Respiratory Syncytial Virus Induces Pneumonia, Cytokine Response, Airway Obstruction, and Chronic Inflammatory Infiltrates Associated with Long-Term Airway Hyperresponsiveness in Mice

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Background. Respiratory syncytial virus (RSV) infection is associated with acute morbidity (e.g., pneumonia and airway obstruction [AO]) and long-term complications (e.g., airway hyperresponsiveness [AHR]). We present a comprehensive evaluation of the acute and chronic phases of RSV respiratory tract infection, using a mouse model.

Methods. BALB/c mice were inoculated with RSV and monitored for 154 days. RSV loads and cytokines were measured in bronchoalveolar lavage (BAL) samples. Pneumonia severity was assessed using a standard histopathologic score, and pulmonary function was determined by plethysmography.

Results. RSV-infected mice exhibited viral replication that peaked on day 4–5 and became undetectable by day 7. These mice developed acute pneumonia (peak days, 4–5) and chronic pulmonary inflammatory infiltrates that lasted up to 154 days after inoculation. BAL concentrations of tumor necrosis factor–α, interleukin (IL)–6, interferon–γ, IL-4, IL-10, KC (an IL-8 homologue), MIG (CXCL9), RANTES, macrophage inflammatory protein–1α, and eotaxin were significantly higher in RSV-infected mice than in control mice. RSV-infected mice developed acute AO during the first week of infection that persisted for 42 days. RSV-infected mice also showed significant AHR in response to methacholine up to 154 days.

Conclusion. This model provides a means to investigate the immunopathogenesis of RSV infection and its association with reactive airway disease.

Respiratory syncytial virus (RSV) is the leading viral respiratory pathogen in infants and young children worldwide. RSV causes both upper and lower respiratory tract infection (LRTI), which includes bronchiolitis and pneumonia. LRTI is also associated with significant short and long-term morbidity. In addition, RSV LRTI has been associated with long-term complications, such as recurrent wheezing, reactive airway disease, and pulmonary function abnormalities [1, 2].

Studies have demonstrated that, in addition to the virus itself, an exaggerated immune response plays a significant role in the clinical manifestations of RSV LRTI. The various inflammatory mediators by which RSV is thought to induce respiratory tract pathology include (but are not limited to) Th1 and Th2 cytokines, chemokines, leukotrienes, neuronal pathways, and nitric oxide. We have previously shown that levels of the cytokines interleukin (IL)–6 and IL-10 and the chemokines IL-8, RANTES, and macrophage inflammatory protein (MIP)–1α were significantly elevated in the respiratory tract of children hospitalized with acute RSV infection and that concentrations of IL-8, RANTES, and IL-10 correlated with disease severity [3].
Several animal species have been used for studying the pathogenesis of RSV disease [4]. The mouse model of RSV infection was established during the 1980s [5]. Young adult BALB/c mice have been frequently used to characterize the acute phase of the disease, because they develop a robust LRTI after RSV infection.

We have adapted the mouse model to analyze the pathogenesis of both the acute and chronic manifestations of RSV disease. This approach allows us to quantitate the virus and inflammatory mediators in the lower respiratory tract and to assess disease severity by means of histopathologic abnormalities, using a standardized scoring system and assessing the overproduction of mucus, and by the degree of airway obstruction (AO), using whole-body plethysmography. Most previous studies of mice that analyzed the ability of RSV to induce airway hyperresponsiveness (AHR) used a combination of antigen sensitization and RSV infection to achieve this response. Because the focus of our studies is to define the role of RSV alone in the development of long-term AHR, we chose to inoculate mice once with a large RSV inoculum (~10^7 pfu). After clearing the virus and recovering from acute disease, the mice progressed into a chronic phase that was characterized by AHR and persistent airway inflammation. These results demonstrated that the mouse model could be tailored to study the immunopathogenesis of RSV-induced long-term AHR. In addition, they provided an experimental confirmation to the well-described clinical association between RSV LRTI and recurrent wheezing and AHR.

**MATERIALS AND METHODS**

**Mice.** Female, pathogen-free BALB/c mice, 8–10 weeks old, from Charles River Laboratories were maintained in filter-top cages and routinely monitored for mouse hepatitis virus, Sendai virus, pneumonia virus of mice, reo-3 virus, mouse encephalomyelitis virus, mouse rotavirus, minute virus of mice, Mycoplasma pulmonis, pinworms, and fur mites. The study was approved by the Institutional Animal Care and Research Advisory Committee at the University of Texas Southwestern Medical Center at Dallas.

**Virus.** RSV A-2 strain, which was maintained in our laboratory and routinely monitored for viral and bacterial contamination, was grown in a HEP-2 cell line. Results of plaque assays were reported in log_{10} plaque-forming units per milliliter, with 1.7 log_{10} pfu/mL as the lowest limit of detection. UV-inactivated RSV was generated by exposing the RSV A-2 stock virus to UV radiation (UV Crosslinker; Fisher Biotech) for 40 min. Plaque assays confirmed the absence of viable virus.

**Inoculation.** Mice were lightly anesthetized with inhaled methoxyfluorane before intranasal inoculation with 10^{-7} pfu of live or UV-inactivated RSV in 100 μL of Eagle minimal essential medium (EMEM) supplemented with L-glutamine, HEPES, penicillin, streptomycin, and 10% heat-inactivated fetal calf serum. Control animals were sham-inoculated with 100 μL of sterile 10% EMEM. Animals were allowed 30 s to aspirate the inoculum while being held upright and were observed until they fully recovered from the anesthetic. A total of 668 mice were used in these experiments.

**Sample collection.** Mice were anesthetized with an intraperitoneal injection of 75 mg/kg of ketamine and 5 mg/kg of acepromazine before they were killed by exsanguination. Bronchoalveolar lavage (BAL) specimens were obtained by infusing 0.5 mL of 10% EMEM through a 25-gauge needle into the lungs via the trachea, followed by aspiration of this fluid into a syringe. Virus loads and cytokine concentrations were measured in these samples. Whole-lung specimens were collected and fixed with a 10% buffered formalin solution for histologic evaluation. Samples were obtained on days 0 (within 2 h of inoculation), 1, 2, 3, 4, 5, 6, 7, 8, 10, 11, and 14 for the study of acute disease and on days 21, 28, 35, 42, 50, 70, and 154 for the study of long-term disease.

**Histopathology.** The histopathologic score (HPS) was determined by a pathologist who was unaware of the infection status of the animals. This HPS system assigned values of 0–21 (the higher the score, the greater the inflammatory changes in the lung). Formalin-fixed lungs were embedded in paraffin, sectioned, and stained with hematoxylin-eosin (H-E). Lungs were evaluated for inflammatory infiltrates and graded according to a scheme similar to that described by Cimolai et al. [6]. Each section was graded on the basis of a cumulative score from 5 categories: (1) peribronchiolar and bronchial infiltrates, (2) bronchiolar and bronchial luminal exudates, (3) perivascular infiltrates, (4) the number of monocytes, and (5) parenchymal pneumonia. This scoring system has been previously validated in other mouse models of respiratory infections [7–10]. Lung sections were also stained with the periodic acid–Schiff (PAS), to identify mucus-producing cells.

**Cytokines found in BAL specimens.** BAL specimens were analyzed by ELISA (R&D Systems) for concentrations of tumor necrosis factor (TNF)–α, IL-6, interferon (IFN)–γ, IL-4, IL-10, KC (an IL-8 homologue), MIG (monokine induced by IFN-γ; CXCL9), RANTES, MIP-1α, and eotaxin. Samples were tested for up to 70 days after inoculation, except for KC and IL-6, which were tested for up to 14 days after inoculation.

**Plethysmography.** Whole-body, unrestrained plethysmography (Buxco Electronics) was used to monitor the respiratory dynamics—specifically, the enhanced pause (Penh)—of mice in a quantitative manner with and without methacholine exposure. Penh is a dimensionless value that represents a function of the ratio of peak expiratory flow to peak inspiratory flow and a function of the timing of expiration, and it correlates with pulmonary airflow resistance. Penh, as measured by plethysmography, has been previously validated in animal models.
of AHR [11, 12] and infection-associated AO [7–9, 13]. We have chosen to describe the spontaneous baseline airway resistance (with or without infection) as AO and the transient airway resistance in response to methacholine as AHR. Baseline Penh was evaluated initially both before and after inoculation, to measure AO. Penh after methacholine challenge was measured to assess AHR. Before exposure to methacholine, mice were allowed to acclimate to the plethysmograph chamber, and then baseline readings were recorded to determine AO. After initial methacholine dose-response experiments, which showed a 50 mg/mL concentration to result in the most consistent AHR, mice were exposed to 50 mg aerosolized methacholine/mL (Sigma). Plethysmograph readings were recorded again to determine AHR. Groups of infected and control mice were always evaluated in parallel at all time points during the entire study.

Statistical methods. Calculations were made with the statistical software package SigmaStat (SPSS). For data that were normally distributed, a t test was used to compare different groups of animals at the same time point. For data that were not normally distributed, the Mann-Whitney rank sum test was used for comparisons. Spearman’s rank order test was used for correlations, because all the data, taken together, were not normally distributed.

RESULTS

RSV inoculum selection. Mice were inoculated intranasally with 10^6 and 10^7 pfu of RSV in 100 μL of 10% EMEM. There was a clear dose-dependent effect of inoculum on BAL virus load, pulmonary HPS, and AO (figure 1A–C). Because the most significant changes were observed when the 10^7-pfu inoculum was used and one of our goals was to establish a model with long-term abnormalities, we used the 10^7-pfu inoculum for further experiments.

Dynamics of RSV replication in BAL samples. RSV loads in BAL samples obtained from mice 2 h after live RSV inoculation were significantly higher (figure 1A), representing the large inoculum used. Virus load declined significantly within 24 h and then showed a progressive increase, which indicated active replication; it peaked at days 3–5 and decreased to below the limit of detection by day 7 (limit of detection, log_{10} 1.7 pfu/mL). BAL virus loads remained undetectable, according to plaque assay, for 7–154 days after inoculation (data not shown). These results were consistent, regardless of the size of RSV inoculum used.

Acute and chronic pulmonary inflammatory infiltrates and mucus overproduction. Compared with the H-E–stained lungs of control mice, those obtained from live RSV–infected mice showed a persistent progression of the severity of pneumonia, as determined by an increasing HPS, which peaked between days 4 and 5 (figures 1B and 2). RSV-induced histo-
Figure 2. The effect of live and UV-inactivated respiratory syncytial virus (RSV) on acute and long-term histopathologic score (HPS) and mucus overproduction. A, Serial formalin-fixed lung samples were obtained between days 0 (+2 h) and 154 after inoculation with live RSV (black circles) or sterile 10% Eagle minimum essential medium (black triangles), as shown in the composite graph. Lungs from UV-inactivated RSV-inoculated mice (white circles) were harvested between days 0 and 7, as shown in the 0–14-day graph insert. HPS is given as mean ± SE. *P < .05, comparison between live RSV–inoculated and sham-inoculated control mice. ‡P < .05, comparison between live RSV–inoculated and UV-inactivated RSV-inoculated mice, by t test when data were normally distributed and by Mann-Whitney rank sum test when data were not normally distributed. B, Lung sections from sham-inoculated mice on days 5 and 154 (I and II, respectively) showed rare, small lymphocytic infiltrates that were unchanged over time. Acute and chronic inflammatory infiltrates were seen in RSV-inoculated mice, here shown on days 5 and 154 after inoculation (III and IV, respectively). C, Periodic acid–Schiff stain for mucus-producing cells (arrows) showed increased mucus production, compared with control mice, on both days 5 and 14 after inoculation.

Pathologic abnormalities gradually decreased during the first week after inoculation. However, during the several weeks after inoculation, RSV-infected mice continued to demonstrate significantly greater HPS than the sham-inoculated controls, for up to 154 days (figure 2A and 2B).

During the acute phase, changes included perivascular edema and the margination of neutrophils and mononuclear cells (day 1), which progressed to a sparse perivascular infiltrate and, later, to dense perivascular and peribronchial/peribronchiolar inflammatory infiltrates that were composed of mononuclear cells and scattered neutrophils. During the peak of the acute phase (days 4–7), these inflammatory infiltrates extended into surrounding alveolar septa in a stellate manner, with patchy involvement of the parenchyma and abundant macrophages and occasional lymphocytes and neutrophils in alveolar spaces (figure 2B, III). No intraluminal exudates were identified in the
airways. The persistent changes (days 14–154) included chronic inflammatory infiltrates located around vessels and airways that consisted of lymphocytes, macrophages, and scattered plasma cells but no neutrophils or eosinophils. Occasional hemosiderin-laden macrophages were also seen. No involvement of alveolar septa or air spaces was seen during the chronic phase (figure 2B, IV). With time (days 21–154), the inflammatory infiltrates changed from circumferential to partial involvement around vessels or airways, and there was a tendency for involvement of the larger central vessels and airways rather than the smaller peripheral bronchovascular bundles. Control mice infiltrates changed from circumferential to partial involvement (figure 2B, I and II) demonstrated only rare, small lymphocytic infiltrates that involved only a small portion of the circumference of central vessels or airways.

With regard to mucus overproduction, infected mice on day 5 had increased numbers of PAS-positive cells in the central and peripheral airways, compared with control mice (figure 2C, I and 2C, III). These cells appeared to be hypertrophic, compared with cells in control mice. On day 14, there were more mucus-producing cells in the central airways of infected mice than in control mice (figure 2C, IIIIV). No PAS-positive cells were seen in peripheral airways on day 14.

**Acute and persistent AO.** Similar to the HPS, Penh values representing AO followed the dynamics of virus load and peaked around day 5. AO decreased markedly by day 10 after inoculation (figures 1C and 3A). Despite this reduction, AO remained significantly higher in RSV-infected mice than in uninfected control mice for up to 42 days after inoculation (figure 3A). Measurements were performed daily in 5–8 animals/group/time point between days 0 and 14. From days 14 to 42, 16–40 mice/group/day were tested, and 3–8 mice/group/time point were tested on days 49–154, for the determination of AO. Each experiment was repeated at least 3 times, to ensure reproducibility.

**AHR during primary RSV infection.** Mice were challenged with aerosolized methacholine to measure AHR—initially on day 5, 7, 10, and 14; then, serial weekly measurements were performed up to 154 days after inoculation. RSV-infected mice showed significantly greater Penh values after methacholine was administered than control mice, and this was consistently observed up to 42 days after RSV inoculation (n = 16–40 mice/group/data point). After day 42, RSV-infected mice intermittently showed significantly greater AHR values than control mice for up to 154 days (n = 3–8 mice/group/data point; figure 3B). Results are presented as ΔPenh in figure 3B, which shows the difference between the baseline and postmethacholine Penh as representing the net AHR. ΔPenh values were lower in RSV-infected mice on days 5 and 7 because of the higher baseline Penh at the peak of pneumonia (figures 1C and 3A). Peak postmethacholine AHR values in 6 separate experiments were consistently detected on days 14–21.

**Pulmonary cytokine response.** Compared with those from control mice, BAL samples from RSV-infected mice showed significantly elevated concentrations of TNF-α, IL-6, IFN-γ, IL-4, IL-10, KC, MIG, RANTES, MIP-1α, and eotaxin at different time points during the acute phase of the disease (figure 4A–J). However, no differences were observed between RSV-infected and control mice after day 14 and up to day 70 (data not shown).

**Lack of significant pulmonary disease with UV-inactivated RSV.** Mice inoculated with UV-inactivated RSV showed a response similar to that in control mice inoculated with sterile medium, in terms of virus load, HPS, AO (figures 1 and 2), and cytokine profiles (figure 4A–J), which suggests that the changes in mice inoculated with live RSV were induced by actively replicating virus.

**Correlations among inflammatory markers, HPS, and clinical indices of disease severity.** On days 0–14 during the acute phase of disease (table 1), Spearman’s rank order analysis of data from RSV-infected mice demonstrated a significant correlation between HPS and AO. In contrast, HPS and AO did not correlate significantly with the dynamics of virus load. BAL concentrations of TNF-α and KC correlated with RSV load, whereas concentrations of IFN-γ, IL-10, MIG, and eotaxin correlated with markers of disease severity (i.e., HPS and AO). IL-6, IL-4, RANTES, and MIP-1α concentrations correlated with both virus load and disease severity (table 1). On days 14, 28, and 35 after inoculation in RSV-infected mice, we observed a significant correlation between HPS and AO, as defined by baseline Penh values (day 14, r = .749, P = .01, and n = 10; day 28, r = .693, P = .01, and n = 12; and day 35, r = .772, P = .025, and n = 7).

**DISCUSSION**

We have presented a mouse model of RSV infection established in our laboratory with the objective of better understanding the different components of the inflammatory response to this virus, during both the acute and the chronic phase of the disease, that determines the characteristic clinical manifestations of this infection—namely, AO and AHR. We used methodologies that increase the yield of data from each individual mouse (e.g., BAL to measure virus load and cytokines), which enabled us to evaluate the histopathology in the intact lung from the same animal. Using noninvasive whole-body plethysmography to assess pulmonary function, the results of which strongly correlated with our standardized HPS system, to assess the severity of pulmonary inflammatory infiltrates potentially reduces the need to kill mice to assess pulmonary disease.

In agreement with the results of other studies [13], we did not find evidence of RSV replication, as measured by plaque assay, after 7 days of infection. Other, more-sensitive methods
RSV Induced Acute and Long-Term LRTI

Figure 3. Respiratory syncytial virus (RSV)-induced acute and long-term airway obstruction (AO) and postmethacholine airway hyperresponsiveness (AHR). A, AO: groups of mice were monitored up to day 154 after sham inoculation (white squares) or live RSV inoculation (black circles), to measure enhanced pause (Penh) without methacholine challenge using whole-body plethysmography. B, AHR data, presented as ΔPenh, which is the difference in Penh before and after the administration of methacholine for each group of mice, in sham-inoculated (white rectangles) and RSV-inoculated (black rectangles) mice, using whole-body plethysmography from days 5–154. Penh values are presented as mean ± SE for 16–40 mice/group (days 0–42). Later values are for 3–8 mice/group. Data shown are the result of 6 separate experiments. *p < .05, comparison by t test when data were normally distributed and by Mann-Whitney rank sum test when data were not normally distributed.

to detect the presence of RSV are under investigation, although, at present, there is no evidence that supports the possibility of persistent, active replication after the acute phase of the disease.

At present, there is no uniformly accepted method to evaluate histologic lung inflammation in mouse models of RSV infection. We have presented a scoring system modified from a method we used in a mouse model of Mycoplasma pneumoniae lung infection [6–10]. The majority of the earlier studies described inflammatory changes in the lung after RSV infection in mice, but only a few authors used a scoring system to quantify these changes [14–17]. We propose our HPS system to be a detailed, objective, and reproducible method to evaluate both the acute and chronic pulmonary inflammatory response after RSV infection.
Figure 4. Respiratory syncytial virus (RSV)–induced cytokine and chemokine responses in bronchoalveolar lavage (BAL) samples. BAL samples were obtained from live RSV–inoculated (white circles), UV-inactivated RSV–inoculated (black triangles), or sham-inoculated (white squares) mice, to measure concentrations of proinflammatory cytokines (A) tumor necrosis factor (TNF)–α and (B) interleukin (IL)–6; (C) Th1 cytokine interferon (IFN)–γ; (D) Th2 cytokine IL-4; (E) Th3 cytokine IL-10; CXC chemokines (F) KC (an IL-8 homologue) and (G) MIG; and CC chemokines (H) RANTES, (I) macrophage inflammatory protein (MIP)–1α, and (J) eotaxin. Values are presented as pg/mL. *P<.05, comparison between live RSV–inoculated mice and sham-inoculated control mice; †P<.05, comparison between live RSV–inoculated and UV-inactivated RSV–inoculated mice, by t test when data were normally distributed and by Mann-Whitney rank sum test when data were not normally distributed.

Our results demonstrate persistent inflammatory changes in the lungs of RSV-infected mice for up to 154 days after inoculation. Other researchers have described the persistence of lymphocytic infiltrates for up to 14 days after inoculation [18]. In contrast with the results of some investigators [11], we did not find an increased number of eosinophils in the lungs of RSV-infected mice. Other researchers found that RSV in BALB/c mice produces increased numbers of lung lymphocytes even...
if mice were previously sensitized with ovalbumin [19]. Other investigators described peribronchial/perivascular mononuclear infiltrates in mice infected with RSV only that persist for at least 15 days [20, 21]. Lukacs et al. [22] found peribronchial inflammation 42 days after inoculation with 10^5 pfu RSV but no increase in numbers of lung eosinophils without an allergen. Unexpectedly, we found a persistent chronic mononuclear perivascular infiltrate, which included plasma cells, for up to 154 days after inoculation.

Along with our findings of inflammatory infiltrates produced by RSV infection in our mouse model, we confirmed the presence of mucus overproduction for up to 14 days after inoculation. The results of previous studies have also documented the presence of mucus in airways of mice for up to 12 days after inoculation but not after that time point [16, 23]. Other investigators did not find significant changes in goblet cell hyperplasia after RSV infection alone [24]. Excess mucus production is a common feature of many pulmonary diseases, including asthma and bronchiolitis. The overproduction of mucus has been associated with adverse effects, such as mechanical AO; however, normal levels of mucus are thought to play an important role as a protective homeostatic mechanism in the lungs [25]. Cytokines of which we found elevated levels in our model and that have been associated with increased mucus production in the lungs of humans and mice are TNF-α [26], IL-4 [27], IL-5, IL-9, and IL-13 [28].

The possible relationship between these histologic abnormalities and the physiologic consequences manifested by AHR are not clear, as has been shown by Cho et al. [16]. Their results demonstrated that interventions that decreased RSV-induced inflammation and mucus production after RSV infection in mice did not prevent the development of AHR. Further studies are needed to better understand these persistent inflammatory changes and the increased mucus production elicited by RSV.

Peebles et al. [20] found increased baseline airway resistance in mice for up to 15 days after inoculation. Our results demonstrate increased AO for 42 days after inoculation. AO occurred in 2 peaks—on days 1 and 5 after RSV inoculation—at a similar time to that reported by van Schaik et al. [13] with regard to other pulmonary functional changes, such as respiratory rate. A possible explanation for the persistent AO could be the presence of pulmonary inflammatory infiltrates, which significantly correlated with AO for up to 35 days after inoculation.

Schwarze et al. [11] and van Schaik et al. [13] noted that RSV infection alone in mice induced AHR within the first week of infection. Other researchers have found that RSV induces exaggerated AHR that returned to baseline levels by day 21 [12, 22]. In our experiments, AHR consistently lasted for 42 days after RSV infection, with a trend up to day 154. The severity of RSV-induced abnormalities varies according to the age of the mice used [5]; therefore, the differences in AHR may be explained, in part, by age differences among the mice used by different investigators. We always used 8–10-week-old mice and consistently demonstrated the stated results. The long-lasting differences observed in our studies could also be related to the large number of mice included and the use of a large RSV inoculum (10^7 pfu). The results of previous studies have shown that RSV infection in BALB/c mice after presensitization with ovalbumin induced AHR [19], but no AHR was demonstrated in mice inoculated with RSV alone. Even after ovalbumin sensitization, no AHR was reported beyond day 29 after RSV infection [20].

RSV infection in our mouse model was associated with significantly elevated BAL concentrations of the early proinflam-

Table 1. Correlations between histopathologic score (HPS) and airway obstruction with cytokine and chemokine levels on days 0–14 in a study of respiratory syncytial virus (RSV) inoculation.

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<th>IL-6</th>
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<th>IL-4</th>
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NOTE: Correlations were derived by Spearman’s rank order analysis, because data were not normally distributed. Statistically significant correlations (P<.05) are given in bold. IFN, interferon; IL, interleukin; MIP, macrophage inflammatory protein; Penh, enhanced pause; TNF, tumor necrosis factor.
matory cytokines TNF-α and IL-6; Th1 cytokine IFN-γ; Th2 cytokine IL-4; Th3 cytokine IL-10; CXC chemokines KC and MIG; and CC chemokines RANTES, MIP-1α, and eotaxin, compared with controls and mice inoculated with UV-inactivated RSV. These results clearly suggest that RSV initiates a complex and often redundant inflammatory response that is most likely responsible for acute and long-term disease. Of interest are the differences in the dynamics of these inflammatory mediators.

Levels of TNF-α and IL-6 peaked early, and levels of IL-6 remained higher, but in lower concentrations, up to day 5, which clearly confirms that both of these cytokines are some of the most proximal inflammatory mediators triggered by RSV infection [29, 30]. Levels of TNF-α correlated with virus load dynamics, whereas levels of IL-6 correlated with both virus load and the severity of pneumonia. Levels of IFN-γ peaked between days 3 and 8, around the same time that pneumonia and AO peaked, whereas IL-4 concentrations were elevated during the initial 5 days of infection and therefore correlated with virus load and the severity of pneumonia. These data are clearly in accordance with the observations that IFN-γ was associated with disease severity in mice [31] and in children [32] with RSV infection. Levels of IL-10 peaked on day 5 but showed sustained low levels up to day 11 and correlated with AO and the severity of pneumonia, similar to the clinical observation that IL-10 correlated with RSV disease severity in children [3].

As the most potent chemoattractant of neutrophils, IL-8 is involved early in the inflammatory response [33–36]. In our model, levels of the CXC chemokine KC peaked very early as well.

The novel CXC chemokine MIG is expressed by macrophages, lymphocytes [37], and dendritic cells [38]. MIG is a chemoattractant for activated B cells [38], CD4+ T helper cells [39], CD8+ memory T lymphocytes [37, 40], monocytes [41], and CD56+ NK cells [37]. It promotes Th1 responses by serving as an antagonist to IL-4/Th2 responses [42]. Our data suggest that MIG plays a role in the pathogenesis of RSV, especially during the first 10 days of infection, which may explain the potential link between the acute RSV inflammatory response, which is predominantly innate, and the long-term/chronic response, which is mostly adaptive via the Th1/Th2 pathway.

The CC chemokines RANTES, MIP-1α, and eotaxin have all been of interest in the pathogenesis of RSV because of their effect on lymphocytes and eosinophils. RANTES and MIP-1α have been shown to be associated with the severity of RSV disease in children [3, 43–45]. All 3 of these chemokines, along with MIG, had the most sustained elevated concentrations in our model and correlated with disease severity, which suggests that these molecules play a fundamental role in RSV disease.

The dynamics of the cytokines and chemokines studied appeared to follow the observations made in clinical studies, making this a clinically relevant model. Therefore, this model is suitable for further studying the role of these inflammatory mediators to understand the pathogenesis of RSV disease.

In conclusion, we have demonstrated that RSV infection without allergic presensitization is capable of inducing both AO and AHR and that these functional changes last for at least 42 days. In addition, these alterations in pulmonary function are present in the context of persistent histopathologic abnormalities.

The role of different cytokines in the development of AHR is still under intense investigation; it is likely that there is not a single mediator but, rather, multiple ones that participate in the pathogenesis of RSV-induced long-term physiologic consequences. Despite the fact that specific cytokine receptors have been found to participate in the development of AHR after RSV infection in mouse models [23], the cytokine-receptor network is known to be redundant. The influence of the complex interaction among the components of the cytokine/chemokine milieu on the development of long-term AHR appears to be an early phenomenon triggered by RSV, independent of allergic sensitization, in this murine model. The persistence of some of these components beyond the phase of viral replication (days 0–7) may account for the alterations in pulmonary function and histopathologic abnormalities that we have observed in our studies. A better characterization of the immune mechanisms that are responsible for the development of AHR should provide new therapeutic targets aimed at reducing the long-term consequences of RSV infection in children.

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