Maternal immunization might protect infants from severe disease due to respiratory syncytial virus (RSV). Guinea pigs are susceptible to infections with RSV and transfer antibodies to their offspring prenatally. Pregnant guinea pigs were immunized by infection with RSV and their offspring were challenged intranasally with RSV. Pulmonary viral replication was compared among the pups born to immunized mothers (group A) and the pups from nonimmune mothers (group B) in two studies. Mean ($\pm$SD) log$_{10}$ virus titers were, in study 1, group A, 2.3 $\pm$ 0.8 pfu/g of lung ($n = 10$); group B, 3.6 $\pm$ 1.5 pfu/g ($n = 13$) ($P = .0058$); and study 2, group A, <1.69 pfu/g ($n = 8$); group B, 3.4 $\pm$ 0.9 pfu/g ($n = 6$) ($P = .0002$). Thus, immunization of pregnant guinea pigs resulted in a significant reduction in viral replication in the lungs of their offspring. Guinea pigs should be useful for the study of maternal immunization against RSV.

Respiratory syncytial virus (RSV) is the most common viral agent of lower respiratory tract infections in infancy. No vaccine is available. Challenges to vaccine development include the fact that most severe infections occur in infants 2–7 months of age. Studies in animals and in humans have demonstrated the protective capacity of serum antibodies against RSV [1]. Higher titers of transplacentally acquired antibodies are associated with protection against severe disease. Maternal immunization with an immunoaffinity-purified fusion (F) protein of RSV is being investigated in humans. Immunization of pregnant women with the F protein should stimulate the production of neutralizing antibodies, which will raise the titers of antibody in the infant and contribute to protection against severe RSV infections [2].

Although maternal immunization is an attractive approach, most experimental work with RSV has used small animals, which have more postnatal than prenatal transfer of antibodies and thus do not mimic the human situation [3]. Infant cotton rats acquire protection against RSV infections from the intake of colostrum and milk from immune mothers [4]. Influenza virus antibodies are transferred mainly by breast feeding in mice [5]. In ferrets, there is a transfer of protection against RSV from mother to neonate by the products of lactation [6]. The guinea pig may provide a useful alternative for studies of maternal immunization. The guinea pig has a placental architecture with similarities to that of humans, and there is a prenatal passage of antibodies to the fetus. Antibody uptake postnatally by enteric routes is minimal if it occurs [3]. Guinea pigs infected with RSV develop a neutralizing antibody response, and the lungs demonstrate viral replication and histopathologic bronchiolitis [7, 8]. In the work described here, we examined the use of guinea pigs as a model for the study of maternal immunization against RSV infections in young infants.

Methods

Cells and viruses. HEp-2 cells, (TK$^{-}$)143 cells, and the RSV strain A2 were grown as described [9]. Mock preparations of uninfected HEp-2 cells were similarly prepared. Virus titers were measured by plaque assay in Vero cells. Endotoxin assays were done by the limulus lysate method at the Media Preparation Shared Facility of the University of Alabama at Birmingham. Animal studies. Pregnant Hartley guinea pigs were obtained from Charles River Laboratories (Portage, MI). Anesthesia for intranasal virus administration and for sacrifice was provided with intramuscular ketamine/xylazine. Two studies were performed. In study 1, guinea pigs were obtained at 25–35 days and in study 2 at 35–45 days of gestation. The anesthetized females were immunized with live RSV intranasally (10$^6$ pfu in a 100-$\mu$L volume), immunized with a mock preparation, or left unimmunized. Their anesthetized offspring were challenged within 72 h of birth with the same amount of live virus intranasally. The pups were anesthetized and killed at 4 days (study 1) or 5 days (study 2) after infection. The right lungs were taken for determination of virus titers, and the left lungs were fixed for histopathologic analysis [9]. Blood was obtained from study 2 females at 0, 8, and 16 days after infection, females in both studies at the time of pup challenge, and pups at the time of lung harvest.

Antibody assays. Serum antibodies were measured by ELISA using lysates from mock-infected or A2 strain virus–infected (TK$^{-}$)143 cells as antigens [9]. A complement-enhanced plaque-reduction neutralization assay was performed against A2 strain virus using a modification of a described technique, and 60% plaque-reduction titers were determined [4]. Heat-inactivated sera

The Guinea Pig as a Model for the Study of Maternal Immunization against Respiratory Syncytial Virus Infections in Infancy

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Table 1. Maternal immunization against RSV in guinea pigs: lung virus titers and serum antibody titers.

<table>
<thead>
<tr>
<th>Group (n)</th>
<th>Immunogen</th>
<th>Challenge</th>
<th>Lung titer*</th>
<th>Mothers (n)</th>
<th>Pups</th>
<th>Neutralizing antibody³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A (10 pups)</td>
<td>RSV</td>
<td>RSV</td>
<td>2.3 ± 0.8</td>
<td>3.8 ± 0.4 (3)</td>
<td>4.0 ± 0.2</td>
<td>4.5 ± 1.9 (3)</td>
</tr>
<tr>
<td>B (13 pups)</td>
<td>None</td>
<td>RSV</td>
<td>3.6 ± 1.5</td>
<td>&lt;1.5 (3)</td>
<td>&lt;1.5</td>
<td>&lt;3.3 (3)</td>
</tr>
<tr>
<td>C (5 pups)</td>
<td>RSV</td>
<td>Mock</td>
<td>&lt;1.5</td>
<td>3.7 (1)</td>
<td>4.0 ± 0.3</td>
<td>5.2 (1)</td>
</tr>
<tr>
<td>Study 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A (8 pups)</td>
<td>RSV</td>
<td>RSV</td>
<td>&lt;1.69</td>
<td>3.9 ± 0.5 (3)</td>
<td>3.8 ± 0.1</td>
<td>4.5 ± 0.5 (3)</td>
</tr>
<tr>
<td>B (6 pups)</td>
<td>Mock</td>
<td>RSV</td>
<td>3.4 ± 0.9</td>
<td>&lt;1.5 (2)</td>
<td>&lt;1.5</td>
<td>&lt;3.3 (2)</td>
</tr>
</tbody>
</table>

NOTE. Differences were measured by Student’s t test: Study 1, unpaired t test, \( P = .0058 \) for group A vs. B lung titers; study 2, unpaired t test, \( P = .0002 \) for A vs. B lung titers.

* Mean ± SD of pfu/g of lung tissue (log₁₀). Lower limit of detection, 50 pfu or 1.69 log₁₀, was used for calculations for lungs with no detectable virus.

² ELISA serum antibody titers to RSV from mothers at time of pup challenge and from pups at time of lung harvest are shown as reciprocal titer (log₁₀), mean ± SD. Lowest antibody dilution tested by ELISA was 1:30, with <1.5 log₁₀ used for samples with no detectable antibody.

³ Plaque-reduction neutralizing antibody titers are shown as reciprocal of titer in log₂, with lowest dilution tested, 1:10 (3.3 log₂), used for samples with no detectable antibody. Pups samples were pooled and tested as single sample per litter.

were tested in 3-fold dilutions beginning at 1:10. Mean lung titers and antibody titers were compared using unpaired two-tailed Student’s t tests.

Results

In study 1, we determined whether neonatal guinea pigs were susceptible to infection with RSV and whether immunization of pregnant guinea pigs could modify viral replication in the infected neonate. Pregnant females at 25–35 days of gestation were infected with RSV (group A, 4 females) or were not inoculated (group B, 3 females). Pups were born 35–40 days after immunization of the mothers. Of the 15 pups born to the group A females, 10 pups were challenged with RSV (group A) and 5 pups received a mock infection (group C); pups from 3 litters were nursed by a single female). Of the 13 pups born to the group B females, all were challenged with RSV.

Some of the anesthetized adult and neonatal animals were noted to have spontaneous activity and swallowing motions. For the adult animals, we used a dose of ketamine ranging from 10 to 13 mg/kg, and for the neonatal animals the doses ranged from 7 to 30 mg/kg. Ketamine doses of 30 to 100 mg/kg have been given along with xylazine in other studies in guinea pigs [10]. We chose lower drug doses due to concern about anesthesia in the pregnant female and the lack of information about appropriate dosing of these drugs in neonatal guinea pigs. These low doses of ketamine may have resulted in anesthesia that was inadequate for some animals and reduced the amount of the infectious inoculum that reached the lower airways [11, 12].

Pups born to immunized mothers had a significant (95%) reduction in lung virus titers compared with pups of unimmunized mothers (study 1, table 1). Three pups in group A and 3 pups in group B had no virus detectable in their lungs at the time of harvest. This initial study thus demonstrated that RSV could replicate in the lungs of neonatal guinea pigs and that pups born to immunized mothers had less viral replication than did pups born to naïve mothers.

In study 2, we sought to optimize the conditions for infection of the guinea pigs and to determine the reproducibility of the observations from study 1. Study 2 differed from study 1 in the following features. The pregnant guinea pigs were immunized later in gestation, at 35–45 days. The anesthesia dose used for immunization of the pregnant females was increased to range from 16 to 35 mg/kg ketamine. The group A females (3) received live RSV and the group B animals (2) were given a mock immunization intranasally. Pups received an increased amount of anesthesia, with the ketamine component ranging from 25 to 36 mg/kg. The increased anesthesia resulted in less spontaneous activity among the anesthetized animals. The lungs were taken from the pups at 5 days after infection.

The results of study 2 were similar to those of study 1 (table 1). The offspring of RSV-immunized females (group A) were protected from viral replication in the lungs, and no virus was detected in the lungs of these animals (≥98% reduction in titers). All but 1 of the offspring of the mock-immunized females (group B) had virus recovered from their lungs. The higher doses of anesthesia used in study 2 may have allowed a greater proportion of the group B pups in study 2 than in study 1 to become productively infected.

The kinetics of the antibody response in the immunized females in study 2 was evaluated by ELISA. Of the 3 group A mothers, none had antibodies at the time of immunization, 2 had antibodies detected at 8 days, and all 3 had antibodies at 16 days after immunization and at the time of pup challenge (23–27 days after immunization). The 2 group B mothers in study 2 were tested for antibodies at these same time points and had no antibodies detected by ELISA. The mean antibody titers as determined by ELISA for the study 1 and study 2 mothers at the time of pup challenge were very similar to
those of the pups at the time of harvest (table 1). Neutralizing antibody titers were also measured for the mothers at the time of pup challenge and the pups at the time of harvest (table 1). All of the group A (immunized mothers) pup samples were found to have neutralizing activity. The neutralizing antibody titers of the pups in study 1 were almost identical to those of the mothers. The study 2 neutralizing antibody titers were lower in the pups than in the mothers but remained within one dilution of the titers of the mothers. The group B (mock or unimmunized) mothers and pups had no detectable antibodies to RSV by ELISA or neutralization.

Lung histopathology was evaluated from a limited subset of animals. All had at least some inflammatory changes, including evidence of multifocal interstitial pneumonitis, bronchiolitis, or both. Because of the observation of histopathologic changes in the mock challenge animals (group C), the mock and viral challenge samples were tested for the presence of endotoxin and found to be positive. Because of the effects endotoxin may have on pulmonary tissues [13], we did not attempt further histopathologic analysis of these samples. It was also possible that the observed changes were due to the presence of HEP-2 cellular components in both the immunizing and challenge virus preparations [14].

Discussion

Maternal immunization against RSV may offer a means of providing young human infants with protective levels of neutralizing antibodies against RSV. The studies reported here demonstrated that neonatal guinea pigs were susceptible to pulmonary infection with RSV. In addition, pups born to immune mothers had serum neutralizing antibodies to RSV and showed significant protection against viral replication in the lungs compared with the pups of nonimmune mothers. These factors, combined with the fact that in guinea pigs there is a prenatal transfer of antibodies to the fetus, suggest that the guinea pig may serve as a model for the study of maternal immunization against RSV.

The most interesting aspect of these studies was the protection against viral replication provided to neonates born to immunized mothers. In both studies, there was a significant reduction in pulmonary replication of virus (95%–98%) compared with that in control animals. The observation that the pups born to immune mothers and that received a mock inoculation (study 1, group C) after birth had RSV antibodies indicates that there was an acquisition of antibodies from their mothers. The design of these studies did not directly address the timing of the transfer of these antibodies from mother to pups. However, guinea pigs are known to have a prenatal transfer of antibody, with little or no postnatal transfer [3]. In a preliminary experiment, we examined the sera obtained from 2 pups within 2 h of birth to an immune mother. Both pups possessed neutralizing antibodies to RSV (data not shown). Studies of guinea pig cytomegalovirus have revealed that newborns born to seropositive mothers acquire detectable cytomegalovirus antibodies prenatally and not via breast milk [15]. It seems likely that the majority of the protection demonstrated in this study was transferred prenatally in the form of antibody. The timing (as to prenatal or postnatal) of the transfer of protective immunity, and ascertaining whether the protection was in fact mediated by serum antibodies, may be addressed experimentally.

Viral replication in the lungs and histopathologic bronchiolitis occurs in RSV-infected juvenile guinea pigs [8]. The day of peak viral replication was not reported in the previous studies, and we have not yet defined this parameter for the neonatal guinea pigs. We chose 4 and 5 days for harvests on the basis of studies in mice, cotton rats, and ferrets that suggested these days should be near the peak time for viral replication [11, 12, 16, 17]. Dose-response characteristics have also not been defined, and the virus dose is a critical determinant of the virus titers, pathology, and illness seen in a mouse model of RSV infections [11]. We were unable to consistently detect signs of clinical illness among the infected pups.

There are drawbacks to the use of guinea pigs, such as the fact that the reagents available for the analysis of guinea pig immune responses are not as extensive as those for mice. There is also relatively little known about antibody transfer from pregnant guinea pigs. However, the guinea pig provides a unique opportunity to explore aspects of the maternal transfer of immunity using an intact maternal-fetal unit. In addition, the relatively long gestation (~63 days) of guinea pigs facilitates immunization studies, which require time for the development of the antibody response. The guinea pig should be a useful tool for the study of maternal immunization against RSV.

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References

Fractionation of Hepatitis C Virus Hypervariable Region 1 in Immunocompromised Patients

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The viral variability of 5 hepatitis C virus (HCV)–infected immunocompromised patients was analyzed and compared with that in isolates from immunocompetent subjects. The patients were followed longitudinally with regard to changes in hypervariable region 1 (HVR1) of HCV using a direct DNA sequencing approach. For the immunocompromised patients, viral nucleotide sequence variability was markedly lower than in immunocompetent HCV-positive patients. For 1 agammaglobulinemic patient and 1 AIDS patient, no variation in the major amino acid sequence of HCV HVR1 could be observed, while another agammaglobulinemic patient exhibited transient variations and amino acid substitutions despite the lack of functioning humoral immune response. The study supports the general hypothesis of humoral immune selection as the main force of sequence variation in the HVR1 region but suggests that other selection mechanisms may contribute to modulation of the composition of the viral population.

Hepatitis C virus (HCV) is the major causative agent of non-A, non-B posttransfusion hepatitis and has a positive single-stranded RNA of ~9.4 kb with a quasispecies nature [1]. An isolate of hepatitis C is most accurately described as a population of closely related viral variants, for which, in theory, each genome can be unique due to the error-prone nature of the viral RNA–directed RNA polymerase. Both positive and negative selection as well as random sampling events will influence the viral population and the resulting consensus sequence [2].

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