Med Microbiol Immunol
(1985) 174: 15–24

Medical Microbiology and Immunology

Influence of maternal immunity on the outcome of murine coronavirus JHM infection in suckling mice

K. Pickel*, M.A. Müller, and V. ter Meulen

Institute for Virology and Immunobiology, Versbacher Str. 7, D–8700 Würzburg, Federal Republic of Germany

Abstract. Adult C3H mice are resistant to intraperitoneal infection with murine coronavirus JHM, whereas suckling offspring of non-immune females are susceptible. Resistance can be conferred on suckling C3H mice by postnatal transmission of maternal immunity, if transfer preceeds infection. Suckling mice succumb to infection even when they receive maternal antibodies within 1 day after infection. Prenatal transmission alone without subsequent postnatal transmission of maternal immunity is not sufficient to provide resistance. Persistence of virus without clinical consequences was observed when the supply of breast milk anti-JHMV antibodies was terminated 5 days before infection. Immune reactions restricted by histocompatibility antigens do not play a crucial role in bestowing resistance. As neutralizing anti-JHM serum antibody titers of adult mice only rise sharply 5 to 7 days after infection, these results indicate that infection of adults can be arrested by immunological means but that, in addition, the rate of virus dissemination must be limited by other non-immunological mechanisms.

Introduction

Transmission of passive immunity from mother to offspring plays an important role in neonatal life. For protection against infection the newborn animal depends heavily upon the supply of maternal antibody. In the mouse a small but significant amount of immunoglobulin is transmitted *in utero* to the fetal circulation, the greater part, however, is transmitted after birth via colostrum and milk [3]. In the present study the effect of maternal immunity on the outcome of intraperitoneal infection with murine coronavirus strain JHM in suckling mice was investigated. JHM-virus is a neurotropic variant of mouse hepatitis virus. Intracerebral injection of the virus may lead to various forms of central nervous system diseases. Acute disease always occurs when either intraperitoneal or intracerebral infection is shortly after birth and subacute or chronic dis-

*Corresponding author

orders develop as a consequence of infection later in life [10, 11, 17]. An age-related development of resistance to peripheral intraperitoneal infection with JHM-virus has been shown [13] and the acquirement of resistance parallelled in time the acquirement of immunological competence. It was therefore of interest to investigate further, whether the difference in resistance between suckling and adult mice was solely attributable to their different immunological conditions.

Materials and methods

Mice. C3H mice were purchased from Bomholtgard (Ry, Denmark). All mice used were specific-pathogen free and kept in laminar flow hoods. Neutralizing antibodies against JHM-virus were not found in these mice before experimental manipulation.

Mating was performed with 2–4 months old mice. Adult females were immunized intraperitoneally with 10^5 PFU of JHM-virus before mating or after delivery as described for each experiment. Immune foster mothers were immunized by 3 weekly intraperitoneal injections with 10^5 PFU of JHM-virus. They were mated 10–30 days after the last injection. Suckling mice were infected with JHM-virus intraperitoneally as described for each experiment. The liver of those mice which died after infection was homogenized, and aliquots were assayed for JHM virus by plaque formation, and its inhibition by anti-JHM-antiserum, on L929 cell monolayers.

Virus. JHM-virus was propagated in the mouse fibroblast cell line L929 to titers of $1-5 \times 10^5$ plaque forming units (PFU) per ml as published elsewhere [13]. The titer was evaluated by plaque assay on L929 cells.

Sera. Mice were bled from the tail vein. The sera of adult mice were assayed individually. The sera of suckling mice were prepared individually, mixed together in equal proportions and assayed as a pool. As JHM-virus used for injections was produced in L929 cells all anti-JHM-antisera were absorbed with uninfected L929 cells.

Serum anti-JHM antibody assay. Aliquots of 50 µl of antiserum of various dilutions were incubated with 100 PFU JHM-virus in 50 µl of minimal essential medium in microtiter plate wells for 20 min at room temperature; 5×10^4 L929 cells in 50 µl of medium were added to each well and plaques were counted after an incubation period of 15 h at 37°C. The titer of the serum is described as the reciprocal dilution which produced 50% plaque reduction.

UV-inactivation of JHMV. Virus preparations containing 5×10^5 PFU/ml were UV-irradiated with 30.000 erg/mm^2. No residual infectivity could be detected by plaque assay.

Results

Relationship between the outcome of infection with JHM-virus in suckling mice and the immune status of their mothers

In the experiments summarized in Table 1 female mice were immunized with infectious JHM-virus at different times before mating or after delivery. The offspring were

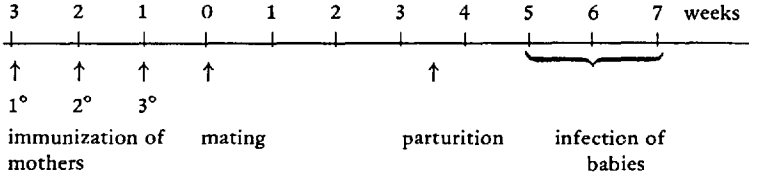
Table 1. Dependence of the outcome of JHMV infection in suckling mice on the immune status of their mothers

Mothers		*Offspring*				
Time of immunization	Dose of virus PFU/mouse	Age of babies at time of infection (days)	Interval between immunization of mother and infection of babies	Survivors/ total group		Occurrence of death days p.i.[a] (average)
(i)						
3X with 10^5 PFU:	2×10^1	14	6–8 wk	10/10		–
3, 2 and 1 wk	2×10^3			5/ 5		–
before mating	2×10^5			5/ 5	100%	–
	2×10^1	6		7/ 7		–
	2×10^1	3		5/ 5		–
3X with UV-JHM[c]	2×10^1	14		0/10	0%	4–7 (5)
(ii)						
1X with 10^5 PFU:						
4 days p.p.[b]	2×10^1	14	10 days	9/ 9		–
	2×10^4			7/ 7	100%	–
5 days p.p.	2×10^1	14	9 days	14/14		–
7 days p.p.	2×10^1	14	7 days	8/12	67%	6–22 (12)
9 days p.p.	2×10^1	14	5 days	3/11	34%	4– 5 (5)
10 days p.p.	2×10^1	14	4 days	4/10		7–12 (9)
11 days p.p.	2×10^1	14	3 days	0/10	0%	4–14 (7)
12 days p.p.	2×10^1	14	2 days	0/11		5– 8 (6)
4 days p.p.	no virus	–	–	6/ 6	100%	–
9 days p.p.	no virus	–	–	7/ 7		–

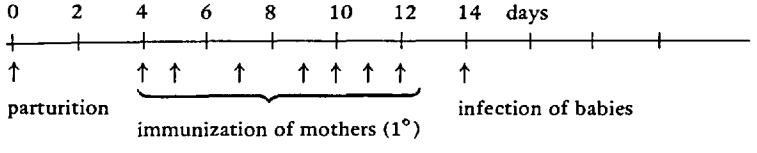
a p.i. post infection
b p.p. post partum
c 5×10^5 PFU before UV-inactivation

Time schedule to Table 1:

(i)



(ii)



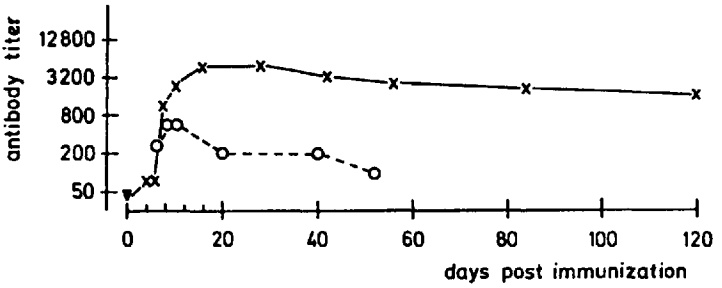


Fig. 1. Titers of neutralizing anti-JHM antibodies in the serum of adult C3H ♀ mice after immunization. x——x Mice were injected intraperitoneally with 10^5 PFU JHM-virus at the age of 8 weeks. o- - - - o Mice were injected intraperitoneally with UV-inactivated JHMV (5×10^5 PFU before inactivation) 3 × in weekly intervals. Serum was taken from the tail vein at various intervals after injection and assayed for anti-JHM antibodies

not clinically affected by this treatment. The offspring of these mothers were infected with JHM-virus at various intervals after immunization of the mother and were found to resist infection to various degrees, independent of the dose of virus used for infection. Full protection was given to litters of mothers which were immunized before mating and protection could also be provided by mothers which were immunized after delivery. In this case a period of at least 9 days between immunization of the mother and infection of the babies was neccessary to provide protection for all offspring and partial protection of the litter was achieved when an interval of 4—7 days was allowed. A period of 3 days or less, however, was not sufficient to confer resistance on any of the offspring.

Figure 1 shows the titer of neutralizing antibodies in the serum of one respresentative female at various periods after a single immunization with 10^5 PFU of JHM-virus. The kinetics of antibody production were the same in all (more than 20) animals tested, although the antibody level reached at the plateau varied to some degree. A notable increase of titer occurred between days 5 and 7. This increase would correlate well with the survival of babies which were infected with JHM-virus 7 days after immunization of their mother. A 3-fold immunization with UV-inactivated JHMV (5×10^5 PFU before inactivation) did not produce comparable antibody titers.

The influence of breast milk anti-JHM antibodies on JHM-virus infection in suckling mice

Having confirmed the ability of immune mothers to confer full resistance on their own offspring, babies born to non-immunized mothers were transferred to JHM-immunized lactating foster mothers at various times before or after infection (Table 2(i)). One day of suckling on the immune foster mothers rendered more than 80% of the babies of non-immune mothers resistant. Transfer to immune foster mothers at the time of infection improved the chances of survival but transfer after infection could not prevent a lethal outcome. Results were identical whether the mice were infected at 5—7 or 11—14 days of age.

Table 2. Relevance of time of maternal antibody supply to the outcome of JHMV infection in suckling mice

(i)

Transfer of offspring of non-immune mother to immune foster mothers[a]	age of babies at at time of infection[b] (days)	survivors/ total group		occurrence of death days p.i. (average)
3 days *before* infection	5– 7	18/19	95%	13
1 day *before* infection	5– 7	21/25	84%	6–14 (10)
at time of infection	5– 7	6/17	35%	4–15 (6)
1 day *before* infection	11–14	13/16	81%	5–13 (9)
at time of infection	11–14	5/14	36%	4–15 (7)
1 day *after* infection	11–14	1/13	8%	4– 9 (5)
2 days *after* infection	11–14	0/ 8	0%	4– 5 (5)
offspring of immune foster mothers	14	33/33	100%	–

a foster mothers were immunized 3X before mating
b 50 PFU/mouse

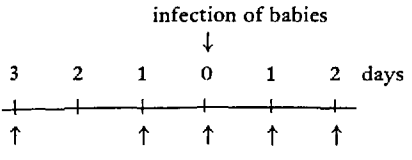
(ii)

Age of babies at time of transfer from immune mothers[a] to non-immune foster mothers (days)	age of babies at time of infection with 50 PFU/ mouse (days)	interval between transfer and infection (days)	survivors/ total group		occurrence of death days p.i. (average)
1–2	6	5	0/ 3		8 (8)
	5	3	0/14	0%	4–12 (7)
	3	1	0/ 7		4– 6 (5)
5	15	10	6/16	38%	4–12 (6)
	12	7	8/13	62%	6–12 (8)
	10	5	11/14	79%	4–12 (7)
	8	3	7/ 7	100%	–
8	15	7	8/14	57%	4– 9 (7)
	13	5	14/18	78%	4–15 (9)
	10	2	11/11	100%	–
	4	-4[b]	20/20	100%	–
babies of immune mothers	2–8	no transfer	27/27	100%	–

a original mother was immunized 3X before mating
b Mice were infected *before* transfer

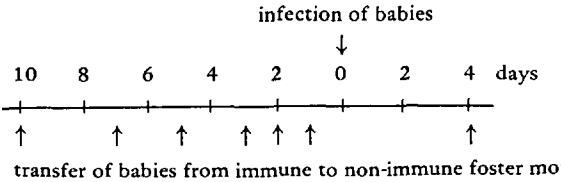
Time schedule to Table 2

(i)



transfer of babies from non-immune to immune foster mothers

(ii)



transfer of babies from immune to non-immune foster mothers

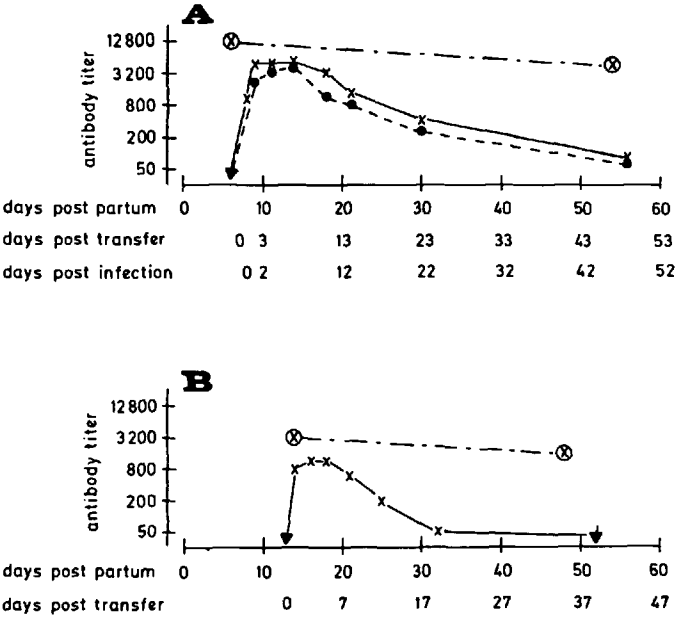


Fig. 2. Appearance of neutralizing anti-JHM antibodies in the serum of C3H baby mice born to non-immune mothers and transferred to immune foster mothers. A. A litter of 6 pups was transferred at day 7 post partum, 3 of the pups were infected with 50 PFU JHM virus 1 day after transfer. B. A litter of 5 pups was transferred at day 13 post partum. Anti-JHM antibodies were determined in adult and suckling mice as described in Methods. x——x uninfected baby mice; •----• infected baby mice; ⊗----⊗ immune mother

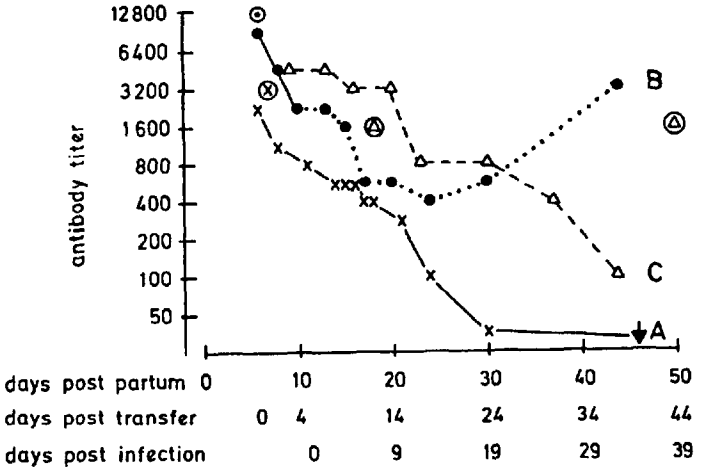


Fig. 3. Presence of neutralizing anti-JHM antibodies in the serum of mice born to immune mothers. x—x A. The litter was transferred to a non-immune mother 7 days p.p. •----• B. The litter was transferred to a non-immune mother 6 days p.p. and infected with 50 PFU JHM virus 5 days after transfer. △----△ C. The litter stayed with the immune mother. Encircled symbols give the antibody titers of the appropriate mother. Sera of the babies were prepared individually, and equal volumes of the sera were pooled per group.

These results correlate well with the finding that a significant level of neutralizing anti-JHM antibodies is transferred to the serum of the suckling mice (Fig. 2) within one day of transfer to immunized foster mothers. The antibody titer declined after day 18 p.p. and eventually reached the level of non-immune mice. Infection of the suckling mice after transfer did not change this antibody profile.

In a second set of experiments the protection of babies born to immune mothers, and foster nursed by non-immune mothers for various times before infection was investigated. As shown in Table 2 (ii) the period of protection varied depending on the age of the babies at the time of transfer. When offspring were removed from their immune mothers within the first 2 days after birth, and were transferred to normal foster mothers protection had not been conferred on these animals. However, when babies were allowed to suckle from their immune mothers for 5 days or longer before being transferred, protection against infection lasted for at least 3 to 5 days, and some of the babies were protected for 10 days. As shown in Fig. 3, the titer of neutralizing antibodies in the serum of babies born to immune mothers and transferred to non-immune mothers 6 days post partum (p.p.) (A) declined slowly, reaching the level of non-immune babies 30 days p.p. However, when the baby mice were infected 5 days after transfer (11 days p.p.) (B) their antibody titer continued to decrease for only a few days and remained at a relatively high level for about 2 weeks before rising again. Figure 3 shows for comparison also the antibody profile of mice born to and left with an immune mother (C).

Influence of histoincompatibility between mother and offspring

Babies born to non-immune C3H ($H2^k$), DBA ($H2^d$) and C57 Bl/6 ($H2^b$) mothers are fully susceptible to JHMV infection (Table 3). When foster nursed by C3H mothers which were immunized 3 times with 10^5 PFU of JHMV, all babies survived infection, independently of whether they were histocompatible or incompatible with the foster nurse.

Table 3. Influence of histocompatibility on protection by maternal immunity

Mouse strain		Age of babies at time of infection (100 PFU/Mouse)	Interval between transfer and infection	Survivors/ Total group
Foster mother	Babies[a]			
C3H ($H2^k$) (immunized 3X with 10^5 PFU before mating)	C3H	5–14	–	30/30
	DBA	10–14	4–6	8/ 8
	C57Bl/6	10–14	4–6	12/12
DBA ($H2^d$) (non-immune)	DBA	14	–	0/ 6
C57Bl/6 ($H2^b$) (non-immune)	C57Bl/6	14	–	0/ 6

a Babies were born to non-immune mothers

Discussion

We have investigated the action of maternal immunity during the interval before the neonate initiates an active immune response of its own. It was shown that babies born to JHM-immune mothers and allowed to suckle for 5 days are protected against lethal JHM-virus infection and this protection lasted for a further 5 days when those babies were transferred to non-immune foster mothers. However, when neonates from immune mothers were transferred within the first 2 days after birth they did not resist a subsequent infection. Mothers which were immunized after delivery could also confer resistance to their offspring and babies which were born to non-immune mice and foster nursed by immune mothers could be rendered resistant against lethal infection. These results show that transfer of maternal immunity against JHM-virus *in utero* is not sufficient to confer protection, whereas protection can be mediated post partum via the breast milk. Similar conclusions have been reached for the infection of neonatal mice with Friend virus [7, 8].

It has been shown by Brambell [3] that a small but significant amount of immunoglobulin is transmitted from mother to fetus prenatally, whereas most of the immunoglobulins found in the blood of suckling mice are acquired from maternal colostrum and milk after birth. IgA, the main immunoglobulin component of the breast milk is not resorbed but remains fixed at the surface of the epithelium of the gastrointestinal tract and only immunoglobulins of the IgG class are transported across the epithelial barrier to the systemic circulation [1, 2, 4, 9, 12]. Therefore maternal IgG is the most likely candidate for conferring protection against the fatal outcome of infection after peripheral inoculation of JHM-virus. It has, however, also been shown that milk lymphocytes and macrophages penetrate in a functional state to the newborn recipients' circulation [14, 15] and a contribution of these cells to the functional immunity detected in our experiments cannot be ruled out. Yet, babies transferred to histoincompatible immune mothers showed the same pattern of resistance as babies nursed by histocompatible mothers. This suggests that at least H2-restricted immune reactions provided by, for instance, cytolytic T-cells do not play a critical role. Our own earlier finding [13] that intraperitoneal injection of spleen cells from an immune donor into normal baby mice 1 day after infection provides protection against a lethal outcome taken together with our observation here that antibody supply by an immune foster mother 1 day after infection did not confer resistance, suggests that under certain conditions cellular mechanisms may play a crucial role. It can, however, be argued that intraperitoneal administration of antibody-producing cells provides a better and faster systemic antibody distribution in the baby mouse than passage of antibodies via breast milk.

Neutralizing antibodies against JHM-virus in the serum of adult C3H mice could not be detected earlier than 4 days after infection. The titer inclined until 16 days after infection, showing a drastic increase between day 5 and 7. It stayed on a high level for more than 4 months. In line with this observation is the fact that mice, when immunized with JHM-virus after delivery, could not confer resistance to any of their offspring when a period of only 2 or 3 days was allowed between immunization of the mother and infection of the babies, but that the ability to confer resistance was enhanced when this period was extended. These results explain our former finding [6]

that injection of immunocompetent spleen cells from non-immune adult donors into immunoincompetent baby mice did not render the babies resistant to a subsequent JHM-virus infection.

Babies born to non-immune mothers and transferred to immune foster mothers showed significant serum antibody titers 12 h after transfer. The titer increased until 2 days after transfer and decreased when the mice were 18–20 days of age as a consequence of gut closure which terminates the capacity to resorb antibodies from the milk 14–16 days post partum [5, 6]. Such babies resisted infection 1 day after transfer. Partial resistance of the litter was achieved when infection and transfer were arranged simultaneously, but no protection was provided by a transfer 1 day after infection. These findings show again that a lethal outcome of infection in baby mice can only be prevented, when the infecting virus is encountered by antibodies immediately. By contrast, adult C3H mice are not harmed by JHM-virus infection although they do not develop significant antibody titers until 4–5 days after infection. Therefore, not only the immune system but also non-immunological factors have to be considered to be the basis of the age dependent difference in resistance to JHM-virus infection. One such mechanism could be the interferon system, which has been shown to be incompletely developed in suckling mice [16], or alternatively a change of target cells for JHM-virus might occur with age.

Finally, our results are also relevant to the question of JHM persistence. In vivo persistence has been demonstrated for JHM-virus in various systems, in some cases leading to clinical symptoms (reviewed in [18]). From our results, development of virus persistence might be assumed because of two observations. Firstly, adult C3H mice maintain anti-JHM-antibodies at a high level for more than 4 months after a single immunization with infectious JHM-virus without additional experimental challenge, while immunization with UV-inactivated JHMV, even after several boosts, never leads to comparable antibody titers. Secondly, when 11-day-old babies, which had been transferred from an immune mother to a non-immune foster mother 5 days after birth, were infected with JHMV there was initially no increase of antibody titers, but when the animals achieved immune competence at about 20 days of age, a significant increase in antibody titer was observed. As this increase must have been caused by the infants' own immune system it must be assumed that, under these counterbalancing conditions between virus and antibody, the virus must have persisted in the animal for a period of at least 2 weeks without causing any clinical symptoms. Infectious virus could be detected in peritoneal exudate, liver and spleen of adult animals only until day 7 or occasionally until day 14–16 post infection and in passively immunized baby mice until day 3–5 post infection. Whether the virus which gives rise to antibody production persists in an infectious form at other sites than those examined, or in a non-infectious form, cannot be decided. However, it has to be assumed that at least viral antigens which induce neutralizing antibodies have to be present at sites which are easily accessible to the immune system. Further, as UV-inactivated JHMV fails to induce a successful immunization it must be proposed that these viral antigens have been processed and presented in a highly immunogenic form.

Acknowledgements. This work has been supported by the Deutsche Forschungsgemeinschaft. The autors thank Drs. S. Siddell and E. Wecker for helpful discussion, Christine Wehr for expert technical assistance, and Helga Kriesinger for typing the manuscript.

References

1. Borthistle BK, Kubo RT, Brown WR, Grey HM (1977) Studies on Receptors for IgG on Epithelial Cells of the Rat Intestine. J Immunol 119:471–476
2. Brambell FWR (1966) The Transmission of Immunity from Mother to Young and the Catabolism of Immunoglobulins. Lancet 1087
3. Brambell FWR (1970) The Transmission of Passive Immunity from Mother to Young. In: A Neuberger and EL Tatum (eds) North Holland Publishing Co. Amsterdam, London
4. Guyer RL, Koshland ME, Knopf PM (1976) Immunoglobulin Binding by Mouse Intestinal Epithelial Cell Receptors. J Immunol 117:587–593
5. Halliday R (1955) The Absorption of Antibodies from Immune Sera by the Gut of the Young Rat. Proc R Soc B 143:408–413
6. Halliday R (1959) The Effect of Steroid Hormones on the Absorption of Antibody by the Young Rat. J Endocr 18:56–66
7. Mathot C, Scher S (1968) Transmission through Milk of Antibodies against the Friend Virus in Mice. Nature 219:82–83
8. Mirand EA, Grace JT, Buffett RF (1966) Passive and Active Immunity to Friend Virus Disease. Nature 209:696-698
9. Morris IG (1964) The Transmission of Antibodies and Normal Globulins across the Young Mouse Gut. Proc R Soc B 160:276
10. Nagashima K, Wege H, Meyermann R, ter Meulen V (1978) Corona Virus Induced Subacute Demyelinating Encephalomyelitis in Rats: A Morphological Analysis. Acta Neuropathol 44:63–70
11. Nagashima K, Wege H, Meyermann R, ter Meulen V (1979) Demyelinating Encephalomyelitis Induced by a Long-term Corona Virus Infection in Rats. Acta Neuropathol 45:205–213
12. Nagura H, Nakane PK, Brown WR (1978) Breast Milk IgA Binds to Jejunal Epithelium in Suckling Rats. J Immunol 120:1333–1339
13. Pickel K, Müller MA, ter Meulen V (1981) Analysis of Age-Dependent Resistance to Murine Coronavirus JHM infection in Mice. Inf Immun 34:648–654
14. Pittard WBIII, Polmar SH, Fanaroff AA (1977) The Breast Milk Macrophage: A Potential Vehicle for Immunoglobulin Transport. J Reticuloendoth Soc 22: 597–603
15. Seelig LL Jr. Beer AE (1978) Transepithelial Migration of Leukocytes in the Mammary Gland of Lactating Rats. Biol Reprod 17:736–744
16. Taguchi F, Yamada A, Fujiwara K (1979) Factors Involved in the Age-dependent Resistance of Mice Infected with Low-virulence Mouse Hepatitis Virus. Arch Virol 62:333–340
17. Wege H, Koga M, Wege H, ter Meulen V (1982) JHM Infection in Rats as a Model for Acute and Subacute Demyelinating Disease. Adv Exp Med Biol 142:327–340
18. Wege H, Siddell S, ter Meulen V (1982) The Biology and Pathogenesis of Coronaviruses. Curr Top Microbiol Immunol 99:165–200

Received October 29, 1984