, baboons were lightly anesthetized with ketamine (10 mg/kg) and acepromazine (1 mg/kg). On these occasions, measurements of body temperature, respiratory rate, heart rate, and oxygen saturation (O2sat) were obtained, and auscultation of the chest was performed. Venous blood was drawn for complete blood counts and antibody determinations. Animals were then intubated with a 3.0 endotracheal tube (ETT), and bronchoalveolar lavage (BAL) was performed by inserting tygon tubing through the ETT and gently wedging it into the terminal airway. After instilling 0.5-ml aliquots of sterile PBS in the lung, BAL fluid was recovered by suctioning. Recovered fluid (generally 50–60% of the volume instilled) was centrifuged to sediment cells and then frozen for further analysis.

RSV infection of baboons. After obtaining BAL fluids on day 0, baboons were infected with the A2 strain of human RSV. The RSV inoculum contained $2 \times 10^7$ plaque-forming units (pfu)/ml of RSV grown in HEp-2 tissue culture cells. Intranasal infection (n = 2 baboons) was performed by inserting a 22-gauge catheter in the nares and instilling 0.5 ml of inoculum in the nasal cavity. This was repeated in each nas, for a total inoculum of 2 ml. Intratracheal infection (n = 13) was performed by placing 4 $\times$ 1 ml aliquots of inoculum in the lung via tubing inserted through a cuffed ETT and wedged in the distal lung. For each of the four doses, the baboons were repositioned so upper and lower quadrants of each lung were targeted. Sham infection (n = 3) was carried out by inoculating 4 $\times$ 1 ml aliquots of uninfected HEp-2 cells processed in a similar fashion as for the preparation of the RSV inoculum. This preparation was also administered via a cannula passed through the ETT and wedged in the lung.

BAL cell counts and virology. The number of leukocytes recovered from BAL fluid was counted in a hemocytometer. Leukocyte differentials were determined by a pathologist, who generally counted 200 cells. Supernatant fluid was then frozen at $-80^\circ$C. Virus titration was completed using published methods (7).

Serology. RSV-specific antibodies were measured by two methods. First, total RSV-specific IgG was determined using an immunofluorescent bead assay. Ultraviolet-inactivated RSV in 0.1% Tween 20 was coupled to Bio-Plex Pro magnetic carboxyl beads (Bio-Rad) using the Bio-Plex Aamine Coupling Kit (Bio-Rad). Sera were incubated with 2,000 coupled beads for 1 h at room temperature in the dark, with shaking at 300 revolutions/min (rpm) in a flat-bottom 96-well plate. Beads were then washed with the Bio-Plex Pro wash station (Bio-Rad) and incubated with biotinylated goat anti-human IgG (Vector Laboratories) for 45 min at room temperature in the dark, with shaking at 300 rpm. The beads were washed again and incubated with streptavidin-PE for 10 min. Beads were washed again and resuspended in 125 μl of PBS by shaking at 1,100 rpm. Data were collected using the Bio-Plex 200 multiplex bead array system (Bio-Rad).

We also determined titers of RSV-neutralizing antibody (NA) in baboon sera using published methods (10). An RSV stock was diluted to contain 500 pfu/ml, and 0.5 ml of this stock was incubated with 0.5 ml of twofold dilutions of baboon sera in growth medium. The neutralization titer was determined to be the highest serum dilution resulting in a 90% reduction in the number of plaques.

Pathology and immunohistochemistry. Baboons infected intratracheally with RSV were humanely killed on days 1 (n = 2), 3 (n = 3), 5 (n = 2), 8 (n = 1), and 13 (n = 1) after infection; four other baboons survived beyond day 21. One intranasally infected baboon was humanely killed 13 days after infection, and another survived the protocol. Lung tissues from the baboons undergoing necropsy were fixed in formalin, and lung tissue slices were processed for routine histology [hematoxylin and eosin (H&E) staining]. Similar tissues from each of these baboons were processed using immunohistochemical (IHC) techniques for detection of RSV antigen and for enumeration of CD4 and CD8 cells (37). To determine the number of CD4 and CD8 cells present in lung tissues, a pathologist counted the number of cells in 10 high-power microscopic fields (HPF) and averaged the results. Lung tissue from two control baboons with neither RSV nor sham infection was analyzed similarly.

Statistical analysis. Statistical analysis was carried out with the guidance of the institutional biostatistician. Differences in respiratory rates and O2sats between RSV and sham-infected baboons were determined by combining all observations obtained over the indicated intervals. Because values were not normally distributed, medians were compared using the Mann-Whitney nonparametric test. Differences in other group mean outcomes listed below were also assessed for normality and, since the values had a normal distribution, means were evaluated using t-tests. Correlations of BAL leukocyte numbers with disease outcomes were analyzed by calculating coefficients of correlation. The numbers of CD4 and CD8 cells accumulating in the lung over the course of infection were compared by ANOVA. Data are expressed as means ± 1 SE.

RESULTS

Clinical observations. The mean respiratory rate in sedated baboons on day 0, before any procedures were performed, was 44.8 ± 2.5 breaths/min (Fig. 1A). All eight baboons infected intratracheally and allowed to survive for adequate intervals developed marked tachypnea, with respiratory rates 1.5–2.5 times above baseline rates. Peak respiratory rates were observed from days 5 to 8 following infection (Fig. 1A) with daily mean respiratory rates ranging from 64 to 108/min over this interval. Respiratory rates in these baboons remained elevated above preinfection rates for up to 15 days following infection, before returning to baseline values on days 21–22. In sham-infected baboons, mean daily respiratory rates did not exceed 45/min at any point. Respiratory rates in baboons infected intratracheally were higher over the interval from days 1 to 8 (P = 0.0007) and days 1 to 15 (P < 0.0001) than those in sham-infected baboons over the same intervals. Animals infected intratracheally often exhibited labored abdominal breathing with mild-moderate chest wall retractions. Auscultation of the chest revealed unequivocal wheezing in one baboon and slight harshness of breath sounds on expiration in another.
intervals illustrated, the reduced oxygenation could not be Boons received the same degree of sedation at each of the time sham-infected baboons over the same intervals. Because ba-

\[ P \] values in RSV-infected baboons (B), A (culture cell preparations). For respiratory rate values in RSV-infected baboons obtained from 13 baboons infected it and 3 sham infected (uninfected tissue rates and reduced oxygenation in infant baboons. Values illustrated were Fig. 1. Respiratory syncytial virus (RSV) infection causes increased respiratory

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Fig. 1. Respiratory syncytial virus (RSV) infection causes increased respiratory rates and reduced oxygenation in infant baboons. Values illustrated were obtained from 13 baboons infected it and 3 sham infected (uninfected tissue culture cell preparations). For respiratory rate values in RSV-infected baboons (A), n = 12 on day 0, n = 3–6 from days 1 to 8 and days 2 to 3 thereafter. For sham-infected baboons, n = 2–3 at all points. For oxygen saturation (\( O_2\text{sats} \)) values in RSV-infected baboons (B), n = 12 on day 0 and n = 3–6 at all other points. For sham-infected baboons, n = 2–3 at all points. Each point represents the mean ± SE. The horizontal axis represents days after inoculation of baboons. Respiratory rates determined in it-infected baboons from days 1 to 8 and from days 1 to 15 differed significantly from those in sham-infected baboons over the same intervals (\( P = 0.0007 \) and \( P < 0.0001 \), respectively). \( O_2\text{sats} \) measured from days 1 to 8 and from days 1 to 15 also differed between it-infected and sham-infected baboons (\( P < 0.048 \) and \( P = 0.025 \), respectively).

Three of 7 baboons infected intratracheally and tested for oxygenation developed reduced oxygenation, with \( O_2\text{sats} \) of <97% from days 4 to 7 (Fig. 1B). Daily mean \( O_2\text{sats} \) (all 7 baboons included) ranged from 93.5 to 95.5% from days 4 to 7 after infection and did not return to preinfection values of 99–100% until beyond 15 days after infection. Sham-infected baboons maintained mean daily \( O_2\text{sats} \) of >97.5% throughout the entire study period (Fig. 1B). Mean \( O_2\text{sats} \) in baboons infected intratracheally were lower over the interval from days 1 to 8 (\( P = 0.048 \)) and days 1 to 15 (\( P = 0.026 \)) than those in sham-infected baboons over the same intervals. Because ba-

Boobs received the same degree of sedation at each of the time intervals illustrated, the reduced oxygenation could not be attributed to sedation alone.

Boobs infected intranasally experienced only intermittent tachypnea during the 1 wk after RSV infection, and \( O_2\text{sats} \) did not fall below 96% over this interval (data not shown).

Viral replication. We performed serial BAL on 11 baboons (8 infected intratracheally, 3 sham infected) to determine the pattern of viral replication after infection (Fig. 2). In two intratracheally infected baboons, BAL was performed at 6, 12, 24, 48, and 72 h after infection to determine the pattern of early virus replication. Sham-infected animals were negative for RSV on all occasions. Baboons receiving RSV were culture-negative at \( t = 0 \) (preinfection). By 6 h after inoculation, >10\(^{10} \) pfu/ml of RSV was recoverable in BAL fluid samples. The titer of recoverable virus increased to a peak of >10\(^{9} \) pfu/ml of BAL fluid at 12 and 24 h after inoculation and then remained fairly constant at >10\(^{9} \) pfu/ml from 2 to 5 days after inoculation before declining on day 8 and being undetectable on day 12 following infection. All baboons inoculated with RSV intratracheally had virus recoverable in BAL fluid on at least one occasion. Five baboons infected either intranasally or intratracheally were studied before we had developed our technique for obtaining BAL fluids and thus did not have results of viral titrations available.

Antibody responses. The serum antibody response following inoculation with RSV was determined by measuring the titers of total anti-RSV IgG and of RSV NA. Before inoculation, anti-RSV IgG was generally not present, but total RSV-IgG responses were detectable in RSV-infected baboons on days 14 and 21 (Fig. 3A). No anti-RSV IgG was found throughout the study period in sham-inoculated controls.

All baboons were seronegative for NA to RSV before infection. Each of four baboons inoculated intratracheally with RSV developed NA in serum by day 21 after infection, with titers ranging from 4 to 256 (mean = 70, Fig. 3B). Sham-infected baboons remained seronegative for NA to RSV throughout the 21-day period.

Leukocytes in BAL fluid. The total number of leukocytes in BAL fluid was 84,293 ± 4,740/ml at baseline and remained fairly constant in sham-infected baboons (Fig. 4A). In intratracheally infected baboons, the number of leukocytes in BAL fluid rose ~10-fold by 24 h after infection and then declined on days 2 and 3 after infection. Thereafter, total leukocyte counts gradually increased again in BAL from day 4 through day 12 before declining by day 15. Neutrophils were the predominant cell type in BAL fluids during the first 3 days following infection, comprising ~85% of cells present. Macrophages became the predominant cell type from days 4 to 8 following infection and were still present in high numbers through day 12. Lymphocytes began to increase slightly in BAL populations on day 8 and were slightly more numerous than macrophages on day 12 after infection (Fig. 4, B–D). Neither the total number of leukocytes nor the number of any specific cell type correlated in a statistically significant fashion with respiratory rates or \( O_2\text{sats} \) (each probability >0.27).

Routine pathological analysis. Groups of baboons were hu-

manly killed on various days following infection (see Methods), and lung tissues were processed for routine H&E staining. Lung tissues from central and peripheral portions of the lung were analyzed in all cases. There was approximately equal involvement grossly of central and peripheral areas of the lung. Pathological findings at necropsy are illustrated in Fig. 5. The external appearance of the lungs on days 1, 3, and 5 following infection is illustrated in Fig. 5, A–C. On day 1 (Fig. 5A), the external appearance of the lung showed evidence of mild vascular congestion, and the cut surface was essentially normal. On day 3 (Fig. 5B), the external surface showed marked vascular congestion in

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nearly all areas of the lung, and the cut surface confirmed the presence of marked vascular congestion without internal hemorrhage in the lung. The lungs were grossly edematous. On day 5 (Fig. 5C), vascular congestion and edema were still prominent on the external lung surface, and there was consolidation of part of one lobe. The two cut areas revealed marked congestion internally without hemorrhage. Vascular congestion and edema were still prominent on gross examination of the lungs of animals undergoing necropsy on day 13 (data not shown).

Lung slices were also processed for routine H&E staining. Figure 5, D–I, illustrates the pathological findings observed in lung tissue obtained on days 1, 3, and 5 after RSV infection. On day 1 after infection (Fig. 5D), mild degrees of interstitial inflammation were noted, predominantly in peribronchiolar areas. On days 3 and 5, the degree of inflammation increased, with more intense inflammation of the interstitium (Fig. 5, E and F). Figure 5, G–I, demonstrates progressive bronchiolar epithelial damage and sloughing over the course of infection, leading to partial to complete obstruction of some bronchioles by a mixture of fragmented epithelium and mononuclear inflammatory cells. Loss of the bronchiolar epithelium was noted in several areas of all specimens obtained on each day following infection, and proliferative changes were often noted in the bronchiolar epithelium that had otherwise remained intact. Inflammatory changes remained quite prominent in necropsy tissues obtained on days 8 and 13 after infection (data not shown).

The extent of lung involvement varied from mild to severe among individual animals. The distribution of pathology was patchy in some animals and more diffuse in others. Changes were observed in both central and peripheral areas of the lung. Figure 5 illustrates the close similarity of pathological findings in baboons to those occurring in humans with RSV infection.

Figure 6 demonstrates areas of the baboon lung in which RSV infection caused the fusion of epithelial cells into characteristic syncytiotrophoblastic-like syncytia. Pale pink viral inclusion bodies were also observed in some areas.

IHC staining for RSV antigen. Figure 7 illustrates the histochemical findings following IHC staining of the lung for RSV antigen. Figure 7, A–C, demonstrates that RSV antigen is present in ciliated epithelial cells early in the course of infection (Fig. 7A), with staining of primarily basal epithelial cells later (Fig. 7C), presumably after apical ciliated epithelial cells have sloughed as a result of infection. RSV antigen was detected in bronchiolar epithelium in tissues from all animals tested.

Figure 7, D–F, illustrates the progression of RSV infection over the first 5 days following infection. In Fig. 7D, infected cells are observed in the bronchiolar epithelium and in the interstitium of alveoli that are adjacent to the bronchioles. In Fig. 7E, RSV antigen is detected in alveolar interstitium in areas more distant from the bronchioles. Finally, in Fig. 7F, the distribution of RSV antigen is more diffuse, and there is a marked increase in the degree of leukocyte infiltration of the interstitium, with greater thickening of the interstitial walls. RSV infection therefore apparently originates in bronchiolar epithelial cells and then spreads to the interstitium over the first few days of infection.

RSV antigen could not be detected by IHC in tissues obtained on day 8 or day 13 from RSV-challenged baboons.

IHC staining for CD4 and CD8 lymphocytes. Severe RSV infection in human infants is characterized by replication of RSV in bronchiolar and alveolar epithelium with an absence of cytotoxic CD8 lymphocyte responses in the lung (37). To determine if severe RSV infection in infant baboons is also characterized by reduced CD8 responses, we performed IHC for CD4 and CD8 lymphocyte antigens on lung tissues from baboons infected intra-tracheally at 1, 3, 5, 8, and 13 days after infection, comparing results with those in two uninfected control baboons (Fig. 8). The number of CD4-positive cells was 2.2 ± 0.4/HPF at baseline and rose to ~7/HPF over the course of infection (P < 0.0001). The number of CD8-positive cells was 0.3 ± 0.2/HPF at baseline and did not increase in a statistically significant fashion (P = 0.86) during the course of infection. The number of CD8 lymphocytes never exceeded a mean of 1 cell/HPF at any point. CD8+ cells could be detected in the lungs of all baboons tested, indicating that the assay was functional. This suggests that RSV LRTI in infant baboons is associated with a lack of strong CD8 cytotoxic lymphocyte responses, as is the case in human infants with severe disease (37).

DISCUSSION

One of us (Welliver) previously participated in the only study known to us involving RSV infection of infant baboons (23). In
that earlier study, groups of pregnant baboons were immunized with an RSV vaccine or placebo, after which infants born to each group were inoculated with 4 $\times$ 10^7 pfu of RSV. Infants of vaccinated mothers were protected against infection, but the manifestations of illness in infants of unvaccinated mothers were not described in detail. In the present study, we extend these previous results by demonstrating that infant baboons infected with a human strain of RSV develop tachypnea and reduced oxygenation. The degree of tachypnea (daily mean respiratory rates of 64–108/min) and reduced oxygenation (daily mean O$_2$ sat of 93.5–95.5%) observed in infant baboons are meaningful in that they are similar to criteria used by clinicians to hospitalize human infants with RSV infection. The most profound abnormal-

ities of respiratory rate and O$_2$ sat in infant baboons occurred between days 4 and 7 after infection. Differences in these measures from baseline values (and from sham-infected animals) persisted for at least 15 days after infection. This is similar to the case in human infants where signs of LRTI may persist for weeks following some initial improvement (4). The prolonged duration of signs of lower respiratory tract disease in the infant baboon provides an excellent endpoint to assess the effects of antivirals and vaccines in reducing or preventing RSV-related illness. No other nonhuman primate model has been reported to demonstrate tachypnea or reduced oxygenation following infection with RSV (5, 11, 17, 26, 27, 30).

We cultured serial samples of BAL fluid from infected animals to determine the kinetics of replication of RSV in infant baboons. RSV was recovered in culture of BAL fluid from all baboons inoculated by the intratracheal route but was not tested in baboons inoculated intranasally. RSV was present in BAL fluid in low titers at 6 h after inoculation, reached a maximum at 24 h after inoculation, and then remained at fairly constant levels through the next 5 days. These findings clearly demonstrate that RSV was replicating in the lung. RSV was still detectable in BAL fluid samples obtained 8 days after infection but was undetectable in samples obtained 12 days following infection. This pattern of persistent replication is similar to that reported in human infants (22). The degree of replication of RSV in baboons provides another endpoint for evaluation of the efficacy of vaccines and antivirals.

All baboons were seronegative for RSV NA at baseline, and all sham-infected baboons had undetectable NA titers at intervals up to 21 days after inoculation. In contrast, each of five baboons inoculated intratracheally with RSV developed NA responses by day 21 after infection (arithmetic mean titer = 108), suggesting that true infection occurred. NA responses were generally of low magnitude, similar to NA titers in human infants undergoing primary RSV infection (35a). This may be because of an immaturity in the ability to develop antibody responses in infants of each species.

We also evaluated leukocyte responses in BAL fluids obtained from RSV-infected animals. We found that infection was followed by a biphasic inflammatory response. Neutrophils were the predominant cell type in BAL fluid for the first 3 days following infection, before declining sharply in number thereafter. Macrophages became the predominant cell type present from days 4 to 8, a time when illness was most severe. This may suggest that macrophages are the most important cell responsible for eradicating virus and clearing debris from the airway, as has been demonstrated in mouse models of RSV infection (29). Alternatively, it may mean that exaggerated macrophage responses somehow contribute to illness. We plan to study the role of macrophages in modifying RSV infection, hopefully identifying successful therapeutic approaches to the management of RSV infection.

Total BAL leukocyte counts reached a second peak on day 12 following infection, coincident with the appearance of lymphocytes (along with macrophages) in BAL fluids. Studies in human infants similarly demonstrate that RSV-specific cytotoxic lymphocytes do not appear in the airway of infants with bronchiolitis until 10–12 days following infection, when illness has largely resolved (15). A role for lymphocytes in eradicating RSV infection is suggested by their appearance in BAL fluid at the time when the titer of replicating virus begins.
to decline. However, the low numbers of lymphocytes present during the time of maximum illness do not support a role for these cells in the pathogenesis of RSV infection in baboons. This appears to be the case in RSV infection of human infants as well (37).

In this study, total BAL leukocyte counts did not correlate with the severity of illness in infant baboons. Total leukocyte counts have not been demonstrated to correlate with clinical indicators of illness in human infants (13). It is possible that the severity of RSV infection is not the result of the number or activity of a single cell type or cytokine, but the end effects of either airway obstruction, or else sloughing of the bronchiolar epithelium, with the loss of homeostatic factors normally produced by airway epithelial cells (12, 32). These abnormalities may increase the work of breathing necessary for the animal to maintain adequate ventilation.

The histopathological findings in RSV-infected baboons included severe vascular congestion, marked interstitial pneumonitis, extensive sloughing of bronchiolar epithelium, and areas of obstruction of the airway lumen with mononuclear cells and sloughed epithelial cells. These are the characteristic findings in RSV LRTI in human infants (16, 37), again emphasizing the similarity of disease induced in the two species. Serial IHC studies in our baboons revealed that RSV first infected ciliated epithelial cells in the bronchiolar airways followed by infection of basal epithelial cells, since the more apical ciliated cells were sloughed. Infection then appeared to spread first to the pulmonary interstitium in peribronchiolar areas before disseminating to areas of the interstitium located further from the airways. The severity of interstitial involvement seemed to reach a maximum at day 5 following infection, simultaneous with the onset of tachypnea and reduced oxygenation in the animal. This suggests that the degree of pneumonitis may underlie the degree of limitation of gas exchange in the lung.

IHC staining also revealed that CD4 lymphocytes increased approximately threefold in lung tissues over the first 5 days of infection, whereas increases in CD8 cells were minimal in lung tissues through day 13 after infection, another finding that is similar to that of human infants with severe RSV infection (37). A lack of cytotoxic CD8 lymphocyte responses might lead to more extensive viral replication and enhanced disease severity in both species. The significance of the increase in CD4 lymphocytes in
the lung during RSV infection is less clear. The fact that CD4 lymphocytes, but not CD8 lymphocytes, are present in the lung might suggest that, during RSV infection, there is some interference with the ability of CD4 helper lymphocytes to promote the development of cytotoxic CD8 lymphocytes.

The infant baboon model presents the opportunity to examine the comparative roles of viral replication and inflammatory responses in determining the outcome of RSV infection. A variety of antiviral compounds against RSV are in development (36), but there is an understandable reluctance to test new drugs in infants. Immune modulating drugs may conceivably improve the outcome of RSV infection by regulating the inflammatory response, but the safety of this approach (especially in infants) is in question. Baboons are ~95% identical genetically to humans (5, 6). Therefore, establishing the safety and efficacy of such compounds in an infant baboon would suggest that these compounds could also be used safely in human infants.

Fig. 5. RSV infection induces pathological changes in infant baboons that resemble those in human infants with RSV infection. The external appearance of intact, fresh lung blocks recovered from RSV-infected animals on days 1, 3, and 5 following infection is shown in A–C. The cut surface (arrows) reveals the state of the lung parenchyma in each figure. Lungs demonstrate incrementally greater vascular congestion and edema over time. D–F demonstrate interstitial pneumonia with worsening inflammation, epithelial damage, and consolidation over time. The inflammatory infiltrate is more subtle on day 1 and found primarily in a peribronchiolar distribution with multifocal infiltration in the bronchiolar epithelium. Progression to day 5 reveals severe thickening of the interstitium and alveolar collapse as a result of the worsening inflammatory infiltration. H&E, hematoxylin and eosin. In G–I, there is progressive bronchiolar epithelial damage with sloughing, leading to partial to complete obstruction of some bronchioles with fragmented epithelium mixed with mononuclear inflammatory cells (arrows).

Fig. 6. Formation of syncytial cells and appearance of inclusion bodies in lung tissue of RSV-infected baboons. RSV infection of humans results in fusion of epithelial cells into multinucleate syncytia, and in the formation of intracellular inclusion bodies. Arrows indicate areas where these characteristic findings are also present in the lungs of RSV-infected infant baboons.
Regarding RSV vaccines, severe illness at the time of natural RSV (not vaccine-induced) infection appears to be related to a limited release of antiviral Th1 cytokines (1, 7, 13) and a failure to generate CD8 cytotoxic lymphocyte responses in the lung (37), that is, the Th1 cytokine interferon-γ (IFN-γ) is present in infants with milder forms of RSV infection, but is virtually absent in severe forms of disease (1, 7, 13), whereas CD8 lymphocytes are essentially undetectable in the lungs of human infants with fatal RSV infection (37) but were present in an infant with very mild RSV infection who died in an automobile accident (16). We have not yet studied IFN-γ responses in infant baboons, and IFN-γ could be produced by the CD4 cells present in the lung. This will be a point of further study. Nevertheless, the number of CD8 lymphocytes in the lung was quite low in our infant baboons with RSV infection. We believe that the induction of antiviral CD8 lymphocyte responses by an RSV vaccine would protect human infants against severe forms of infection. The infant baboon appears to be an ideal model to study whether RSV vaccines can induce protective antibody and CD8 lymphocyte responses safely and effectively.

In summary, the infant baboon model of RSV infection provides an opportunity to study the relative contribution of virus replicating in the lung vs. the resulting inflammatory response in determining the outcome of RSV infection. Additionally, the model permits the evaluation of the safety and effectiveness of vaccines, antivirals, and immune-modulating agents before progressing to studies in humans. Various theories of pathogenesis could be studied in this model, hopefully providing guidance in the development of new therapeutic agents for use in severe forms of RSV LRTI.

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