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Neonatal Respiratory Syncytial Virus Infection: Role of Transplacentally and Breast Milk-Acquired Antibodies

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The effect of transplacentally and breast milk-acquired antibodies on respiratory syncytial virus infection was studied in neonatal and 2-month-old cotton rats. Adult female rats infected intranasally with live virus regularly produced virus-specific antibodies in the serum, colostrum, and breast milk. By using foster feeding techniques, we showed that both transplacentally and breast milk-acquired antibodies were effective in reducing the replication of respiratory syncytial virus in the lungs of neonatal animals when they were challenged with live virus via the nasal route at 3 days of age. However, the protection provided by these antibodies was rather brief. There was no difference in the replication of respiratory syncytial virus in the lungs of 2-month-old animals that were delivered and nursed by seropositive (immunized) or seronegative (control) cotton rats.

Respiratory syncytial virus (RSV) is one of the most common causes of lower respiratory tract infection in young children (2, 8, 18). Annual outbreaks of RSV infection have been observed in many temperate countries during the winter months. The peak incidence of RSV infection occurs in children between 2 to 6 months of age (8, 18).

The role of passively acquired immunity, either through the placenta or the breast milk, in the pathogenesis of RSV infection has been controversial. Some investigators reported the presence of RSV-neutralizing antibodies in the serum of young infants during the acute phase of infection, suggesting that maternally derived antibodies may not be protective (2, 18). Furthermore, it was observed that children who were immunized with a Formalin-inactivated RSV vaccine suffered significantly more severe pulmonary disease during a community outbreak of RSV infection than did comparable cohorts who were either unvaccinated or who received an inactivated parainfluenza virus vaccine (2, 7, 9, 11). In contrast, several epidemiological studies have suggested that maternally derived passive immunity may be protective to a certain extent against pulmonary disease after RSV infection (4, 6, 8, 12).

To clarify the role of passive immunity in the pathogenesis of RSV infection, we studied the replication of RSV in the respiratory tract of neonatal cotton rats in the presence or absence of maternally derived immunological reactivity.

Adult female cotton rats that were seronegative for RSV were experimentally infected with 0.2 ml of live stock virus (10^7 PFU/ml) intranasally just before mating with male rats of the same age. Uninfected control female rats were similarly mated, but they were kept in a separate isolation room to prevent accidental acquisition of RSV.

At about 4 to 5 weeks after mating, the first litters were born. Samples of serum, colostrum, and breast milk were collected from the lactating mothers after anesthesia (Metofane; Pitman-Moore). RSV-specific antibodies were determined by indirect immunofluorescence technique as previously described (23). None of the uninfected female rats had RSV-specific antibody in the serum or milk samples. Virus-specific immunoglobulin G (IgG) and IgA were detected in samples of serum, colostrum, and breast milk from all the female rats that were infected intranasally with the live virus (data not shown).

To demonstrate the transfer of RSV-specific antibodies via the placenta and breast milk to the neonatal animals, two adult female cotton rats were infected intranasally with live RSV as before and were then separately mated with male rats. At 2 to 3 days before the expected date of delivery, one of the pregnant rats was killed, and hysterectomy was performed to remove the fetuses. Blood was collected and pooled from these fetuses after decapitation. RSV-specific IgG was present in the serum of these fetuses (Fig. 1).

The second immunized female rat was allowed to deliver and nurse the neonates. Blood and breast milk samples were collected from the mother and the neonates at regular intervals after delivery. RSV-specific IgG and IgA were demonstrated in the colostrum and breast milk samples of this lactating rat for as long as 2 weeks after delivery (Fig. 1). It appears that the antibodies were able to cross the gut mucosa of the neonatal rats during the early postnatal period, as evidenced by the appearance of IgA and a rise in titer of IgG in the serum of these animals during the first 2 weeks of life (Fig. 1). Similar absorption of poliovirus-specific IgA from the gut has also been demonstrated in human neonates that were given antibody-rich human colostrum via a nasogastric tube within 24 h after birth (17).

Nursing continued in these neonatal rats for the first 2 weeks of life, when the titer of virus-specific antibodies remained fairly stable. After weaning, the maternal antibodies began to fall steadily so that by 2 months of age, very little RSV-specific IgG or IgA could be demonstrated in the serum of these animals.

The effect of transplacentally and breast milk-acquired immunological reactivity on the replication of RSV in the respiratory tract of neonatal animals was examined by challenging these animals with 3×10^5 PFU (30 μl) of live virus administered intranasally at 3 days of age. At regular

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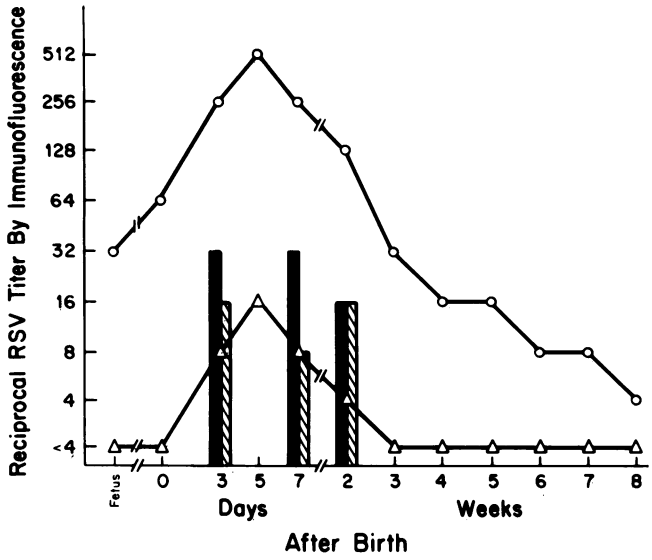

FIG. 1. RSV-specific IgG and IgA in breast milk of lactating animals and serum of fetal and neonatal rats (see text). Symbols: ○, RSV-specific IgG titer in serum; △, RSV-specific IgA titer in serum; ▬, RSV-specific IgG titer in breast milk; ▨, RSV-specific IgA titer in breast milk.

intervals after infection, neonatal rats were killed by asphyxiation with carbon dioxide. After exanguination, the pulmonary circulation of these animals was perfused with balanced salt solution to remove any remaining antibody that might have affected the enumeration of live virus in the lungs. The lungs and nasal tissues were then removed and homogenized separately into 10% suspensions as previously described (23). The amount of live virus in the tissues was assayed in HEp-2 monolayers established in 24-well tissue culture plates.

Replication of RSV in neonatal cotton rats that were delivered and nursed by seronegative control mothers was similar to that observed in adult rats after RSV infection (20; 23). Live virus was recovered from the respiratory tract of these animals during the first week after infection (Table 1). The peak virus titer was present 3 to 5 days after intranasal infection. On the contrary, neonatal rats that were delivered and nursed by immunized rats exhibited a significant reduction in the replication of RSV in pulmonary tissues (Table 1). Only 7 of 16 newborn rats tested between 2 and 5 days after infection had live virus isolated from their lungs, compared with 100% of neonatal rats from seronegative control rats. As reported by other investigators, replication of RSV in the nasal mucosa was not affected by maternally derived immunological reactivities (19).

In an attempt to better define the role of transplacentally and breast milk-acquired immunity in the pathogenesis of RSV infection, foster feeding experiments were carried out by transferring neonates from immune rats to control rats and vice versa. Female rats that were infected with RSV were housed in cages which had wire-mesh beddings once they were found to be pregnant by abdominal palpation. As a result, the neonates were separated from their natural mothers immediately after delivery and before the onset of breast feeding. The neonates were then transferred as soon as possible to uninfected lactating rats that had delivered within the previous 2 days. The immunized rats were allowed to nurse neonates that were delivered by the control rats. Both groups of neonatal animals were challenged with live virus after 3 days of foster feeding.

Neonatal cotton rats that were delivered by immunized mothers and nursed by control lactating animals exhibited significant reduction in the replication of RSV in the lungs after intranasal challenge with live virus (Table 2). Again, there was no protection observed in the nasal turbinates. RSV-specific IgG, but not IgA, could be demonstrated in the serum of these animals at the time of death.

In those neonates that were delivered by seronegative rats and then subsequently nursed by immunized foster rats, RSV-specific antibodies were present in their serum 3 days after initiation of foster feeding and before infection (data not shown). Replication of RSV in the nasal tissue was similar to that observed in neonatal rats delivered and nursed by seronegative rats. However, there was marked reduction in the amount of virus recovered from the lungs of these animals (Table 2). Only 5 of 13 rats examined 2 and 4 days after infection had live virus present in their lungs. RSV-specific IgG and IgA were demonstrated in the serum of these rats at the time of death.

The duration of protection provided by these maternally derived immunological reactivities was rather brief. Cotton rats that were delivered and nursed by seropositive rats were challenged with 100 μl of live virus (10^6 PFU) at 2 months of age. A second group of 2-month-old animals that was delivered and nursed by seronegative control rats was similarly infected at the same time, and the results are shown in Table 3. The protection that was observed during the neonatal period had disappeared. Live virus was recovered in all the animals 2 and 4 days after infection. Furthermore, there was

TABLE 1. Recovery of RSV from neonatal animals delivered and nursed by seronegative (control) and seropositive (immune) cotton rats and then challenged with 3×10^5 PFU of live RSV at 3 days of age

Expt no.	Maternal status	Days after infection	Virus recovery			
			Nasal		Pulmonary	
			No. positive/ total	Log_{10} PFU/g[a]	No. positive/ total	Log_{10} PFU/ g
1	Control	1	ND[b]	ND	3/4	3.3 ± 2.4
		3	ND	ND	3/3	4.7 ± 0.8
		5	ND	ND	5/5	4.0 ± 0.8
		7	ND	ND	1/3	0.9 ± 1.6
	Immune	1	ND	ND	3/4	2.2 ± 1.5
		3	ND	ND	1/3	0.9 ± 1.6
		5	ND	ND	1/2	1.3 ± 1.9
		7	ND	ND	0/2	<1[c]
2	Control	2	2/2	4.9 ± 0.1	2/2	3.8 ± 0.7
		4	4/4	5.1 ± 1.1	4/4	4.0 ± 1.0
	Immune	2	6/6	4.0 ± 0.5	3/6	1.0 ± 1.2[d]
		4	5/5	4.5 ± 0.9	2/5	0.7 ± 0.9[d]
3[e]	control	2	2/2	5.3 ± 0.1	0/2	<1[d]
		4	2/2	5.2 ± 0.2	0/2	<1[d]

[a] Values are mean ± standard deviation.
[b] ND, Not done.
[c] No virus isolated from 10% tissue homogenates.
[d] $P < 0.01$ compared with the control group in experiment 2 that was killed on the same day after infection.
[e] Neonatal animals were nursed by seronegative control rats and received 0.2 ml of adult convalescent serum 1 day after birth (see text).

TABLE 2. Serum antibody titer and recovery of live virus from nasal and pulmonary tissues of neonatal rats after RSV infection in foster feeding experiments

Status of natural mother/foster mother	Days post-infection	RSV antibody titer[a]		Virus recovery			
				Nasal		Pulmonary	
		IgG	IgA	No. positive/ total	Log_{10} PFU/g[b]	No. positive/ total	Log_{10} PFU/g[c]
Immune/control	2	32	<4	3/3	3.5 ± 0.9	0/3	<1
	4	32	<4	5/6	3.3 ± 1.7	3/6	1.7 ± 1.9
Control/immune	2	8	4	7/7	4.7 ± 0.4	3/7	1.8 ± 1.2
	4	16	4	6/6	4.4 ± 2.3	2/6	0.9 ± 1.5

[a] Reciprocal of immunofluorescent antibody titer at the time of death.
[b] Values are mean ± standard deviation.
[c] $P < 0.05$ compared with the control group of animals in experiment 2 of Table 1 that was killed on the same day after infection.

no difference in the amount of virus present in the lungs of those animals who did or did not receive transplacental and breast milk reactivities.

To determine whether the protective effect that was observed in neonatal rats was mediated by virus-specific antibodies, a passive immunization experiment was performed. Adult cotton rats were infected intranasally with 0.2 ml of stock virus and were exanguinated 2 months after infection. The serum samples were pooled, heat inactivated, and stored at −70°C until used. RSV-specific IgG titer in this serum pool was 1:128 by indirect immunofluorescence test. Neonatal animals that were delivered and nursed by seronegative rats were injected with 0.2 ml of this pooled serum intraperitoneally 1 day after birth and were then challenged with live RSV at 3 days of age. As shown in Table 1 (experiment 3), replication of RSV in the lungs of these rats was totally abolished, despite the presence of large amounts of live virus in the nasal mucosa. Therefore, the protection observed in the rats that received maternally derived passive immunity was, at least partially, mediated by virus-specific antibodies. Furthermore, cotton rats that were delivered and nursed by seropositive mothers had significant reduction of maternal antibodies during month 2 of life (Fig. 1), which coincided with the loss of protection that was observed at 2 months of age.

The results of these experiments support epidemiological observations that transplacentally and breast milk-acquired antibodies may be effective in protecting, at least partially, young infants from serious RSV infection (4, 6, 8, 12). It has been observed that severe lung disease after RSV infection is relatively uncommon in children under 1 month old, when transplacental antibodies were still present at high levels (2, 18). Children with culture-proven RSV infection had lower titers of RSV-specific antibodies in the cord sera compared with those in random specimens (8). Furthermore, infants born with higher titers of neutralizing antibodies developed infection at a later age (8), and the severity of pneumonia was inversely related to the level of maternal antibodies (12). It was even suggested that serious respiratory diseases will not occur if maternally derived antibody titer is 1:16 or higher at the time of RSV infection (8).

RSV-specific antibodies have been demonstrated in human colostrum and breast milk (4, 5). Reinfection of lactating females induced a rise in the titer of RSV-specific IgA in the breast milk (5). It has been shown that children who were admitted to the hospital with RSV infection were less likely to have received breast feeding when compared with age-matched control subjects (4). However, the amount of transplacental antibodies was not determined in this study. In the animal model, RSV-specific IgG and IgA could be demonstrated in the colostrum and breast milk of seropositive rats. Some of these antibodies were absorbed through the gut mucosa and resulted in reduced replication of RSV in the lungs of these animals when they were challenged with the live virus at 3 days of age. Furthermore, the amount of virus recovered from the lungs of these animals 4 days after infection was actually less than that recovered on day 2 (Table 2), suggesting that 2 additional days of breast feeding resulted in further reduction of virus replication in the lungs.

Our data do not support speculations that preexisting antibody in the serum is the cause of severe lower respiratory tract disease after RSV infection (2, 9, 11). This speculation was partly based on the observation that children immunized with a Formalin-inactivated RSV vaccine suffered significantly higher morbidity and mortality when they were subsequently exposed to the natural virus (2, 7, 9, 11). All of these children had demonstrable neutralizing antibodies in their serum after vaccination. It was proposed by the investigators that the serum antibody present at the time of infection reacted with RSV antigens in the lungs, resulting in tissue damage (2, 11). However, more recent data have suggested that the aberrant reactions observed in these vaccinees might have resulted from the denaturation of a surface glycoprotein (fusion protein) that has been identified in RSV (21) during the process of Formalin inactivation. A similar fusion protein has also been found in other paramyxoviruses, like the measles virus (14). It has been shown that this fusion protein can be destroyed by Formalin and Tween 80-diethyl ether, both of which have been used for the preparation of inactivated measles virus vaccine (14,

TABLE 3. Replication of RSV in the respiratory tract of 2-month-old cotton rats after intranasal infection with 10^6 PFU of live RSV

Maternal status	Days post-infection	Virus recovery			
		Nasal		Pulmonary	
		No. positive/ total	Log_{10} PFU/g[a]	No. positive/ total	Log_{10} PFU/g
Control	2	4/4	4.9 ± 0.9	6/6	4.4 ± 0.8
	4	4/4	5.1 ± 1.1	7/7	4.5 ± 0.6
Immune	2	4/4	3.3 ± 0.4	4/4	4.3 ± 0.5
	4	3/3	4.6 ± 0.7	3/3	5.6 ± 0.3

[a] Values are mean ± standard deviation.

15, 16). Children who were vaccinated with inactivated measles vaccine lack antibodies directed against the fusion protein (15, 16), thus explaining the unusual disease entity called atypical measles that was observed when these vaccinees were subsequently exposed to the wild virus (1, 3, 10, 13). Furthermore, monoclonal antibodies that were generated against the fusion protein of RSV were effective in reducing the replication of RSV in the lungs of cotton rats experimentally infected with the live virus (22).

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