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Maternal Transfer of Hantavirus Antibodies in Rats

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Hantaviruses (genus Hantavirus, family Bunyaviridae) causing hemorrhagic fever with renal syndrome (HFRS) are widely distributed throughout the world and are capable of infecting rodents and humans (1). The genus Hantavirus comprises approximately 20 species, including those causing Old World hantavirus infections (i.e., Hantaan, Seoul, Puumala, and Dobrova) and hantavirus pulmonary syndrome (HPS), an acute respiratory distress syndrome first recognized in 1993 as being caused by a New World hantavirus (2). The term HFRS denotes a group of clinically similar diseases that occur throughout Eurasia and adjoining areas. Hantavirus pulmonary syndrome has been reported in North and South America, and the virus involved causes an acute respiratory tract disease (3). Capillary leakage is localized exclusively in the lungs, and death occurs from shock and cardiac complications (4). Rodents serve as the natural reservoirs of these viruses, whereas humans are incidental, end-stage hosts.

To study the mechanism of viral infection in rodents, we used a foster nursing method to study the effects of specific antibodies against hantavirus, Seoul type B-1 strain, transferred from dams to neonatal rats. Results of our study indicated that maternal antibodies transferred through the milk were capable of protecting neonatal rats against hantavirus infection, as was the case with fetal rats in utero. We also documented that IgG and IgA are transferred in utero and by breast feeding (5).

Two- to 3-month-old, 200- to 250-g F344/Jcl rats were purchased from Japan Clea (Osaka, Japan) as specific pathogen free, were inoculated intraperitoneally with the B-1 virus prepared in Vero E6 cells (5, 6), and were housed in autoclaved polycarbonate cages with wood tips and kept in isolators equipped with a HEPA filter at 24 to 26°C and 50 to 80% humidity. Rats were kept on a 12-h light cycle, with lights off at 1800 h. All rats were fed a standard laboratory diet (solid feed CMF; Oriental Yeast Co., Ltd., Tokyo, Japan) and sterilized tap water ad libitum. Sera were obtained from newborn rats and dams to test for antibodies against hantavirus. All animal experiments were carried out in a room with P3 facilities and conformed to the established guidelines for animal use and care (7). All procedures using animals were approved by the Institutional Animal Care and Use Committee.

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Exsanguination from the heart (newborn) or tail vein (adults) was performed on rats under ether anesthesia. The indirect immunofluorescent antibody (IFA) test was carried out to detect serum antibodies. Sera were heat-inactivated at 56°C for 30 min and were assayed for hantavirus antibodies by use of the indirect IFA test (5). Fluorescein isothiocyanate (FITC)-labeled goat antibodies to rat IgG, IgM (Cappel Laboratories, Cochransville, Pa.), IgA (anti-α) (The Binding Site, Ltd., Birmingham, England), and IgA (anti-secretary component [SC]; Bethyl Laboratories, Inc., Tex.) were used as secondary antibodies. The IFA titer was expressed as the reciprocal of the highest serum dilution giving specific fluorescence for antigen in Vero E6 cells infected with the B-1 virus (5).

To characterize humoral immunity in rats with hantavirus infection, we performed a cross-fostering experiment: progeny of immune dams were transferred to and nursed by nonimmune dams and vice versa. To induce immunity, five adult female Fischer F344/Jcl rats were inoculated intraperitoneally at 7 and 5 weeks before delivery with a nonlethal dose of the Seoul type B-1 strain of hantavirus. Each inoculum contained titer $>10^3$ 50% lethal doses (5) for newborn rats. The resultant viral infection was not lethal but induced immunity in adult rats. The rats were bred 2 weeks after the second inoculation. Mean ± SEM serum IgA antibody titer at that time was $\log_2$ 12.25 ± 0.25. Newborn rats from immune and nonimmune dams were allocated to two groups. Before suckling colostrum from their dam, neonates born to nonimmune dams were cross-fostered to immune dams, so as to acquire their immunity solely via the milk. Neonates born to immune dams were cross-fostered to and suckled by nonimmune dams before suckling colostrum from their dams, so as to acquire their immunity solely by in utero transfer. Neonates were examined for antibody titer against the virus until they grew to adulthood. For neonates born to nonimmune dams, antibody titer on day 1 was <1. The IgG and IgA titers peaked at 2 to 3 weeks after fostering with immune dams. In contrast, newborn rats born to immunized dams had high titer of antibody transferred in utero on day 1 before suckling, then titer decreased. The half-life of IgG antibody transferred through either route was similar, approximately 10 days (5). However, the half-life of IgA antibody transferred in utero (5 days) or by milk (10 days) differed (Figure 1A). The IgA antibody transferred via breast feeding has longer duration than that transferred in utero. Thus, IgA anti-

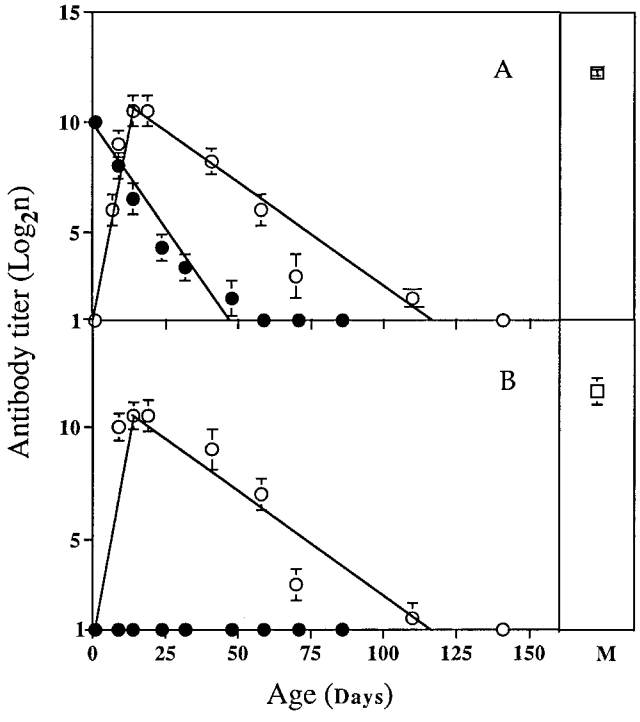

Figure 1. Changes in serum indirect immunofluorescent antibody (IFA) titers of IgA antibodies in neonates foster-nursed by immune or nonimmune dams. **(A)** Immunoglobulin A detected by fluorescein isothiocyanate (FITC)-labeled anti-α antibody; **(B)** The IgA detected by FITC-labeled secretory component (SC) antibody. Titers in immune dams are indicated in the right column (M on the right side, square symbols). Data are expressed as mean ± SEM. Ordinate: Antibody titers expressed as the reciprocal of the highest serum dilution giving specific fluorescence. Neonates born to immune dams and foster-nursed by nonimmune dams; antibodies were transferred in utero (closed symbols [●]). Neonates born to nonimmune normal dams and foster nursed by immune dams; antibodies were transferred by milk (open symbol [○]).

bodies transferred in utero may differ from antibodies transferred by breast feeding.

To investigate the reason for the difference, we compared the titers of IgA antibodies to hantavirus, using two specific antibodies, FITC-labelled anti-SC and anti-α, as secondary antibodies in the IFA test. Kinetic variables of B-1 virus antibody measured in breast-fed rats, using SC antibody, were identical to those detected using α antibody. However, for rats that underwent in utero transfer, specific B-1 virus antibody could not be detected using anti-SC (Figure 1B). These results indicate that IgA antibodies transferred in utero have no or little SC component, compared with those transferred by breast feeding. After 140 and 50 days, antibodies were not detected in the sera of rats suckled by immune dams and of rats born to immune dams, respectively. Furthermore, in neither group of neonatal rats were IgM antibodies detected (data not shown). These results indicate that all antibodies were passively transferred from dams to neonates and were not produced by neonates infected with virus.

We have documented that maternal antibodies of IgG and IgA classes can be detected in the serum of neonatal rats (5). For IgG transport, there is a receptor in the intestine of neonatal mice (8) and rats (9). However, it is not clear whether such receptors for IgA transport exist in the intestine of neonates or whether there is a specific mechanism for IgA transfer during gestation. Although passive transfer of maternal antibody occurs exclusively in utero in humans (10) and other primates (11), postnatal transfer of maternal antibody has been reported to occur by milk in the rat (12), mouse (8), ferret, and mink (13). Others have reported that pre- and postnatal transfer of maternal antibodies occurs in rats when dams are infected with rickettsia (14) or streptococci (9). In all those reports, however, the type of antibodies and their precise kinetics were not studied. We documented that prenatal transfer of such protective antibody occurs in rats in the case of hantavirus infection and that IgG and IgA are transferred in utero and via breast milk (5). The efficiency of prenatal transfer of immunoglobulins is approximately the same as that of postnatal transfer, although the latter mechanism is maintained longer by suckling. These transferred immunoglobulins are likely to offer effective protection to neonatal rats, because these neonates, having high titers of the antibodies, are resistant to viral infection (5) and because the IFA titer is known to be associated with neutralizing antibody titer (15).

The IgA antibodies transferred in utero and by milk were different, although they were transferred efficiently through both routes. The IgA antibody transferred in utero did not contain SC, and its half-life was shorter than that transferred by milk. These results indicate that not only the IgA antibodies but also the mechanisms of IgA transfer from dams to neonates in utero could be different from those associated with the milk. Furthermore, transfer of IgA through the epithelium of the alimentary tract in neonatal rats was efficient, indicating that specific receptors or other mechanisms for transfer may exist. In considering the mechanism for viral infection, hantavirus antibody would not work in the alimentary tract; however, after absorption, it should prevent infection in neonatal rats. Thus, it is likely that rats from seropositive dams are protected from hantavirus infection for extended periods after birth due to immune transfer from dams (5). It is reported that most reproductively active feral rats in Baltimore are seropositive to hantavirus. Thus, infection probably occurs after weaning, which may explain why prevalence of hantavirus antibody in rats increases with age (16).

Antibodies to hantavirus, Seoul type B-1 strain, vertically transferred to neonates prevented infection (5). In cross-fostering experiments, maternal hantavirus-specific antibodies were efficiently transferred to neonates in utero or by milk. In the study reported here, we documented that IgA antibody transferred in utero was found to be defective in a secretary component, which may result in shorter half-life.

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