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Protection against hantavirus infection by dam's immunity transferred vertically to neonates

Brief Report

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Summary. Antibodies to hantavirus, Seoul type B-1 strain, vertically transferred to rat neonates prevented lethal as well as persistent infection. When relatively high titer viruses were inoculated into neonates, the mother's antibodies protected all the neonates from lethal virus infection. However, the antibodies could not protect all of the neonates from persistent infection but only half of them underwent persistent infection. The other half was completely cured but also became persistently infected when rechallenged with the active viruses after reaching maturity.

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Viruses causing haemorrhagic fever with renal syndrome (HFRS) are widely distributed throughout the world and are capable of infecting both rodents and humans [3, 13]. However, rodents serve as the natural reservoirs of viruses whereas humans are incidental, end-stage hosts. Hantaan virus (HV), the aetiological agent of HFRS, was isolated in 1978 from *Apodemus agrarius* [13]. Thereafter, antigenically related viruses were isolated from both rodents and humans [8, 10, 11, 17, 24, 26, 27, 30]. These viruses are members of the family *Bunyaviridae* and belong to a new genus called Hantavirus [5, 6, 15, 19–21, 25]. Serological and genetic data have indicated that these viruses can be classified into at least 7 groups, namely Hantaan, Belgrade, Seoul, Thailand, Puumala, Prospect Hill, and Thottapalayam [18, 21, 24]. Furthermore, there appeared to be yet another. An acute respiratory distress syndrome that has recently been recognized, was caused by a new hantavirus [4, 17].

Some investigators have attempted to infect various rodents with hantavirus [7, 9, 11, 12, 14, 22, 23, 28, 29]. Infection of newborn rats with hantavirus, Seoul type B-1 strain, resulted in growth retardation and death due to viral

replication to high titers in various organs, such as the lung, kidney, spleen and brain [28]. In contrast, the infection of adult rats caused no clinical signs other than persistent infection [14].

A similar challenge to newborn rats from mothers who had been immunized by infection with the same virus did not induce growth retardation and the virus was not found in any organ. The newborn rats from immunized mothers had elevated titers of the maternal antibody responsible for immunity [31]. These results suggested that total protection from viral infection was due to maternal immunity [28, 31].

In our previous study, we used a foster nursing method to study the effects of specific antibodies transferred from mothers to their infants. Our study showed that the maternal antibodies, against hantavirus, Seoul type B-1 strain, transferred to the infant rat through breast-feeding, protected the infants against hantavirus infection [2] as was true of fetal rats in utero. However, it should be noted that the viruses have been isolated from wild rodents [11, 13] and laboratory animals [26] even though they have had relatively high titers of the antibodies for long periods. At present, it remains unclear to what extent humoral antibodies offer effective protection against hantavirus infection.

In this study, we investigated the protective effects of antibodies in rat neonates born to or foster-nursed by immune mothers for the following purposes: to clarify the role of humoral immunity in hantavirus infection, especially of vertically transferred maternal antibodies and to understand the relationship between the titer of viruses infected in the presence of maternal antibodies and the prevalence of infection.

To understand the role of humoral immunity in hantavirus infection, we performed cross-fostering experiments. Newborn rats delivered from immune and nonimmune mothers were divided into three groups: (1) infants born to immune mothers were cross-fostered to and suckled by nonimmune mothers, so as to acquire immunity solely by in utero transfer, (2) before suckling the colostrum of their own mother, infants born to nonimmune mothers were cross-fostered to immune mothers, so as to acquire immunity solely via breast milk, and (3) infants also born to nonimmune mothers were suckled by their own mothers as negative controls (Table 1). Within 24 h after birth, neonates were given intraperitoneally, either vehicle (PBS) or a lethal dose of hantavirus, Seoul type B-1 strain (5×10^3 or 5×10^5 LD_{50} for newborn rats). The infants were then followed up for antibodies to the virus by means of the indirect immunofluorescence antibody test. Their serum antibody titers were measured using fluorescein isothiocyanate (FITC)-labeled goat antibodies to rat IgG or IgM (Cappel Laboratories, Cochranville, PA, U.S.A.) or IgA (anti-α) (The Binding Site Ltd, Birmingham, England) as the secondary antibodies [28]. All animal experiments proceeded in a room with P3 facilities and conformed to the established guidelines for animal use and care (1985) [16].

When neonates carrying the immune mother's antibodies were challenged with sufficient low titer viruses (5×10^3 LD_{50}) to kill rat neonates without the immune mother's immunity, both suppression of subsequent antibody produc-

Table 1. Experimental infection of the neonates immune-transferred from mothers via two routes

Immune transfer	Virus titer used for challenge ($\times 5$ LD_{50})	Lethality	Persistent infection
In utero	$\times 10^3$	0/8	0/8
	$\times 10^5$	0/8	4/8
Via milk	$\times 10^3$	0/8	0/8
	$\times 10^5$	0/8	4/8
None	$\times 10^3$	8/8	–

Female Fischer rats of the F344 strain, 8–10 weeks of age, purchased from Japan Clea (Osaka), were challenged twice intraperitoneally at 7 and 5 weeks before delivery with Seoul type B-1 strain virus (more than 10^3 LD_{50} on newborn rats) to induce immunity. The resultant viral infection was not lethal to adults but induced only immunity [22, 28] and the titers were $\log_2 13.5 \pm 0.5$ for IgG and $\log_2 10.5 \pm 0.5$ for IgA at the time of delivery. The rats were bred 2 weeks after the secondary challenge. Neonates were cross-fostered and challenged with 5×10^3 or 5×10^5 LD_{50} of the B-1 virus within 24 h after birth as described in the text. Persistent virus infection was assessed on the basis of a transient increase in IgM, establishment of high titers of IgA and IgG antibodies against the virus, and the recovery of active viruses

tion [2] and nonlethal infection occurred (Table 1). When the virus titer used for challenging neonates was relatively higher (5×10^5 LD_{50}), two types of chronological changes in the antibody titer in the serum of each infant were evident: (A) In one half of the infants (8/16) who acquired immunity either in utero (4/8) or via breast milk (4/8) (Table 1), both IgA and IgG antibodies decreased daily (Fig. 1a and b). After that, IgM antibody transiently appeared, followed by the re-increase in both IgA and IgG antibodies (Fig. 1a and 1b). This group (A) consisted of individuals persistently infected with the B-I virus, since infectious viruses were recovered from various organs of these rats (data not shown). In the other half (B) of the infants (8/16), who also acquired the maternal immunity either in utero (Fig. 2a) or via breast feeding (Fig. 2b), the titer of both IgA and IgG antibodies decreased daily, as reported [2]. Further studies demonstrated that the above group (B) had no sign of persistent infection since no antibody was detected and no virus was recovered (data not shown) Neither group of rats expressed either clinical signs or lethality (Table 1) even though lethal doses were used for infection. Thus in the rat, serum protective antibodies transferred from the immune mother to the infants were effective either in utero or via breast feeding.

Whether or not virus infection was established appeared to depend upon the titer of the virus used for the challenge (Table 1). During the establishment of persistent infection, the kinetics of IgA and IgG antibody titers reached a nadir at about 1 month for those transferred in utero (Fig. 1a) and about 4 months for those transferred through milk (Fig. 1b). Upon establishing

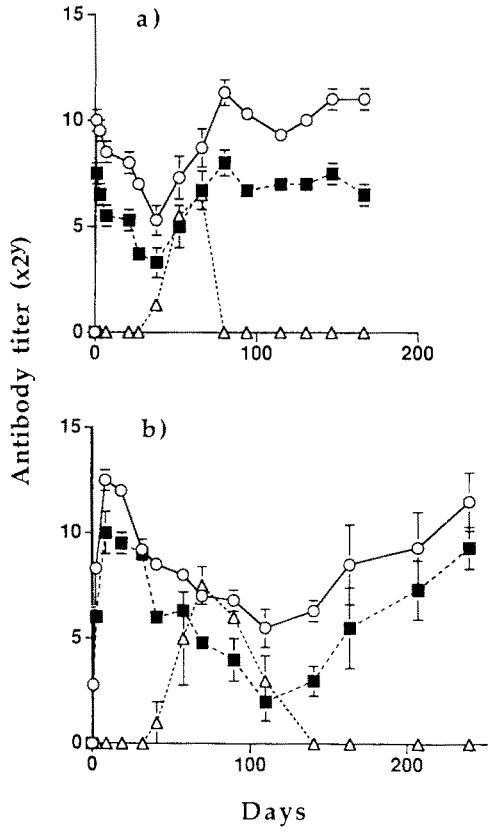

Fig. 1. Changes in serum IFA titers of persistently infected newborn rats foster-nursed by immune or nonimmune mothers. Seoul type B-1 strain virus (5×10^5 LD_{50}) was injected into neonates intraperitoneally on the day of birth (day 0). Exsanguination from the heart (newborn) or tail vein (adult rats) was performed under ether anesthesia. The sera were heat-inactivated (56°C, 30 min) and assayed for the antibody on various days after infection. The following two types of neonates became persistently infected and were studied here; **a** Neonates born to immune mothers and foster-nursed by nonimmune dams. The immune mother's antibodies were transferred in utero to these animals. **b** Neonates born to nonimmune normal mothers and foster-nursed by immune dams. Maternal antibodies were transferred to these neonates by breast feeding. Antibodies examined were IgA (■), IgG (○) and IgM (Δ). Abscissa: days after birth. Ordinate: antibody titers expressed as the reciprocal of the highest serum dilution giving specific fluorescence. Each plot shows the mean ± S.E.M. of more than 4 infants

persistent virus infection, the IgM antibody titer increased in all of the rats before the re-increase in IgG and IgA antibody titers (Fig. 1a and b). Thus, the induction of IgM was always associated with the establishment of persistent infection. However, it was delayed as compared with the control infants who had received no maternal immunity. The peak formation of IgM was around 2–3 months among the neonates with maternal immunity (Fig. 1a and b) as compared with 2–3 weeks in the case of the control infants (data not shown). The second increase in immunoglobulins IgA and IgG also seemed to be very

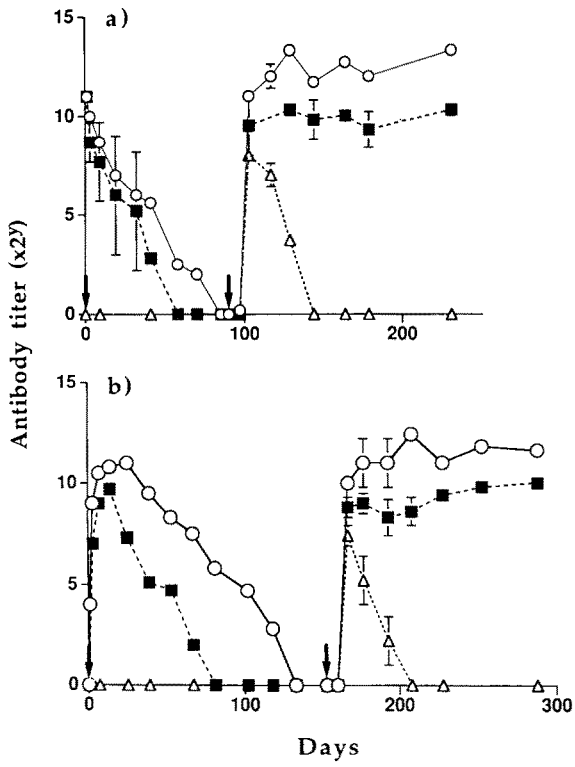

Fig. 2. Rechallenge of rats that were protected from neonatal infection by maternal antibodies with the active virus. Neonates that were completely protected from neonatal virus infection by maternal antibodies transferred in utero (**a**) or by breast milk (**b**) were rechallenged with the same virus 90 (**a**) or 155 (**b**) days after birth. Arrows show the days of virus challenge. The 1st challenge proceeded with the Seoul virus B-1 strain on day 0 after birth. The antibodies examined were IgA (■), IgG (○) and IgM (△)

delayed. This may have been because of the partial suppression of virus replication in the infants due to the transferred immunity.

To understand the immune response of the adult rats that were protected from persistent infection by challenge with high titer active viruses (Table 1), we studied their response to a secondary challenge. The rats were challenged again with the active virus having the titer of 5×10^3 LD_{50}, after disappearance of the immunoglobulins transferred from immune dams namely 90 (Fig. 2a) and 155 days (Fig. 2b) after birth. Within 2 weeks after the secondary intraperitoneal challenge with the active virus, the titer of anti-hantavirus antibodies increased rapidly in all of the rats. IgM peaked at about 3 weeks after virus injection, while the IgG and IgA titers reached stable, high levels after about 1 month. Furthermore, the infected rats maintained a high titer of antibodies for more than 100 days, implying that persistent virus infection had occurred. This was also confirmed by isolating active viruses from these rats (data not shown).

These results indicated that the maternal immunity transferred to neonates was transient, although it protected the neonates from persistent infection at the first challenge with the active virus. However, the adult rats who were neonatally protected from persistent infection did not have a memory of the first challenge. Thus, upon the second challenge with the active viruses they became susceptible to and underwent persistent infection.

In a previous report, we showed that in hantavirus infection, protective antibodies were transferred prenatally in rats, and that both IgG and IgA were transferred to fetal rats in utero and to neonates by breast-feeding [2]. The efficiency of the prenatal transfer of immunoglobulins was about the same as that of postnatal transfer, although the latter was maintained longer due to suckling. Antibodies transferred through both routes offered protection from hantavirus infection.

In this study, we demonstrated that these immunity transfers were not sufficient for complete protection against virus infection. If the titer of the virus used for challenging neonates was high, no persistent infection occurred in one half of the infants who eventually lost the antibodies transferred via both routes (Table 1). The disappearance of the antibodies indicated complete protection against infection. The other half of the infants, however, became persistently infected (Table 1). When these results were evaluated together, they indicated that complete protection from viral infection depends upon both the titer of the virus used for the challenge and the titer of the immunity generated in the neonates. However, in all animals, even those in which persistent infection was established, no lethal effects were apparent (Table 1).

Feral rats become infected with hantavirus in an age-dependent manner that may relate to the time of exposure or susceptibility. It is possible that young wild rats from seropositive mothers are protected from hantavirus infection for extended periods after birth because of immune transfer from dams, as we demonstrated using laboratory rats [2]. In fact, it is reported that most reproductively-active wild rats in Baltimore are already seropositive [1]. Thus, it is possible that their neonates are protected from the virus infection by the mother's immunity as we showed in laboratory rats. Furthermore, this study showed that the immunity transferred from dams was transient, that the mother's immunity did not induce the neonate's immune response to the injected active viruses, and that the mother's transient immunity protected the neonates from persistent or lethal infection (Table 1). Among the neonates protected from persistent infection, those that reached adulthood became susceptible to virus infection after the disappearance of the transient maternal immunity and underwent persistent infection (Fig. 2). Thus, infection starts after weaning. This may explain the increasing prevalence of anti-hantavirus antibodies in older wild rodents [1].

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