An animal model for therapeutic intervention studies of CMV infection in the immunocompromised host

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Summary. An experimental rat model to study acute cytomegalovirus infections is described. Eight-week old male Brown Norway rats, immunosuppressed by total body irradiation, were infected with rat cytomegalovirus (RCMV). The effects of infection were determined by survival rates and the presence of virus or viral components in different organs was assayed by plaque test, immunoperoxidase staining, dot-blot DNA hyridization and in situ DNA hybridization. At days 10-post infection nearly 90% of the animals had died. Spleen, liver and bone marrow were heavily infected. Interstitial pneumonia was observed. Pathological findings strongly resembled the full scale of lesions in human CMV infections. Anti-RCMV hyperimmune serum was effective against mortality from RCMV infection and viral spread to lungs and liver was prevented. This model is appropriate for studies on the pathogenesis and antiviral therapy of CMV infections in the immunocompromised host.

Introduction

The ubiquity of cytomegalovirus (CMV) infections in man is now well established. Although most primary infections are asymptomatic and become latent, it has become clear that in immuno-suppressed patients, including cancer patients receiving chemotherapy, AIDS patients, and transplant recipients, CMV is a frequent pathogen and an important cause of morbidity and mortality. After renal transplantation most patient develop symptomatic CMV disease due to either primary or secondary infections [1].

Clinically these patients present a variety of symptoms associated with virus-induced lesions in almost every organ system. Ultimately, in these severely infected patients CMV is responsible for 15–20% mortality. However, in transplant recipients anti-CMV immune globulin dramatically decreases morbidity and mortality [2, 3].

To further elucidate the pathogenesis of CMV infections, a homologous virus host system in a readily available laboratory animal is of primary importance [4]. In addition, an animal model to study the effect of therapeutic intervention is still needed. In 1982 rat cytomegalovirus (RCMV) was isolated from wild rats [5]. CMV infections in the immunocompetent rat are asymptomatic, as is generally assumed to be the case in man. After primary infections the virus remains in a latent state in the host. Immune suppression leads to reactivation of the latent virus. Passive transfer of RCMV infections by renal transplants in rats occurs with high incidence [6]. However, this model does not reflect the course of a severe CMV infection. Therefore, in an attempt to mimic the conditions in which CMV infections in their most threatening form can be studied, we developed a model of RCMV infection in the immune compromised rat. In this model the pathology of the acute viral infection in different organ systems is described and the effect of anti-CMV immune globulin treatment was studied to evaluate the model for therapeutic intervention studies.

Materials and methods

Animals

Inbred specific pathogen-free (SPF) male Brown Norway (BN) rats were used at eight weeks of age with a total bodyweight ranging from 140 to 180 g. These rats were bred at the Department of Experimental Animal Service at the University of Limburg, Biomedical Centre, Maastricht, The Netherlands.

Immunosuppression

A 5 GY dose of total body X-irradiation (TBI) was administered one day before infection as described earlier [7] or otherwise as indicated.

Virus and infection

The RCMV stock consisted of a pool of salivary glands from acutely infected laboratory rats [8]. The salivary glands were homogenised and injected by the intraperitoneal route as described before [5].

The animals received 10^5 plaque-forming units (PFU) of virus or otherwise as indicated.

Design of the experiments

Groups consisting of a minimum of 5 immunocompromised rats were infected with RCMV one day after TBI. The rats were maintained in separate cages for a maximum of three weeks post infection (p.i.). From rats which had died in the first three weeks after infection, material from the organs was collected for determination of infective RCMV, RCMV-DNA and viral antigens. Bone marrow and brain tissue were obtained for DNA and antigen determination only. Surviving rats were sacrificed after three weeks and samples of the same organs were taken.

The following control groups were used:
a non-irradiated and non-infected group (group A), an irradiated (5 Gy) and non-infected group (group B), and an infected (10^5 PFU RCMV) and non-irradiated group (group C). Half of each control group was killed 8 days p.i. and the other half was killed at three weeks p.i.

To study the effect of anti-RCMV hyperimmune serum (HIS) animals were treated in a one day single dose as described below. The effect was measured by survival rates and virus organ titers 8 days p.i.

Histology

Samples of organs were fixed in paraformaldehyde-lysine-periodate (PLP) and embedded in paraffin using routine histopathological procedures.

Serial sections of 5 μm thickness were cut for routine hematoxylin-eosin staining as well as immunoperoxidase for RCMV antigens and in situ hybridization for RCMV DNA detection.

Monoclonal antibodies

Mouse monoclonal antibodies (McAbs) against RCMV-induced antigens were produced and characterized in our laboratories as described before [9]. In this study two McAbs, which specifically reacted in immunoprecipitation with polypeptides major abundant in virions. McAb 35 directed against a 29 kDa polypeptide of RCMV gives a diffuse immunofluorescence signal in the cytoplasm of RCMV-infected cells. McAb 8, directed against 41, 46, and 91 kDa polypeptides of RCMV [10] gives an immunofluorescence signal in the nucleus of infected cells. None of the McAb reacted with uninfected cells.

Immunohistochemistry

Tissue sections (5 μm) were mounted on glass slides, deparaffinized and blocked for endogenous peroxidase by incubation in 100% methanol containing 0.3% H_2O_2 for 30 min.

The sections were further rehydrated and washed with phosphate buffered saline (PBS) and preincubated with PBS containing 2% bovine serum albumin (BSA) for 30 min, followed by incubation with a mixture of mouse McAbs 8 and 35 for 1 h. The sections were washed in PBS for 5 min, followed by incubation with biotinylated, affinity-purified, sheep anti-mouse Ig (Amersham Nederland B.V., Houten, The Netherlands) 1 : 200 in PBS containing 2% BSA for 1 h, washed again in PBS and incubated with biotin-streptavidin-HRP-complex (Amersham), 1 : 400 in PBS containing 2% BSA for 30 min. After washing in PBS the sections were incubated for 10 min in diaminobenzidin (DAB) substrate 0.05% in Tris-HCl buffer pH 7.4 containing 0.002% H_2O_2. The colour reaction was stopped by washing in PBS. After counter staining during 30 sec in haematoxylin the slides were dehydrated and mounted in Entellan (Merck, Darmstadt, Federal Republic of Germany).

The specificity of the monoclonal antibodies was evaluated with an anti-HCMV McAB and with organ sections obtained from uninfected control rats.

Only McABs 8 and 35 showed reactivity with organ sections obtained from RCMV infected animals.

Probe DNA for in situ and dot blot hybridization

The construction and analysis of recombinant plasmids of RCMV-DNA were performed as described previously [11].

The XbaI fragments C, D, and E were cloned in the p SP62 plasmid and transfected in *Escherichia coli* strain HB101 rec A⁻. Isolation of plasmids was performed according to Birnboim et al. [12].

Plasmids were cleaved by XbaI and subjected to 0.7% agarose electrophoresis.

XbaI fragment C, D, and E were then cut out of the gel and the DNA was recovered using the Biotrap apparatus (Schleicher and Schull, Dassel, Federal Republic of Germany) as described by Göbel et al. [13].

The fragments, accounting for 23.7% of the entire RCMV genome, were mixed in equimolar amounts and labelled with biotin 11-dUTP (Sigma, St. Louis, U.S.A.) using the random primed labelling kit (Boehringer, Almere, The Netherlands).

The specificity of the biotinylated DNA probes was evaluated in uninfected rat embryonal fibroblasts (REF). REF infected with RCMV and organ samples using both dot-blotting and in situ hybridization also were performed without DNA probes to evaluate non-specific reactions.

In situ hybridization

This technique was performed as described earlier [14]. In short, the paraffin-embedded tissue sections were mounted on chronium-alum-gelatin coated, glutaraldehyde-activated slides. Sections were dewaxed and aldehyde groups were inactivated by incubation in 1% ammoniumchloride solution in PBS. Proteolytic digestion was carried out with proteinase K. After washing in glycin PBS buffer the slides were dehydrated in a series of graded alcohols. Tissue sections were then overlaid with hybridization mixture containing the DNA probe, covered with a silicon coated coverslip and sealed to create a constant microenvironment.

Cellular and probe DNA were denatured simultaneously by heating the sections to 80 °C on a heating block of 100 °C for 30 sec followed by immediate immersion in ice water. Subsequently, the hybridization was carried out overnight at 42 °C. Visualization was performed with the BLU-gene™ kit (Bethesda Research Laboratories, BRL, Faithersburg, U.S.A.). RCMV-infected REF and RCMV-infected organ samples showed hybridization reactivity whereas mock infected REF and uninfected organ samples did not show any reactivity at all. HCMV and plasmid probes never showed hybridization.

Dot blot hybridization

Extraction of cellular DNA was performed as previously described [15]. Before spotting on filters the DNA was denatured by incubation for 1 h at 65 °C in 0.35 M NaOH, followed by adjusting the pH to 7.0 and ionic strength to 6 × sodium chloride sodium citrate buffer (SSC). Thereafter the resulting solution was serially diluted in 6 × SSC in order to obtain a final amount of 2.5, 1.25, and 0.6 µg/DNA spot. Using a manifold this DNA was spotted on nitrocellulose filters type BA85 (Schleicher and Schull). To bind DNA to the nitrocellulose, the filters were placed at 80 °C for 1 h.

Prehybridization and hybridization were performed as described earlier [14]. To detect probe target hybrids, filters were incubated in a streptavidin-alkaline phosphatase conjugate (BRL). After extensive washings 5 mm spots were punched out and placed in a 96 wells microtiter flat-bottom plate, filled with 200 µl pNPP (6.6 nM/l in 0.05 M carbonate buffer pH 9.8 containing 0.001 M MgCl$_2$).

After incubation at RT for 2–4 h the reaction was stopped with 1/3 volume of 5 N NaOH and the nitrocellulose discs were removed. Absorbance was measured in a multichannel spectrophotometer (Multiskan, Amstelstad, Zwanenburg, The Netherlands) at 405 nm. The assay was evaluated with DNA extracted from RCMV- and mock infected REF. Only DNA extracted from RCMV infected REF showed hybridization reactivity. HCMV and plasmid probes did not show hybridization.

Virus assay

A quantitative assay for RCMV was performed as described previously [8]. Briefly, samples of spleen, kidney, liver, and lungs were homogenized within 12 h after the death of the animal and diluted 1 : 10 (w/v) in Eagle's Minimal Essential Medium (MEM). Ten-fold dilutions were cultured on REF and the amount of virus was expressed as the number of PFU per gram tissue.

Preparation of hyper immune serum (HIS)

HIS was obtained from immunized SPF male BN rats as follows: eight week old animals were infected intradermally below the popliteal fossa of the posterior legs with 10^5 PFU RCMV, emulsified in Freund's complete adjuvants (FCA). After 4 and 7 weeks the procedure was repeated with RCMV in FCA. Ten days after the last administration of RCMV serum was obtained from the abdominal aorta of the animals. Negative control serum was prepared by administration of a non-infected salivary gland suspension. To test the concentration of neutralizing antibodies in the HIS the plaque-reduction assay was used [16]. In short, 2-fold dilutions of heat-inactivated serum were incubated with 300 PFU RCMV in basal medium Eagle's (BME) + 2% newborn calf serum (NCS) for 1 h at 37 °C. These mixtures were absorbed on a REF monolayer in a 24-wells plate for 1 h at 37 °C. After mixtures were removed the monolayers were overlayed with a BME-agarose mixture. After 7 days the monolayers were fixed in formalin 1.8% in PBS, stained with methylene blue and the plaque reduction titer (ID_{50}) was calculated.

Administration of HIS to RCMV infected rats

To determine the possible efficacy of HIS in modulating the effect of RCMV infection 1 ml of 2-fold dilutions of HIS with a neutralization titer of 160 was injected intravenously into animals that were subsequently challenged with a variable irradiation dose (5 Gy or 6 Gy) and with a variable amount of infectious virus (10^4 PFU to 10^6 PFU RCMV). The day of HIS administration varied in different experiments as defined.

In the control groups the lethally infected rats received negative control serum. In all experiments groups of 10 rats were used.

Statistical analyses

For statistical analyses the Mann-Widney test and Fisher's exact test were used. For comparison of the survival curves the generalized Willcoxon test by Gehan was used [17, 18]. p-Values $\leqslant 0.05$ were considered statistical significant, unless indicated otherwise.

Results

Effect of immunosuppression and RCMV infection on the mortality

To study the effect of RCMV infection on the mortality of immunosuppressed rats (receiving 5 Gy TBI) the animals were infected with 10^2, 10^3, 10^4, or 10^5 PFU RCMV. The animals were observed twice daily for a period of three weeks. In Fig. 1 the survival of these infected rats is shown. All animals receiving 10^4 or 10^5 PFU RCMV died between day 7 and day 13. In contrast, the animals receiving 10^2 or 10^3 PFU virus and the control rats all survived the three week period.

To determine the effect of TBI on the course of RCMV infection 4 groups of 5 rats received 5, 4, 3, or 2 Gy one day prior to infection with 10^5 PFU RCMV. In the 5 Gy TBI group all animals died within 12 days p.i. In the other groups no mortality was observed during the three weeks post infection. Repetitions of this experiment indicated that 5 Gy TBI is the critical dose needed to result in a lethal infection.

Subsequently, we calculated the survival rates from 5 separate experiments with rats, receiving 5 Gy TBI and 10^5 PFU RCMV. As shown in Fig. 2 the
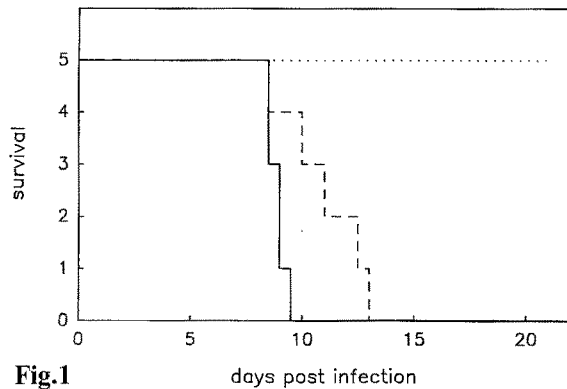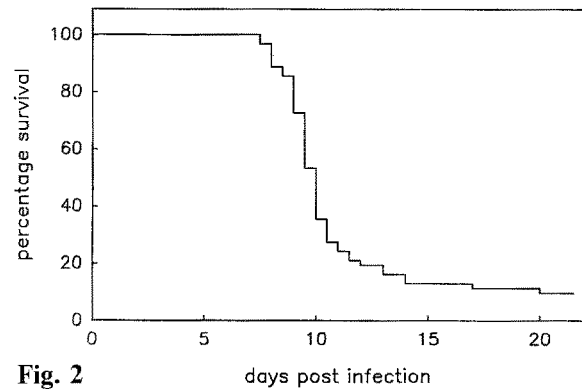
Fig.1   days post infection   Fig. 2   days post infection

Fig. 1. Survival curves of four different groups of immunocompromised rats (n = 5) after intraperitoneal inoculation with 10^5 PFU (————), 10^4 PFU (– – –), and 10^3 or 10^2 PFU (· · ·) RCMV and of control groups A, B, and C (· · ·). These results are representative for 3 separate experiments

Fig. 2. Overall survival rates from 5 separate experiments on 62 consecutive rats receiving 10^5 PFU RCMV i.p. and 5 Gy TBI. The control group A, B, and C showed no mortaility (p < 0.0001)

overall survival rate declines following a sigmoidal curve to 8% at the end of the experiment. Control rats receiving 5 Gy TBI without infection (group B) all survived during the experimental period (p < 0.0001).

Infectious virus, viral genome and antigen in the organs

The presence of infectious virus in the organs of the RCMV infected rats was studied using the plaque assay. In these experiments rats receiving 5 Gy TBI were infected with either 10^2, 10^3, 10^4, or 10^5 PFU RCMV (5 rats per group). Controls consisted of group A, B, and C (see Materials and methods). The animals were killed three weeks post-infection. All rats receiving 10^4 and 10^5 PFU died from infection. They were autopsied within 24 h post mortem and plaque assays were performed. These results are shown in Fig. 3.

In all organs of rats receiving 10^4 or 10^5 PFU RCMV infectious virus could be detected. Virus yields were higher (p < 0.05) in spleen and liver than in kidneys and lungs in both groups (Fig. 3). The virus yields detected in each organ were not significantly different between these two groups (p > 0.05). In the groups receiving 10^3 PFU RCMV, the results were negative (data not shown). Although occasionally, a very low level of virus (about 70 PFU per gram tissue) was found in the spleen. In the control groups, including the infected non-irradiated group (C), no virus could be detected at day 10 or 21 p.i.

The amount of viral DNA as determined by dot blot analysis in various organs is shown in Fig. 3C and D for the groups which received 10^4 and 10^5 PFU virus.
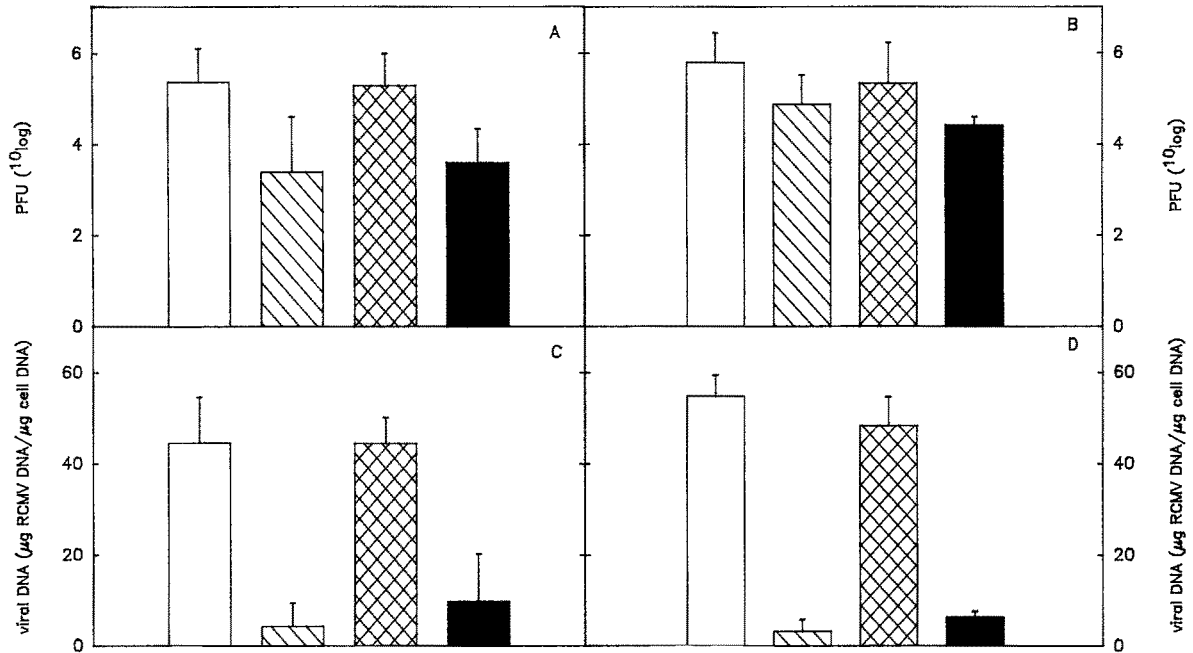
Fig. 3. Yield of infectious virus and viral DNA in spleen (□), kidney (▨), liver (▨) and lungs (■) in rats, challenged with RCMV. Amount of plaque forming units in the group infected with 10^4 PFU RCMV (**A**) and in the group infected with 10^5 PFU RCMV (**B**); amount of viral DNA, detected by dot blot hybridization in the group infected with either 10^4 PFU RCMV (**C**) or 10^5 PFU RCMV (**D**). These data are representative for 2 separate experiments. Each bar represents the mean value of 5 rats ± the standard deviation

In all organs tested viral DNA was detectable. The amount of viral DNA was significantly higher ($p < 0.05$) in the spleen and liver than in the kidneys and lungs. Significant differences were not found between the groups which received 10^4 or 10^5 PFU RCMV ($p > 0.05$) (Fig. 3).

In the groups receiving 10^2 or 10^3 PFU RCMV (data not shown) no or very little viral DNA could be detected (in the order of 1 ng RCMV DNA/µg cellular DNA).

No viral DNA could be detected in all control groups.

In situ hybridization showed that in the organs of the animals infected with 10^5 PFU RCMV, cells containing viral DNA were present (Table 1).

In spleen and liver a significantly higher percentage of cells contained viral DNA than in kidneys and lungs ($p < 0.05$).

In the control groups (A, B, or C) reactive cells were not detected.

The results of the experiments for the detection of viral antigens by immunoperoxidase staining are also shown in Table 1.

The proportion of positive cells in the organs is quite similar to the results obtained with the in situ hybridization.

Table 1. Presence of viral antigens and viral DNA in
the organs of RCMV infected rats[a]

Organ	Viral antigens[b] positive (%) ±sd	Viral DNA[c] positive (%) ±sd
Spleen	69 ± 21	49 ± 28
Kidneys	< 1	< 1
Liver	27 ± 15	29 ± 17
Lungs	3.6 ± 3	< 1

The results are expressed as the percentage of positive cells of at least 10 slides ± standard deviation. Data represent the results of 3 separate experiments

[a] The rats were infected with 10^5 PFU RCMV and received 5 Gy TBI

[b] Viral antigens were detected by the immunoperoxidase technique

[c] Viral DNA was detected by the in situ DNA hybridization test

Pathology

Macroscopic examination

After an incubation period of 7 to 14 days the RCMV infection became symptomatic. The rats showed symptoms, such as brushy hairs, ocular discharges and impaired reactivity to exogenous stimuli. Symptomatic disease progressed within 48 h into death. Since most animals died between day 8 and day 14 (Fig. 2) organ pathology was documented in a transversal study in experimental and control (A, B, and C) rats on day 10 p.i. Fifty percent of the animals died between dy 8 and 10 and the remaining 50% of the animals which survived till the 10th day of infection were then sacrificed. Twenty percent of the rats showed disease and 30% of the rats had no symptoms of infection. All control groups remained asymptomatic. To prove that morbidity and mortality is related to the occurrence of CMV viremia and the presence of extensive organ damage, the spleen, kidneys, liver and lungs were harvested and used for plaque assay (determination of infectious virus) and immune peroxidase staining (detection of viral antigens). The results are shown in Table 2. In asymptomatic animals neither viral DNA nor viral antigens could be detected in any organ tested but the spleen. In the group of animals which died before day 10 infectious virus and viral antigens were detectable in all organs studied. In the animals alive on day 10 post infection and showing signs of infection small amounts of virus and viral antigens were detectable in the organs.

Histopathology

The characteristic histopathological changes in the organs of lethally infected animals can be summarized as follows:

Table 2. Presence of virus and viral antigens in the RCMV infected rats[a].
Transversal study

Organs	Detection of viral antigens[b] in rats			Detection of infectious virus[c] in rats		
	"spontane-ously" dead before d10 50%	with symptoms at d10 20%	symptom-free at d10 30%	"spontane-ously" dead before d10	with symptoms at d10	symptom-free at d10
Spleen	70	50	1	5.8	3.6	1.7
Kidneys	<1	neg.	neg.	4.9	3.1	neg.
Liver	60	20	neg.	5.3	3.6	neg.
Lungs	17	<1	neg.	4.4	1.8	neg.

[a] The rats were infected with 10^5 PFU RCMV and received 5 Gy TBI

[b] Viral antigens are detected by the immunoperoxidase technique. The results are expressed as the percentage of cells giving a positive staining

[c] Infectious virus is detected by the plaque assay and is expressed as ^{10}log PFU RCMV per g organ. The results shown represent the means of 2 experiments

● Liver: The parenchyma showed patchy necrosis (Fig. 4a). Scattered throughout the parenchyma mononuclear cells were seen. Cholestasis was not apparent. Numerous hepatocytes showed characteristic inclusion bodies. By immunohistochemistry these cells as well as many Kupfer cells contained viral antigens (Fig. 4b) and by in situ DNA hybridization: viral DNA (Fig. 4c).

● Spleen: The periarteriolar lymphocyte sheaths had almost completely disappeared. Sinuses were massively congested with macrophages with nuclear inclusions and cytoplasmic hemosiderin pigment. These cells contained viral antigens (Fig. 4d) as well as viral DNA.

● Lungs: In rats inoculated with a high input of virus the interstitium showed congestion with hemorrhages and infiltrating macrophages. Furthermore, numerous alveolar macrophages and erythrocytes were present. The macrophages and occasional pneumocytes were immunoreactive for CMV (Fig. 4e).

● Kidneys: In the kidneys only the glomeruli showed discrete abnormalities. The mesangial cells were swollen and displayed characteristic nuclear inclusions. These cells contained viral antigens (Fig. 4f) as well as viral DNA. Occasional vascular endothelial cells displayed similar findings. Also numerous viral antigen and DNA bearing cells occurred in the mesenchymal cells in the renal capsule.

● Bone marrow: In bone marrow smears intensely immunoreactive mononuclear cells could be detected (Fig. 4g).

● Brain: Histologically the cerebrum and cerebellum did not show signs of infection. Scattered immunoreactive cells were found intravascularly in one rat only.
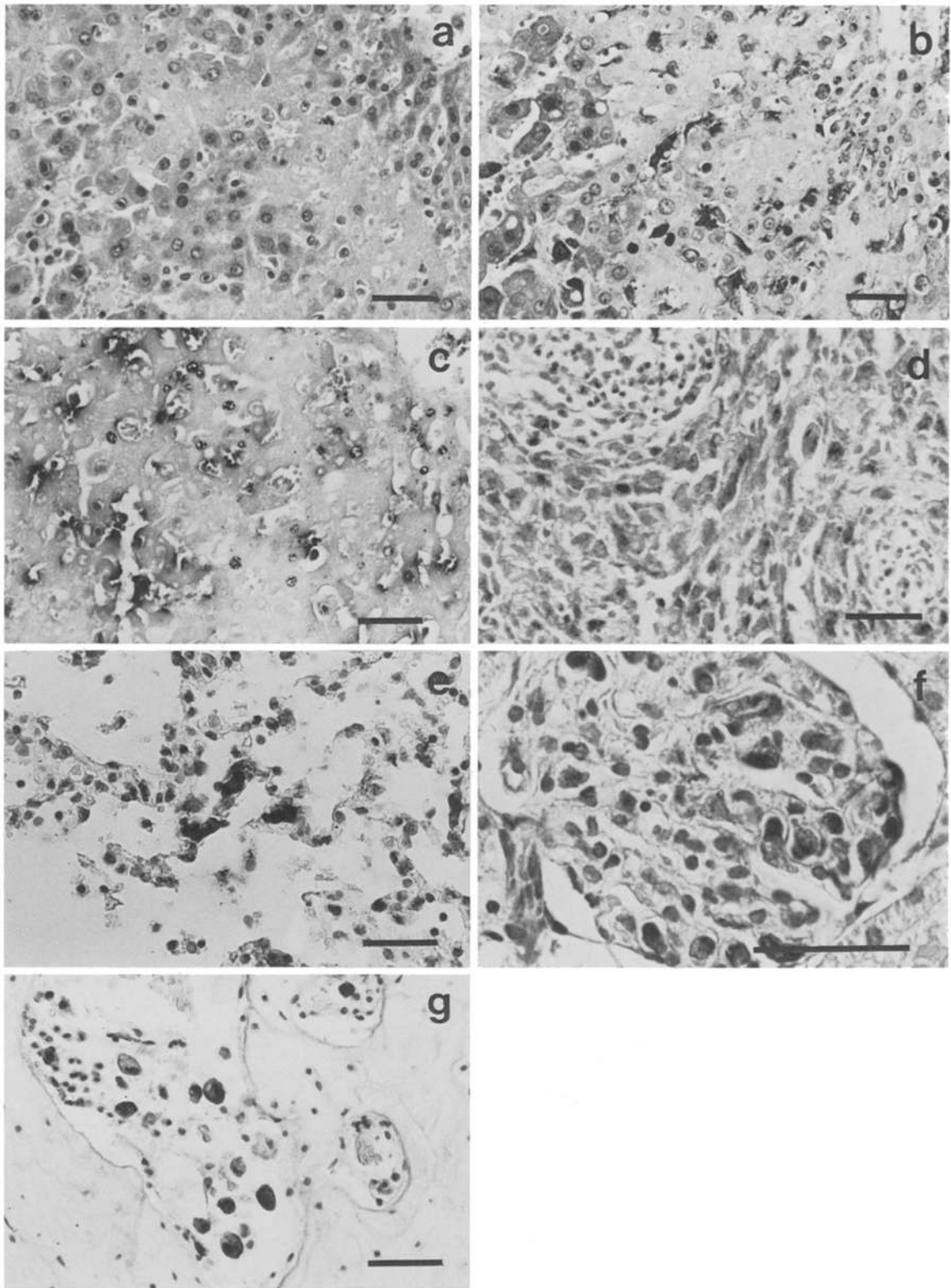
● Eye: In the retina no histological abnormalities were found. By immune peroxidase and in situ hybridization procedures, exclusively blood-borne cells were reactive. Furthermore, immunoreactive cells were visualized in the lacrimal glands.

Viral spread to several organs early in infection

Irradiated RCMV infected rats were killed on day 1, 3, and 7 post-infection. Material from several organs was collected to perform plaque assays and histopathological studies.

As shown in Table 3 infectious virus was first detected in the spleen in small amounts on the third day p.i.

Later in infection kidney, liver, and lungs became infected. Immunoperoxidase and in situ hybridization studies confirmed these results. Furthermore, on the third day p.i. the bone marrow stained positive in both techniques.

Table 3. The presence of infectious virus in several organs from RCMV infected immunocompromised rats[a] on several stages post infection

Days p.i.	Organ			
	spleen	kidney	liver	lungs
1	neg.[b]	neg.	neg.	neg.
3	2.3	neg.	neg.	neg.
7	3.9 ± 1.2	3.2 ± 0.6	2.8 ± 0.8	3.2 ± 0.7

[a] Each group consisted of 5 rats. The animals received 5 Gy TBI

[b] neg. = <2.2. The virus titer is expressed as ^{10}log PFU RCMV per gram organ, mean ± standard deviation

HIS treatment

The efficacy of HIS in modulating the lethal effects of RCMV infection was studied as described in Materials and methods. The administration of a single

Fig. 4. Demonstration of liver damage in serial sections by HE staining (a), immunoperoxidase staining (IPOX) (b), and ISH (c). Note the patchy necrosis and numerous hepatocytes with CMV inclusion bodies which stained positively by IPOX and ISH. IPOX of the spleen (d) shows massive congestion of sinuses with immunoreactive macrophages. IPOX of lungs (e) shows immunoreactive macrophages and pneumocytes. IPOX of the kidneys (f) shows a glomerulus with immunoreactive swollen mesangial cells and characteristic nuclear inclusions. IPOX of the bone marrow (g) shows intensive reactivity of mononuclear cells. Bar: 5 μm

Table 4. The effect of variables in RCMV infection and treatment schedules on the survival of HIS treated rats

Irradiation (Gy)	RCMV (^{10}log PFU)	Therapy day	Neutralization titer[a] of the HIS used				
			5	10	20	40	80
5	5	−1	0	2	8	10	10
5	5	+1	0	0	1	8	10
5	6	−1	0	1	7	10	10
5	4	−1	0	2	7	10	10

Each group consisted of 10 rats

[a] The neutralization titer is expressed as the dilution of serum which corresponds with the ID_{50}. Undiluted serum provided an ID_{50} of 160

Table 5. Effect of HIS administration on the presence of infectious virus in the infected rats[a]

Therapy	Virus titers[b]			
	spleen	kidney	liver	lungs
Control serum	5.6 ± 1.3	3.3 ± 0.8	5.3 ± 1.4	4.5 ± 2.9
HIS	5.3 ± 1.1	2.9 ± 0.6	neg.[c]	neg.[c]

[a] BN rats were infected with 10^5 PFU RCMV one day after X-irradiation (5 Gy). Groups of 10 rats each were treated with 1 ml HIS by i.v. route 1 day prior to infection or with negative control serum. Plaque assay was performed 7 days post-infection

[b] The virus titer in the organs is expressed as the ^{10}log PFU RCMV, mean ± standard deviation

[c] neg. = <2.2. P = 0.01

dose of HIS at appropriate dilution, 1 day prior to challenge with 10^5 PFU RCMV provided full protection to experimental animals (Table 4).

Complete protection was afforded by the antiserum, when it was administered up to 24 h post-challenge, but this effect rapidly waned thereafter (day 2 or later).

Experiments using different infection doses (10^4, 10^5, and 10^6) gave similar results as shown in Table 4.

Furthermore, when immunosuppression increased (6 Gy) no protective effect of HIS was observed even when HIS with a titer of 160 was used.

As expected, in experimental animals receiving negative control serum, survival was low ($^1/_{10}$). Furthermore, rats receiving high dose TBI without treatment (up to 7 Gy) never died. The lethal dose seemed to be 9 Gy.

In addition, in infected animals the effect of HIS therapy on the virus loads of several organs was studied. As shown in Table 5, the number of infective virus particles in the spleen and kidneys was not influenced by HIS therapy, but in the liver and lungs a significant reduction in the number of infectious RCMV was noted (p < 0.01).

Finally, the effect of HIS treatment on organ pathology was examined. On macroscopic examination no symptoms of disease were noted in the HIS treated group. Histopathologic changes of lungs and liver were minimal and resembled those described by Bruning et al. [7] in the immunocompetent host.

Discussion

Like human CMV, RCMV is poorly pathogenic in the immunocompetent host [19] but the virus may cause severe disease under immunocompromised conditions. An inoculum of 10^4 PFU or more of RCMV combined with severe immunosuppression (5 Gy TBI) resulted in symptomatic disease and a high mortality. Neither immunosuppression nor infection by itself caused disease or mortality. The incubation time varied between seven and fourteen days. Disease symptoms correlated with the presence of infectious virus, viral antigens, and viral DNA, which were found in high concentrations in spleen and liver, less in the lungs and bone marrow, even less in kidneys and eyes and sporadically in brain tissue.

Viral antigen detection by immunoperoxidase staining was slightly more sensitive than detection of viral DNA by in situ hybridization, but overall the results of these techniques correlated very well. Furthermore, the percentages of reactive cells using these techniques correlated well with those of the plaque assays and dot blot hybridization.

Early occurrence of RCMV immunoreactive cells, predominantly macrophages, in the spleen was a constant finding in symptomatic infection, which parallels the situation in man where involvement of the spleen is a common finding in CMV infections [20]. Also in mice splenic macrophages seem to play a role in the pathogenesis of lethal infection [21]. In animals which died from infection, the spleens invariably showed extensive necrosis. Numerous monocytes and macrophages showed characteristic inclusions and frequently RCMV-specific immunoreactivity. Furthermore, a reduced volume of white pulp was observed, which is of interest in relation to the described immunosuppressive effect of CMV [1, 22, 23].

In the kidneys RCMV-specific, immunoreactive mesangial cells could be detected in the glomeruli together with some proliferation of mesangium. The renal capsule contained many immunoreactive cells, which might account for relatively high virus yields in the plaque assay. In contrast, in the human kidney CMV localized predominantly in tubular epithelium and hardly ever in the glomerular cells [1]. In immunocompromised patients a symptomatic CMV infection is frequently accompanied by hepatitis [24]. The present findings in

our model do closely resemble the pathological findings in HCMV hepatitis. Histologically, extensive liver damage was apparent with numerous inclusion bodies in hepatocytes, Kupffer cells, and bile duct epithelium and was accompanied by focal liver cell necrosis as described for HCMV hepatitis elsewhere [25, 26].

Pneumonitis is the leading cause of death in CMV infected transplant patients [1]. In RCMV infected rats numerous immunoreactive cells were found in the lungs, including alveolar macrophages and interstitial mononuclear cells, resembling the histopathology of HCMV induced pneumonitis [1]. Although in transplant patients CMV infection of the cerebrum is rare, in other immuno-suppressed conditions, as in AIDS, cerebral involvement occurs frequently in the form of infection of glial cells and neurones [27]. In this study infection of the brain was indeed detectable, albeit at a low level and without histological abnormalities.

In this rat model, viral antigens were present in the retinal pigment layer. Most antigens occurred in blood-borne cells, a finding which supports the hypothesis of a hematogenous spread of infection in CMV retinitis.

In the bone marrow of RCMV infected rats numerous cells displayed signs of CMV infection. This indicates a masive infection of the bone marrow, which may lead to immunosuppression as described in human and mouse studies [28, 29]. The exact nature of infected cells still has to be investigated.

The applicability of this infection model for screening therapeutic strategies in CMV infection was evaluated using preventive antibody treatment. In man preventive administration of HIS in bone marrow transplant patients [2] and renal graft recipients [3] has a benficial effect on morbidity and mortality. In the rat HIS administered prophylactically to animals receiving lethal challenges of virus plus X-irradiation can provide 100 per cent protection against RCMV infection. These results corroborate the findings in man.

Two possible mechanisms by which antibodies exert their protective effect are the neutralization of free virus particles and the elimination of virus-infected cells by antibody-dependent cell-mediated cytotoxicity (ADCC). Results of our experiments suggest that the latter, i.e., the ADCC plays a role in the protection against early RCMV infection.

First, antibodies administered 24 h p.i. still protect the host from acute RCMV infection, although at that time infectious virus particles can be obtained neither from peritoneal washing fluid [30] nor from the blood serum (data not shown).

Second, a 100-fold increase of RCMV inoculum (10^4 PFU to 10^6 PFU) does not require a higher neutralization titer of the HIS to protect against lethal infection (Table 4) suggesting that the neutralization of free virus particles is not involved in our system.

Third, an increase of the irradiation dose (5 Gy to 6 Gy) has a negative effect on the outcome of antibody treatment indicating that dose-related suppression of the cellular immune system influences the protective effect of antibodies.

These results corroborate the findings of Manischewitz et al. [31] and Quinnan et al. [32] indicating that ADCC plays a major role in the early phase (the first week) of acute CMV infection.

However the exact mechanism by which HIS prevents lethal infection in this model remains to be determined.

In HIS treated rats infection of the lungs and the liver was prevented (Table 5). This is in contradiction to the finding in mice that in antibody-treated animals the virus load in the organs was the same as in non-treated animals [33, 34]. At this moment we have no full explanation for the discrepancy in the virus content of the organs in these two animal species.

In conclusion, a model to study acute CMV infection in the immunocompromised host is described. It is characterized by high mortality and the full range of CMV-related lesions. The preference of the virus for different internal organs shows similarity with the situation in immunocompromised man. Furthermore, antibody treatment in the early stage of the infection was effective against mortality and viral spread to different organs.

Finally, this model provides the possibility to study the effect of chemotherapeutic and immunological intervention.

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