INFLUENCE OF THE IMMUNE SYSTEM ON THE COURSE OF INFECTION WITH MURINE CORONAVIRUS JHM IN SUCKLING MICE

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INTRODUCTION

JHM-virus, a neurotropic strain of murine corona virus, has been shown to induce various diseases of the central nervous system in rats which are influenced by the age of the animals at the time of infection (1-3). Infection early in life always results in acute disease while subacute or chronic diseases develop when adult animals are infected. In this connection it appears to be of importance to analyze the factors determining the different reactions of young and adult animals to infection with JHM virus. It has been shown for various viruses, that resistance to infection can occur, based either on the genetics or on the age of the animal (4-11). In this study experiments were carried out to investigate age dependent resistance by analyzing the influence of components of a competent immune system on the course of JHM virus infection in suckling mice.

MATERIALS AND METHODS

VIRUS: JHM-virus, originally derived from suckling mouse brain (1) was propagated in the C3H mouse fibroblast cell line L 929 to titres of 1 -5 x 10^5 plaque forming units (PFU)/ml. The titre was evaluated by plaque assay on L 929 cells. Inactivation of JHM-virus

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was obtained by UV-irradiation of 2 x 10^5 PFU/ml with 30.000 erg/mm^2.

MICE: C3H mice were purchased from Bomholtgart (Ry, Denmark). The expression "baby mice" was used for mice younger than 25 days, "adult mice" for mice older than 2 months.

CELLS: Spleen and thymus cell suspensions were prepared in MEM containing 5 % FCS, and injected intraperitoneally if not quoted otherwise.
Normal spleen cells (NSC) were derived from non-immune adult C3H mice, immune spleen cells (ISC) from adult C3H mice, which were immunized i.p. once with 10^5 PFU of JHM virus. Immune spleen cells were obtained 4 days, 14 -30 days, or 50 - 100 days after immunization. All ISC populations were tested for the presence of infectious virus *in vitro* by plaque assay, and *in vivo* by injecting the cells into susceptible baby mice. Both assays always worked comparably: injection of cell samples which turned out to be positive in the plaque assay killed suckling mice.

DESIGN OF EXPERIMENTS: To circumvent maturation differences between different litters, experiments were set up in the following way: When differently treated groups were compared in an experiment, in which more than one litter was used, the animals of the different litters were dispensed in such a way that each experimental group was represented in each litter. The virus was injected intraperitoneally throughout the experiments.

RESULTS

Age Dependence of the Outcome of JHM-Virus Infection in C3H mice

The outcome of an infection with respect to the age of the animals was followed up by injecting virus into suckling mice at different intervals after birth. As shown in Table 1A it was found that intraperitoneal infection with 20 PFU per mouse was always lethal for mice up to the age of 20 days. The rate of mortality was reduced when mice were 21 or 22 days old. Older animals were resistant to infection. There was hardly any time shift when higher virus doses were given (Table 1B). Table 1A and 1B show the results of distinct representative sets of experiments. Comparing different sets, the occurrence of resistance was shifted by maximally 2 days

days, probably reflecting the variation in the maturation of different litters. These findings demonstrate that natural resistance to JHM infection is an all or none effect. It does neither develop gradually over a longer period of time nor by showing clinical symptoms of changing severity. Paralysed hind legs and impaired balance could only be detected 8 - 4 hours before the animals died.

Table 1. Outcome of JHM Infection in C_3H Mice

A) AGE DEPENDENCE

TIME OF INFECTION (20 PFU[1]/MOUSE) DAYS P.P.[2]	SURVIVORS OF INFECTION / TOTAL GROUP	OCCURRENCE OF DEATH DAYS P.I.[3]
14	0/1	4 - 8
16	0/6	5 - 6
18	0/5	6 - 8
20	0/5	5 - 7
21	3/5	8 - 11
22	7/9	9
23	7/7	-
24	8/8	-

1) PFU plaque forming units
2) P.P. postpartum
3) P.I. post infection

Table 1. Outcome of JHM Infection in C_3H Mice

B) DOSE DEPENDENCE

TIME OF INFECTION DAYS P.P.	PFU/ MOUSE	SURVIVORS OF INFECTION / TOTAL GROUP	OCCURRENCE OF DEATH DAYS P.I.
14	2×10^1	0/10	4 - 8
	2×10^2	0/10	4 - 6
	2×10^3	0/5	4 - 5
22	2×10^2	3/4	7
	2×10^3	2/3	7
	2×10^4	1/3	7
24	2×10^2	3/3	-
	2×10^3	1/2	7
	2×10^4	4/7	7 - 11
25	2×10^5	49/50	18

This development of natural resistance to intraperitoneal JHM virus infection parallels in time the development of competence of the immune system in mice (12). We therefore investigated whether or not immunological factors can influence the course of JHM virus infection in suckling mice.

Influence of Spleen Cells from Adult Mice on the Course of JHM Infection in Suckling Mice

Normal spleen cells from adult mice as a source of mature lymphocytes were injected intraperitoneally into baby mice together with or before application of virus (Table 2). Although up to 6 x 10^7 cells were transferred protection was found only in a few cases.

Table 2. Effect of Normal Spleen Cells on JHM Virus Infection in Baby Mice

TIME OF INFECTION (20 PFU/MOUSE) DAYS P.P.	CELL TRANSFER: DAYS BEFORE INFECTION	NUMBER OF NSC[1] TRANSFERRED	SURVIVORS / TOTAL GROUP	OCCURRENCE OF DEATH DAYS P.I.
14 - 16	0	6 x 10^7	1/6	5 - 8
		3 x 10^7	3/8	5 - 10
	2	6 x 10^7	1/8	5 - 11
		3 x 10^7	1/10	5 - 8
	4	6 x 10^7	0/9	6 - 12
		3 x 10^7	0/5	5 - 7
14 - 16	-	-	0/20	4 - 12

1) NSC NORMAL SPLEEN CELLS

To see if any immune protection at all could occur in suckling mice immune spleen cells from adult mice, which had been immunized with JHM virus for various periods were injected into baby mice on the day of infection (Table 3). By this transfer - in contrast to the transfer of non-immune spleen cells- the majority of baby mice could be protected from death of infection, when at least 10^7 spleen cells were applicated. Protection by these cells did not depend on the priming period of the immune mice. These results show that immunological events can play a protective role , however, priming of the transplanted lymphocytes was a prerequisite for protection.

Table 3. Effect of Immune Spleen Cells on JHM Virus Infection in Baby Mice

TIME OF INFECTION (20 PFU/MOUSE) DAYS P.P.	PRIMING PERIOD IN DAYS	NUMBER OF ISC[1)] TRANSFERRED	SURVIVORS / TOTAL GROUP	OCCURRENCE OF DEATH DAYS P.I.
14	50	3×10^7	2/3	7
		1×10^7	6/8	7
14	14 - 30	3×10^7	5/6	11
		1×10^7	2/3	8
		3×10^6	0/2	5 - 6
14	4	3×10^7	8/8	-
14	-	-	0/15	4 - 8

1) ISC IMMUNE SPLEEN CELLS

Influence of Baby Lymphocytes on the Course of Infection in Adult Mice

As primed lymphocytes were not inhibited to exert their function in suckling mice the question was asked whether the lack of protection by normal spleen cells was due to suppression of their priming by the baby host. Immune suppression by lymphocytes from suckling mice has been shown in several other systems (13-15). As a first approach we tested this possibility by injecting thymocytes or spleen cells of 14 days old mice intraperitoneally or intravenously into adult mice one day before infection with 10^5 PFU per mouse (Table 4). None of the injected animals showed any clinical symptoms. Thus the resistance of adult mice to JHM virus infection could not be abolished by lymphocytes from suckling mice. These findings could suggest that baby lymphocytes did not interfere with the anti-JHM immune response of adult mice. On the other hand it could well be possible that the immune response was affected but the effect was masked because defense mechanisms other than the immune system, or a lack of appropriate target cells prevented the conversion from resistant to susceptible mice.

It further cannot be excluded that lymphocytes from suckling mice were only able to suppress adult spleen cells when they were allowed to operate in their own surrounding, namely the baby's body.

Table 4. Effect of Baby Lymphocytes on the Infection in Adult Mice

CELL TYPE TRANSFERRED	MODE OF CELL TRANSFER	NUMBER OF CELLS TRANSFERRED	PFU PER MOUSE	SURVIVORS / TOTAL GROUP
THYMOCYTES	I.V.[1]	4×10^8	1×10^5	2/2
"	"	2×10^8	"	3/3
"	"	1×10^8	"	2/2
"	I.P.[2]	2×10^8	"	3/3
"	"	1×10^8	"	2/2
SPLEEN CELLS	I.V.	1×10^8	"	3/3
"	I.P.	1×10^8	"	3/3

1) I.V. INTRAVENOUSLY

2) I.P. INTRAPERITONEALLY

Priming of Non-Immune Adult Spleen Cells in Baby Mice

The lack of protection of baby mice by unprimed spleen cells from adult mice as a consequence of suppression by the host was investigated in the following way: normal spleen cells from adult mice were injected into baby mice together with UV-inactivated JHM virus at different times before challenge with live virus. If the antigen-dependent differentiation -induced by the inactivated virus -were suppressed a priming of the injected lymphocytes would not occur. Consequently the rate of survivors should not extend the rate of survivors of those groups which were supplemented with NSC or UV-inactivated virus alone. Table 5 shows that application of UV-inactivated virus did not change the fate of 14 days old baby mice after infection, but that it slightly enhanced the rate of survivors when the animals were infected at the age of 19 - 21 days. Thus priming of the babies' own

Table 5. Effect of UV-Inactivated JHM-Virus on JHM-Virus Infection in Baby Mice

TIME OF INFECTION (20 PFU/MOUSE) DAYS P.P.	APPLICATION OF UV-JHM DAYS BEFORE INFECTION	SURVIVORS / TOTAL GROUP		OCCURRENCE OF DEATH DAYS P.I.
14 - 16	0	0/10		5 - 10
	2	1/8		4 - 8
	4	0/6		4 - 9
19 - 21	0	3/4		11
	1 - 2	6/10		6 - 13
	3 - 4	5/17	16/31	4 - 12
	5 - 6	6/8		5 - 13
14 - 16	-	0/15		5 - 8
19 - 21	-	6/18		5 - 12
NO INFECTION	14 - 16 DAYS P.P.	7/7		

lymphocytes did not occur in 2 weeks old animals, but seemed to become possible in 3 weeks old mice. On the other hand, when UV-inactivated JHM-virus was injected into 14 - 16 days old mice together with spleen cells from adult mice (Table 6) more than 50 % of the baby mice were protected against death of a subsequent infection. It made no difference whether the mice were challenged with live virus 2 or 4 days after cell transfer.

CONCLUSION

The experiments suggest that the lack of protection by normal spleen cells alone is not due to suppression by the host, because normal spleen cells can be primed in suckling mice. Thus the discrepancy is still unexplained that the presence of normal spleen cells in adult mice

Table 6. Priming of Normal Spleen Cells from Adult Mice in Baby Mice

TIME OF INFECTION (20 PFU/MOUSE) DAYS P.P.	CELL TRANSFER AND APPLICATION OF UV-JHM (DAYS BEFORE INFECTION)	NUMBER OF CELLS TRANSFERRED	SURVIVORS / TOTAL GROUP	OCCURRENCE OF DEATH DAYS P.I.
14 - 16	2	6×10^7	4/7	5 - 8
	2	3×10^7	4/8	4
	2	1×10^7	2/5	7 - 9
	3	6×10^7	2/3	7
	3	3×10^7	8/12	4 - 10
	4	6×10^7	6/7	5
	4	3×10^7	9/14	7 - 8
	4	1×10^7	5/10	4 - 6
	4	3×10^6	0/5	5 - 6
14 - 16	-	-	0/20	4 - 9
NO INFECTION	14 - 16 DAYS P.P.	6×10^7	5/5	-

is sufficient to cope with a JHM virus infection, while suckling mice, when supplemented with normal spleen cells from adult mice, are not able to control an infection successfully. It is therefore suggestive that the different course of infection with JHM virus in suckling mice and in adult mice is not only due to the different stages of immune competence, but that additional factors must play a role.

It is well known that dissemination of virus in the adult animal can also be prevented by non-immunological defence mechanisms. These might not yet be fully developed in a baby mouse. Findings reported by Taguchi et al. (16) for the interferon system would be in line with these interpretations. Comparing suckling and weanling mice these authors showed that, after infection, the production of interferon was delayed in suckling mice. In addition, target cells for JHM-virus might change during development. Baby mice might have relatively more target cells for JHM-virus than adult mice, and the change during maturation might reflect a

reduction of target cells by loss of virus receptors, or by alteration of the cellular competence for virus replication. An age-related conversion from susceptibility to resistance has been shown for infection of mouse fibroblasts with Sindbis virus (10), and for infection of mouse macrophages with MHV 2 (17). In addition, the relevant cells, namely the potential target cells, might change with respect to their importance for the organism.

SUMMARY
The course of infection with murine corona virus JHM in C3H mice depends on the age of the animals. Mice up to 20 days of age are fully susceptible while mice older than 23 days resist the infection. Protection of suckling mice from death of infection can be provided by intraperitoneal administration of immune spleen cells but not by non-immune spleen cells from adult mice. The immune spleen cells can be generated by priming adult mice, or by priming non-immune spleen cells from adult mice in baby mice with inactivated JHM virus. Thus the immune system might well be involved in the different outcome of infection with JHM-virus in suckling and adult mice, but it does not seem to be the exclusive factor responsible for the achievement of natural resistance.

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