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Modulation of lethal and persistent rat parvovirus infection by antibody

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Summary. Two day-old athymic (rnu/rnu) and euthymic (rnu/+) rat pups nursing immune or non-immune dams were inoculated oronasally with the Yale strain of rat virus (RV-Y). All athymic and euthymic pups (57/57) from immune dams remained clinically normal, whereas 51 of 66 athymic and euthymic pups from non-immune dams died within 30 days. Infectious RV was detected by explant culture in 12 of 15 surviving pups of both genotypes from non-immune dams 30 days after inoculation, but in none of the 57 surviving pups from immune dams. RV-Y DNA was detected by Southern blotting in kidneys of surviving athymic pups from non-immune dams but was not detected in pups from immune dams. Euthymic pups from immune dams appeared not to produce endogenous antibody to RV after virus challenge, whereas euthymic pups from non-immune dams produced high-titered RV immune serum. Pups of both genotypes given immune serum prior to or with RV were fully protected from disease and persistent infection, whereas pups given immune serum 24 hours after RV were partially protected. These studies show that RV antibody offers significant protection against lethal and persistent RV infection.

Introduction

Rat virus (RV) is a common parvovirus [5] that can cause lethal infection in rat pups [4, 8], whereas infection of juvenile or adult rats is usually asymptomatic [2]. Rat pups that survive acute infection can, however, develop asymptomatic, persistent infection [7]. Rat pups born in an enzootically infected colony develop asymptomatic infection as adults [11]. This implies that maternal antibody protects rats from lethal RV infection during their period of maximal susceptibility. Our previous studies showed that antibody-positive dams delivered clinically normal pups that did not develop an RV antibody

response after decay of maternally transmitted antibody [6]. This result suggested that the pups were not persistently infected with RV. However, challenge experiments were not done, so it was not clear if passively immunized pups were protected from lethal or persistent RV infection after inoculation of virus. We have recently shown that endogenous immunity to RV begins within 1 week after virus inoculation of non-immune pups, but frequently fails to protect them from persistent infection [4]. To investigate further the role of passive immunity in modulating RV infection, athymic and euthymic rats were challenged with RV before, during or after passive immunization. Athymic rats were included because they are more susceptible to RV-induced disease and persistent infection and do not develop humoral immunity to RV [3].

Materials and methods

Animals

Specific pathogen-free athymic (rnu/rnu) and euthymic (rnu/+) rats (*Rattus norvegicus*) and Sprague Dawley (SD) 4 week-old female rats (Animal Genetics and Production Branch, National Institutes of Health, Bethesda, MD) were housed in Micro-isolator cages (Lab Products, Maywood, NJ) as previously described [4]. Athymic male rats were bred to euthymic heterozygous female rats to produce mixed litters of athymic and euthymic pups for virus inoculation. Randomly selected rats from each group were tested for antibodies to common murine viruses and *Mycoplasma pulmonis* by immunofluorescence assay (IFA) [12]. Rats tested prior to inoculation with RV, sham-inoculated dams and uninoculated control rats were seronegative. Rats tested after inoculation were seropositive only for RV.

Virus

The RV-Y strain of rat virus [2] was used for all experiments. Virus was propagated, quantified and stored as previously described [4].

Tissue collection and assay for infectious virus

Surviving pups, their dams and SD sentinel rats were euthanatized with carbon dioxide gas and blood was collected by cardiocentesis. Tissues were examined macroscopically and samples were immediately stored for DNA extraction ($-70\,^{\circ}C$). Serum was stored at $-20\,^{\circ}C$ for serology. Kidney and spleen were assayed for infectious virus by explant culture [9].

Serology

Individual sera, diluted 1:10, were tested for antibody to RV by IFA [10]. IgG antibody titers were determined by an enzyme immunoassay (EIA) [4].

Detection of RV DNA in tissues

DNA was extracted from tissue frozen in liquid nitrogen and pulverized while frozen [7]. Southern analysis was performed using 10 μg of PstI digested DNA which was hybridized to a ^{32}P-labeled RV probe [7]. Positive and negative control samples were included on each filter and 1 pg and 10 pg dilutions of gel purified RV DNA excised from plasmid were included to estimate sensitivity.

Statistical methods

Differences in proportions were analyzed by χ^2. Antibody titers were coded and analyzed by the unpaired T test or by analysis of variance followed by post-hoc tests to compare means. A P of $\leqslant 0.05$ was accepted as significant.

Design of experiments

RV challenge of rats with maternally-acquired antibody

Heterozygous (rnu/+) euthymic female rats, lightly anesthetized with ether, were immunized by oronasal inoculation with 80 k $TCID_{50}$ of RV-Y. A second inoculation was given 16 days later. Sixteen days after the second inoculation, each female was caged with 2 sentinel SD females to test for RV-Y transmission. Sentinels were removed 3 weeks later, and tested for RV-Y antibody. All immunized dams were non-transmitting when they were bred to athymic males. When litters were 2 days old, the dams and 2 pups from each were tested for antibody to RV by EIA. Non-immune euthymic dams were sham-inoculated twice with 20 µl of phosphate-buffered saline and subsequently bred to athymic males.

Two day-old pups from immune or non-immune dams were inoculated oronasally with 2 k $TCID_{50}$ RV-Y. Pups were observed daily for clinical signs and deaths were recorded. Moribund pups were euthanatized and necropsied. Thirty days after inoculation, surviving pups were euthanatized and tested for infectious virus, viral DNA and antibody to RV. Blood was collected from the dams and they were euthanatized.

RV challenge of rats given immune serum

Sterile, pooled RV-immune serum of known EIA titer (1:51,200) was injected intraperitoneally into 2 day-old pups from 3 litters. Pups were given 0.05 ml of serum and were euthanatized at 1 h, 1, 3, 7, 10, 14, or 30 days. Antibody titers were determined to establish a standardized dose for passive immunization.

To test the temporal effect of antibody on RV infection, pups were inoculated intraperitoneally with 0.05 ml of RV-immune serum 1 day before, concurrently with or 1 day after inoculation of RV-Y at 2 days of age. Clinical signs and deaths were recorded and moribund pups were euthanatized and necropsied. Surviving pups were necropsied 30 days after inoculation with RV. Spleens and kidneys were tested for infectious virus and serum was assayed for RV antibody.

Results

Effect of maternally-acquired antibody on RV infection

Two day-old pups nursing immune dams had EIA antibody titers equivalent to their dams (Table 1). The increase in serum antibody titers among immune dams may have been due to virus exposure from cleaning pups immediately after virus inoculation. Mean titers in uninoculated control pups from such dams were equivalent at the beginning (day 0) and end (day 30) of the experiment (data not shown).

RV-inoculated pups of both genotypes from immune dams were fully protected from illness and death, whereas pups from non-immune dams had high mortality (Table 2). Six pups born to non-immune dams were cannibalized and were not included because their genotype could not be determined. Signs and lesions in affected pups and survivors were consistent with RV-Y infection [4].

Table 1. Reciprocal of geometric mean EIA antibody titers (± S.D.) to RV in sera of dams and their pups

	Titer on day 0	n	Titer on day 30	n
Immune dams	673 ± 233	4	2,263 ± 122	4
Pups[a] from immune dams				
athymic	200 ± 88	5	295 ± 104	25
euthymic	528 ± 93	5	645 ± 88[b]	32
Pups[a] from non-immune dams				
athymic	not done		< 50	5
euthymic	not done		12,800 ± 696[b]	10

[a] Pups inoculated on day 0 with 2k $TCID_{50}$ of RV-Y

[b] Significant difference between euthymic pups from immune and non-immune dams, $P \leqq 0.001$

Table 2. Mortality ratios and isolation of infectious RV-Y 30 days after virus challenge of rat pups born to immune and non-immune dams

		Pups from immune dams	Pups from non-immune dams
Athymic	deaths	0/25	27/32
	virus[a]	0/25	5/5
Euthymic	deaths	0/32	24/34
	virus[b]	0/30	7/10

[a] Kidneys and spleens of pups from immune dams were all RV-negative and from pups of non-immune dams were all virus-positive

[b] Kidneys and spleens of pups from immune dams were all RV-negative. Among pups from non-immune dams, 7/10 kidneys and 2/10 spleens were RV-positive

Surviving athymic pups from non-immune dams did not have antibody at 30 days, and their spleens and kidneys contained infectious virus (Tables 1 and 2). Surviving euthymic rats from non-immune dams had a substantial immune response to RV and 7 of 10 rats were virus-positive (Tables 1 and 2).

Southern blots detected RV-Y DNA in 2 of 2 kidneys of surviving athymic pups from non-immune dams (Fig. 1). Band patterns were indicative of the replicating form of RV DNA. RV-Y DNA was not detected in spleens of athymic pups from non-immune dams (data not shown), or kidneys and spleens of euthymic pups from non-immune dams (Fig. 1 and data not shown). However, 2 of 3 tested kidneys and 1 of 4 tested spleens of euthymic pups nursing non-

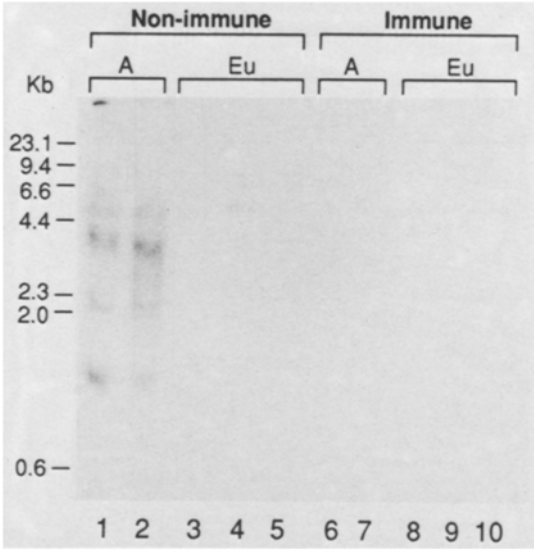

Fig. 1. Southern blot of RV DNA from kidney 30 days after inoculation of athymic and euthymic pups from non-immune dams (*1–5*) or immune dams (*6–10*). RV-specific bands occur at 2.1 and 1.3 kbp. Bands at 4.4 kbp are PstI fragments of rat genomic DNA that hybridized to small amounts of plasmid vector in the RV probe as described elsewhere [7]

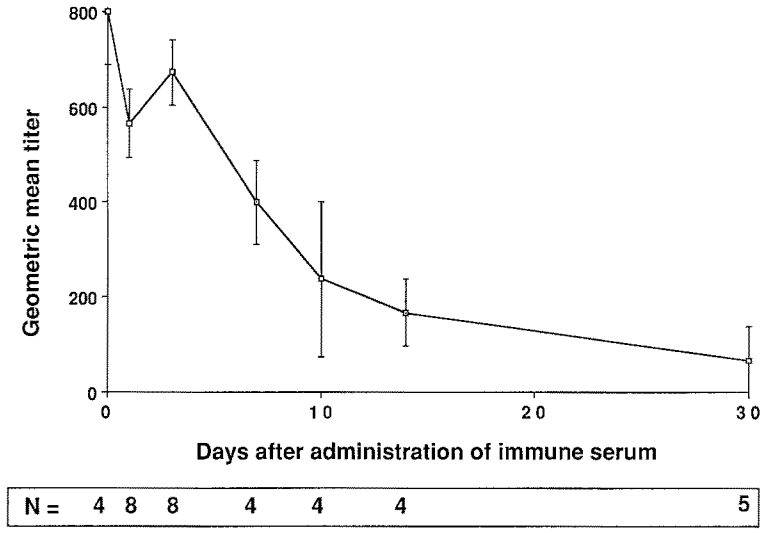

Fig. 2. Reciprocal of geometric mean EIA antibody titers ± S. D. in non-infected rat pups after administration of RV-immune serum

immune dams contained infectious RV-Y even though the tissues were negative for RV DNA.

Neither infectious RV-Y nor RV-Y DNA were detected in the kidneys or spleens of pups from immune dams (Table 2, Fig. 1 and data not shown). Pups from immune dams did not appear to produce a primary humoral immune response to RV challenge (Table 1).

Effect of passively administered immune serum on RV infection

Uninfected pups given 0.05 ml of RV-immune serum had EIA titers comparable to those of pups nursing immune dams (Fig. 2). Antibody concentrations de-

Table 3. Mortality ratios of rats given RV immune serum before, with, or after inoculation of RV-Y[a]

Serum on day	Euthymic	Athymic
− 1	0/20	0/10
0	0/18	0/9
+ 1	0/22	2/13
None	14/21	11/12

[a] Inoculated on day 0 (2 days of age) with 2 k $TCID_{50}$ of RV-Y

Table 4. Detection of RV-Y in rats given immune serum before, with, or after inoculation with RV-Y[a]

	Genotype and tissue[b]					
	Euthymic			Athymic		
Serum on day	spleen	kidney	total	spleen	kidney	total
− 1	0/20	0/20	0/20	0/10	0/10	0/10
0	0/18	0/17	0/18	0/9	0/9	0/9
+ 1	5/20	5/22	6/22	0/11	0/11	0/11
None	3/7	7/7	7/7	1/1	1/1	1/1

[a] Inoculated on day 0 (2 days of age) with 2 k $TCID_{50}$
[b] Number positive/number tested

Table 5. Reciprocal of geometric mean EIA antibody titers at day 30 after inoculation of RV-Y[a] in rat pups given RV immune serum before, with, or after virus

Serum on day	Genotype	No. seropositive[b]	Titer
− 1	athymic	6/10	50 ± 0
	euthymic	14/20	52 ± 60
0	athymic	3/9	50 ± 0
	euthymic	7/18	55 ± 65
+ 1	athymic	8/11	55 ± 64
	euthymic	16/22	57 ± 66
None	athymic	0/1	< 50
	euthymic	7/7	7,052 ± 93

[a] 2 k $TCID_{50}$ at 2 days of age
[b] Number positive/number tested

clined slowly and mean antibody titers were equivalent to pups with maternally acquired antibody for at least 2 weeks after administration of immune serum.

Immune serum given a day before or concurrently with RV protected athymic and euthymic pups from disease (Table 3). Infectious virus was not detected in spleens and kidneys collected 30 days after inoculation (Table 4). Immune serum given a day after RV significantly reduced illness and deaths compared to rats that were not given immune serum (Table 3). Among euthymic rats, RV was recovered from only 27 percent of survivors given immune serum compared to 100 percent of survivors not given immune serum (Table 4). Tissues from pups given RV-Y immune serum were not tested for RV DVA. Pups given immune serum did not produce endogenous antibody in response to RV challenge (Table 5).

Discussion

Acquisition of RV antibody from immune dams or by injection of RV-immune serum completely protected rat pups from disease after oronasal challenge with virulent RV. The efficacy of protection was further demonstrated by showing that it applied to both athymic and euthymic pups. Pre-existing or concurrent passive immunity also appeared to protect pups from persistent RV infection, which commonly develops among pups that are not immune at the time of virus inoculation [7]. Spleens and kidneys from passively immunized pups failed to yield infectious virus after explantation and RV DNA was not detected in these tissues. Failure to detect infectious virus or viral DNA does not rule out infection, but makes it unlikely since the kidney and spleen are common sites of RV infection [7].

The mechanism of protection by passive immunization was not determined, but several findings suggest that it occurred during early stages of infection. First, protection against lethal or persistent infection was fully effective only if antibody was given before or during viral challenge. Passive immunization as little as 24 hours after virus challenge resulted in persistent infection in about 25 percent of euthymic pups and death in about 15 percent of athymic pups. Second, there was no evidence of a primary humoral immune response to RV among passively immunized euthymic rats. Although the latter result could be explained by interference from passively acquired antibody, the time-dependency of full protection coupled with the lack of active endogenous immunity suggests that antibody prevented infection or eliminated virus rapidly.

Passive immunization after virus challenge provided some protection, since deaths and persistent infections were substantially reduced. However, virus was not recovered from surviving athymic rats, while 27 percent of their euthymic littermates became persistenly infected, despite the fact that there were no significant differences in antibody titers between the two genotypes or between virus-positive and virus-negative euthymic pups. This result seems paradoxical, but suggests that the timing of antibody acquisition after virus inoculation is critical to the course of infection. Thus, antibody administered early in infection

may block the replication and spread of virus in some rats, but not in others. Progressing infection in immunodeficient (athymic) rats would lead to illness or death whereas euthymic rats would enlist immune defenses capable of aborting lethal infection, but not persistent infection. This scenario suggests that T cell-mediated defenses exclusive of humoral immunity modulate resistance to lethal RV infection. Closer examination of early events during RV infection will help to clarify these issues.

This study reinforces the concept that pre-existing antibody can provide protection against infection for rats born in an enzootically infected colony [8]. It also suggests that vaccination of dams and sires prior to breeding could be useful for prevention, control or elimination of RV infection. Inactivated vaccines have been employed successfully to prevent parvovirus infection in other species [1, 10], but the possibility of vaccination with an attenuated rat parvovirus or a heterologous rodent parvovirus of low virulence such as minute virus of mice [13] should also be considered.

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