Pathogenesis of Cytomegalovirus Infection. Distribution of Viral Products, Immune Complexes and Autoimmunity During Latent Murine Infection

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SUMMARY

During studies on the mechanisms of virus latency, reactivation and resultant tissue injury in mice infected with murine cytomegalovirus (MCMV) in utero or at birth, we found the occurrence of three distinct pathological groups. In the first group, mice died within 4 weeks of exposure to virus and showed evidence of tissue injury due to MCMV in multiple tissues and organs of the body. The second group consisted of mice which survived the initial infection and was composed of a minority (about 25%) which shed virus (chronically infected). The third group (about 75%) consisted of mice in which shedding of virus could not be detected (latently infected). Study of the latter group indicated that virus was not detected in brain, thymus, liver, kidneys, urine or serum by co-cultivation techniques or by cellular DNA-MCMV DNA hybridization. In contrast, virus could be activated from spleen cells by co-cultivation with allogenic but not syngeneic feeder cells and MCMV-DNA was detected in amounts equivalent to 3 to 4 virus genomes per 100 spleen cells. In both the latently infected and chronically infected mice, in all strains studied evidence of virus-antivirus immune complex deposits in the renal glomeruli occurred. Only one of the six infected strains (C57 Br/cdJ) studied showed manifestations of autoimmune disease with the formation of antibodies to nuclear antigens, DNA and soluble nucleoprotein.

INTRODUCTION

Cytomegalovirus infection is worthy of biomedical study for several reasons. First, this infection plays a significant role in causing multiple human birth defects, tissue injury, and, in some, death within a few years of birth (Weller & Hanshaw, 1962; McCracken, Shinefield & Cobb, 1969; Stern et al. 1969; Krech, Jung & Jung, 1971). Second, cytomegalovirus infection and associated tissue injury frequently occur after transplantation (Craighead, Hanshaw & Carpenter, 1967) or open heart surgery (Kaariainen et al. 1966; Lang, Scolnick & Willerson, 1968; Caul et al. 1971), after multiple blood transfusions and after immunosuppressive drug treatment of patients with terminal leukaemias and lymphomas (Duwall et al. 1966). Third, understanding cytomegalovirus infection should shed light on the biological principles which govern virus latency and reactivation, conditions that frequently relate to this infection (reviewed Fenner et al. 1974).
Several animal species become infected with cytomegalovirus which is relatively specific for that host (Fenner et al., 1974), including guinea pigs, hamsters, rats, mice and humans. Because cytomegalovirus infections in mice (MCMV) and in humans are very similar, because immunological and virological manipulations are relatively simple in mice, and since inbred strains of mice are available, we have begun to study the pathogenesis of MCMV infection. Here we report the initiation of MCMV infection in several mouse strains, the distribution of virus products in various tissues and the immunopathological consequences of that infection.

**METHODS**

**Hosts.** Male and female C3H/HeJ, C57Br/cdJ, C57BI/6, and SWR/J mice were obtained from Jackson Laboratories, Bar Harbor, Maine; C3H/St, BALB/cSt and Ha/ICR mice from L. C. Strong Research Foundation, Del Mar, California; New Zealand white (NZW) mice from the Laboratory Animal Centre, Medical Research Council, Surrey, England. Nude mice having BALB/c and nude mice having CBA backgrounds were obtained from Jacques Miller, Walter and Eliza Hall Research Institute, Melbourne, Australia. Subsequently, all mice except C3H/St, BALB/cSt and Ha/ICR mice were inbred in our institution. Mice of each strain were tested randomly and found to be free of lymphocytic choriomeningitis virus (LCMV), lactic dehydrogenase virus (LDV) and polyoma virus infections. The major H-2 alleles of the mice: C3H/HeJ, C3H/St, C57 Br/cdJ and nude mice (CBA) are H-2 kk; BALB/cSt, C57 BI/6 and nude (BALB/c) mice are H-2 dd; SWR/J are H-2 qq, and NZW are H-2 zz. The Ha/ICR are outbred.

Pregnant mice were injected intraperitoneally (i.p.) with 1000 tissue culture infectious dose (TCD₉₀) of a stock MCMV pool 3 to 5 days before delivery and, after delivery, nursed their own babies. In other experiments, newborns were infected i.p. with 100 TCD₉₀ of the virus within 24 h of birth. Newborn C3H/St, and Ha/ICR were nursed by their own mothers, but C3H/HeJ, SWR/J, C57 Br/cdJ, C57BI/6, NZW and nude mice by Ha/ICR foster mothers.

**Virological studies.** Seed MCMV was originally provided by Dr Richard I. Carp, Department of Microbiology, New York State Institute for Research in Mental Retardation, Staten Island, N.Y., and was passaged, titred and maintained in our laboratory as previously reported (Olding, Jensen & Oldstone, 1975). The MCMV was characterized as being similar to Smith strain by neutralization tests, passed through CFW mouse embryo cells as described (Kim & Carp, 1971) and passed three times through BALB/St embryo fibroblasts in our laboratory. To obtain purified MCMV we infected confluent monolayers of mouse embryo cells grown in either T75 flasks or roller bottles with MCMV at a multiplicity of infection (m.o.i.) of approx. 0·2. Marked cytopathic effects (c.p.e.) occurred 3 to 4 days later. The supernatant fluid was removed and freed of cellular debris by centrifuging at 700 g for 15 min. Virus was pelleted at 60000 g for 60 min, resuspended in tris-EDTA buffer (10 mM-tris-HCl, 1 mM-EDTA, pH 7·7), centrifuged to equilibrium in a Csat gradient with a mean density of 1·3 g/ml for 48 h at 44000 rev/min, and the opaque virus band collected.

For hybridization studies, confluent monolayers of mouse embryo cells in T75 flasks were simultaneously incubated with 1000 TCD₉₀ of MCMV and 750 μCi of ³H-methyl-thymidine (40 to 60 Ci/mmol) in 7 ml minimal essential medium (MEM) containing 10% foetal calf serum (heated at 56 °C for 30 min), L-glutamine and antibiotics (growth medium). After c.p.e. appeared approx. 3 days later, virus was obtained from supernatant fluids by first pelleting and then banding in CsCl equilibrium gradients as described above. The virus harvested was disrupted by treatment with 1% Sarkosyl at 60 °C for 10 min and centrifuged...
to equilibrium in CsCl gradients with a mean density of 1.72 g/ml. The resulting virus DNA band was collected and dialysed against 1 mM-EDTA, pH 8.0. Cellular DNA was extracted from disrupted mouse tissues by phenol-chloroform extraction as described by Randall & Gafford (1969). The DNAs were then sheared to produce pieces approx. 350 nucleotides in size by using a micro probe in a Bronson sonicator. The procedures for hybridization and analysis of the DNA hybrid on hydroxyapatite columns have been reported elsewhere (Britten & Kohne, 1968; Kohne, 1968; Kingsbury & Lerner, 1974).

To recover MCMV from fluids or tissues of infected mice we cultured samples on confluent monolayers of either BALB/c or HA/ICR mouse embryo cells supplemented with growth media. Urine was collected in vials from mice housed in metabolic cages and was kept chilled on ice. The urine was cleared of sediment by centrifuging at 700 g for 15 min, passed through a 0.45 μm Millipore filter, diluted 1:2 and 1:10 in growth media and then inoculated on to mouse embryo cells. Additional urine samples were inoculated directly on to cells without centrifuging. Fresh serum or plasma was diluted 1:2 and 1:10 in MEM before culturing. Salivary glands, kidney, liver and lung tissues were minced into small pieces with sharp scalpels, allowed to adhere to 60 × 15 mm plastic Petri dishes for 30 min at 37 °C after which 1 to 2 × 10^5 embryo fibroblasts were added. In other experiments fibroblasts were obtained from the various tissues to be assayed and then co-cultivated with uninfected embryo feeder cells. Single cell suspensions from spleens and thymuses were prepared and co-cultured with embryo fibroblasts as reported by Olding, Jensen & Oldstone, 1975. Growth medium was changed twice weekly, non-adhering cells were returned and cultures assayed daily for the appearance of c.p.e. Once c.p.e. occurred, the virus was identified as being MCMV by immunofluorescence and neutralization tests as previously described (Olding, Jensen & Oldstone, 1975). Polyoma virus was purified according to the methods of Crawford & Winacour (Crawford, 1969).

Immunological studies. The presence of antibodies to MCMV in serum or plasma of mice was tested by indirect immunofluorescence, using infected mouse embryo fibroblasts, fixed with ether-alcohol, as targets, and rabbit antibody to mouse Ig conjugated to fluorescein isothiocyanate as described (Oldstone & Dixon, 1969; Olding et al. 1975). In addition, antibody binding to surfaces of living culture cells previously infected with MCMV at an m.o.i. of 1 was determined by indirect immunofluorescence (Oldstone & Dixon, 1971a).

Antinuclear antibodies (ANA) were detected by incubating decomplemented plasma on acetone-fixed sections of normal mouse kidney (Tonietti, Oldstone & Dixon, 1970). Plasmas were tested undiluted, diluted 1:5 and twofold thereafter until the end point dilution was reached. In selected plasma samples we tested for antibodies to double-stranded and single-stranded DNA and for antibodies to soluble nuclear protein (sNP) using radio-immune assay (Dixon et al. 1971); Oldstone & Dixon, 1972; Robitaille & Tan, 1973). Antibodies to two other nuclear antigens, the Sm antigen and ribonuclear protein (RNP) were assayed with passive haemagglutination (Northway & Tan, 1972). The Sm antigen was previously characterized as a non-histone nuclear protein devoid of nucleic acids (Northway & Tan, 1972). Tests for anti red blood cell antibodies were done by the direct and indirect Coombs tests (Tonietti et al. 1970).

We evaluated immune complex deposition in mice infected in utero or at birth with MCMV and sacrificed at varying times after infection. Their kidney tissues were snap frozen in liquid nitrogen after which 4 μm sections were cut in a cryostat, fixed in ether-alcohol and examined for host IgG, C3 (third component of complement), albumin and fibrinogen by direct immunofluorescence using monospecific antibodies conjugated to FITC. Details of preparation and handling of tissues and staining procedure used have been published.
(Oldstone & Dixon, 1969). Sections were also stained with a monospecific rabbit antibody to MCMV conjugated to FITC which had been previously absorbed with both normal mouse kidney tissues and cultured normal mouse embryo cells. Kidneys from the MCMV-infected mice showing moderate to heavy glomerular deposits of IgG were pooled, and homogenized. The IgG was eluted with glycine HCl buffer, 0.02 M, pH 2.8 (Oldstone & Dixon, 1969; Oldstone, Del Villano & Dixon, 1976) and analysed by using radial immunodiffusion assay (Mancini, Carbonara & Heremans, 1965). Using similar techniques, IgG could be eluted from the glomeruli of mice persistently infected with LCMV and analysed. Immunoglobulins from the sera or plasmas of these or similar mice were purified by Pevikon gel electrophoresis (Müller-Eberhard, 1960).

In order to assay their sp. act., immunoglobulins eluted from the renal glomeruli or obtained from sera or plasmas were concentrated to 500 μg/ml of MEM and absorbed with either (a) uninfected, non-disrupted or disrupted (by freezing and thawing 3 times or sonication) cultured mouse embryo cells, (b) MCMV infected, non-disrupted and disrupted cultured mouse embryo cells, (c) LCMV infected, non-disrupted and disrupted cultured mouse embryo cells, (d) purified MCMV, and (e) purified polyoma virus. In all instances fractions to be tested were absorbed by various tissue or virus preparations at 37 °C for 30 min and at 4 °C for 30 min. The sediment formed by centrifuging was removed and the supernatant fluid tested for concentration of IgG by radial immunodiffusion assay.

**Histopathological studies.** Tissues from brain, thymus, spleen, lymph nodes, liver, heart, salivary gland, lung and muscle were fixed in Bouin's solution, embedded in paraffin, fixed by routine histological techniques and stained with haematoxylin and eosin, and periodic acid-Schiffs.

**RESULTS**

**Response to MCMV infection**

We studied over 25 male and 25 female mice of each strain infected in utero or at birth with MCMV. Usually, more than 75 % of the mice infected in utero survived the first month of life. In contrast the survival rate for mice infected at birth was significantly lower and differed among the various strains: 50 to 55 % C3H/St, BALB/c St and Ha/ICR mice survived the first month, while only 32 % of NZW mice, 12 % of C57 Br/cdJ mice and 10 % of C3H/HeJ mice survived. Uniformly, all nude mice infected in utero or at birth died within the first 2 to 3 weeks of life.

At sacrifice, moribund mice had widespread viraemia and intranuclear virus inclusion bodies in several tissues (Fig. 1 to 3). In addition, necrosis and inflammatory exudate occurred in most tissues and was especially prominent in the liver. Blood-tinged ascitic fluids were also found. In contrast, the tissues of surviving mice infected in utero or at birth had no viral inclusions and no significant inflammatory changes when sacrificed at one month of age or later.

We looked for antibodies to MCMV in the circulations of 10 or more MCMV infected mice of each strain sacrificed at 2 to 3 months of age. Specific antibodies to MCMV were present in the circulations of all tested C3H/St and C3H/HeJ mice, and in more than 75 % of Ha/ICR and SWR/J, and in 60 % of C57 Br/cdJ mice. Titres of antibody to MCMV ranged from 1:8 to 1:64. In contrast, MCMV antibodies were not found in 10 uninfected matched controls from each strain. Owing to the occurrence of ANA in some mice, we had to absorb decomplemented plasma with nuclear protein prepared from calf thymus nuclei (Tan, 1967) to remove the ANA in order to see the intranuclear virus inclusions of MCMV-infected target cells. Nuclear protein was added to a final concentration of 500 μg/ml and the precipitate
Fig. 1. Histological section of liver tissue taken from a C3H/St mouse infected with MCMV at birth and sacrificed when moribund 3 weeks later. Virus inclusions (arrows) and inflammatory exudate are seen. PAS stain, magnification × 300. Surviving mice sacrificed at 6 to 12 weeks of age showed no detectable MCMV genome in liver tissue by hybridization or co-culture studies (see Table 1, 2).

Fig. 2. Histological section of heart tissue taken from a C3H/HeJ mouse infected with MCMV at birth and dying at 2 weeks of age. Presence of intranuclear virus inclusions (arrows) and necrosis in myocardial fibres is seen. PAS stain, magnification × 300.

Fig. 3. Histological section of kidney tissue taken from a nu/nu mouse (off BALB/c cross) infected with MCMV at birth and dying at 10 days of age. Presence of virus inclusion (arrow) and hyperplasia of cells within the renal glomerulus is seen. H and E stain, magnification × 300.
Fig. 4. Demonstration of MCMV antigens on the surface of infected murine fibroblasts. Embryo fibroblasts infected with MCMV were stained with a monospecific rabbit antibody to MCMV conjugated with fluorescein isothiocyanate. Uninfected fibroblasts were not stained with this reagent while fluorescein conjugated rabbit antibody to LCMV or VSV failed to stain the infected cell preparation.

Table 1. Recovery of MCMV (murine cytomegalovirus) from viable spleen cells of mice 2 to 5 months old infected in utero or at birth

<table>
<thead>
<tr>
<th>Expt. no.</th>
<th>Strain</th>
<th>Tissue</th>
<th>Treatment</th>
<th>MCMV recovered after co-culture with embryo fibroblasts from*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C3H/HeJ</td>
<td>Kidney</td>
<td>None</td>
<td>C3H/HeJ (H-2 kk) o/4† BALB/c St (H-2 dd) o/4 SWR/J (H-2 qq) o/4</td>
</tr>
<tr>
<td>1</td>
<td>C3H/HeJ</td>
<td>Liver</td>
<td>None</td>
<td>o/4 o/4 o/4</td>
</tr>
<tr>
<td>1</td>
<td>C3H/HeJ</td>
<td>Spleen</td>
<td>None</td>
<td>o/4 o/4 3/4</td>
</tr>
<tr>
<td>1</td>
<td>C3H/HeJ</td>
<td>Spleen</td>
<td>Sonicated</td>
<td>o/4 o/4 o/4</td>
</tr>
<tr>
<td>1</td>
<td>C3H/HeJ</td>
<td>Spleen</td>
<td>F x T †</td>
<td>o/4 o/4 o/4</td>
</tr>
<tr>
<td>1</td>
<td>C3H/HeJ</td>
<td>Thymus</td>
<td>None</td>
<td>o/4 o/4 o/4</td>
</tr>
<tr>
<td>2</td>
<td>SWR/J</td>
<td>Kidney</td>
<td>None</td>
<td>o/4 o/4 o/4</td>
</tr>
<tr>
<td>2</td>
<td>SWR/J</td>
<td>Spleen</td>
<td>None</td>
<td>4/4 4/4 o/4</td>
</tr>
<tr>
<td>2</td>
<td>SWR/J</td>
<td>Thymus</td>
<td>None</td>
<td>0/4 0/4 o/4</td>
</tr>
<tr>
<td>2</td>
<td>SWR/J</td>
<td>Sera</td>
<td>None</td>
<td>Not done o/4 o/4</td>
</tr>
<tr>
<td>2</td>
<td>SWR/J</td>
<td>Urine</td>
<td>None</td>
<td>Not done 0/4 o/4</td>
</tr>
</tbody>
</table>

* Virus identified as MCMV by immunofluorescence and neutralization assays.
† Number of cultures from which MCMV was recovered over total number of cultures used. Similar results were obtained from six additional experiments.
‡ F x T: frozen and thawed.

formed removed by centrifuging at 1000 g for 20 min prior to testing the supernatant fluid for antibodies to MCMV. In addition sera tested from over 20 mice having antibodies to intranuclear virus inclusions of MCMV also contained antibodies to MCMV expressed on the surface of fibroblast cells infected with MCMV (Fig. 4). Sera not containing antibodies to intranuclear inclusions similarly did not contain antibodies to MCMV cell surface antigens.
In less than 25% of adult mice surviving infections with MCMV in utero or at birth could we recover free infectious virus by standard co-cultivation techniques on syngeneic cells. In all these animals virus had been recovered commonly from the salivary glands but rarely from spleen cells (less than 5% of mice). These mice chronically shed virus and were not included in further studies reported here. In the remaining 70% of infected mice we could find no evidence of the shedding of infectious virus by co-culture of brain, thymus, liver, kidney, salivary glands, plasma or urine on either syngeneic or allogeneic mouse embryo fibroblast cells. However, when spleen cells from virus non-shedders were co-cultured with allogeneic mouse embryo feeder cells, MCMV was recovered in 8 out of 8 experiments using individual mice. Table 1 shows the recovery of virus with allogeneic co-cultivation and the absence of MCMV in the kidneys, livers, thymuses, sera or urine of these mice in two such experiments. We saw no virus-induced c.p.e. in non-splenic tissues co-cultured with either syngeneic or allogeneic feeder cells even after 8 weeks. In cultures with c.p.e., the initiating agent was identified as MCMV by both direct immunofluorescence and neutralization tests (Fig. 5). MCMV was recovered only from viable spleen cells whereas spleen cells either sonicated or freeze-thawed yielded no virus. The repeated inability to detect infectious virus from non-viable spleen cells removed from MCMV infected mice and co-cultured with feeder cells, together with the ease of recovery MCMV from viable spleen cells of the same mice under the same conditions, indicates that the virus was present but hidden in a latent form.
Table 2. Demonstration of MCMV-DNA in tissues by hybridization and recovery of infectious MCMV from tissues of adult mice infected in utero or at birth with MCMV*

<table>
<thead>
<tr>
<th>Strain</th>
<th>Inoculated with MCMV</th>
<th>Age (weeks)</th>
<th>Tissue</th>
<th>Cot† of found in Virus DNA</th>
<th>Viral DNA found in hybrid form‡ (%)</th>
<th>Virus genomes per 100 cells</th>
<th>MCMV recovery in tissue culture§</th>
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<tbody>
<tr>
<td>C3H/St</td>
<td>Yes</td>
<td>7</td>
<td>Thymus</td>
<td>3880</td>
<td>0</td>
<td>0/4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>7</td>
<td>Spleen</td>
<td>5046</td>
<td>0</td>
<td>4/6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>7</td>
<td>Liver</td>
<td>4120</td>
<td>0</td>
<td>0/4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>7</td>
<td>Kidney</td>
<td>3260</td>
<td>0</td>
<td>0/4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>7</td>
<td>Salivary gland</td>
<td>3010</td>
<td>4</td>
<td>2/4</td>
<td></td>
</tr>
<tr>
<td>C3H/St</td>
<td>Yes</td>
<td>12</td>
<td>Thymus</td>
<td>4960</td>
<td>0</td>
<td>0/4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>12</td>
<td>Spleen</td>
<td>3986</td>
<td>10</td>
<td>4/4</td>
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<tr>
<td></td>
<td>Yes</td>
<td>12</td>
<td>Liver</td>
<td>3870</td>
<td>0</td>
<td>0/4</td>
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<tr>
<td></td>
<td>Yes</td>
<td>12</td>
<td>Kidney</td>
<td>3050</td>
<td>0</td>
<td>0/4</td>
<td></td>
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<tr>
<td></td>
<td>Yes</td>
<td>12</td>
<td>Brain</td>
<td>2680</td>
<td>0</td>
<td>0/4</td>
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<tr>
<td>C3H/St</td>
<td>No</td>
<td>6</td>
<td>Spleen</td>
<td>3820</td>
<td>0</td>
<td>Not done</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>6</td>
<td>Liver</td>
<td>3340</td>
<td>0</td>
<td>Not done</td>
<td></td>
</tr>
<tr>
<td>BALB/cSt</td>
<td>MCMV infected embryo fibroblasts in tissue culture</td>
<td>2.90</td>
<td>51</td>
<td>10⁵</td>
<td>4/4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Mice infected within 24 h of birth with 10⁰ TCD₅₀ of MCMV were sacrificed when 7 or 12 weeks old. Tissues from 4 to 6 mice were pooled.
† Cot is the product of the initial DNA concentration in mol/l and the time of incubation in seconds. Cot values expressed here are equivalent units to reactions performed in 0.12 M-phosphate buffer. The reactions were actually performed in 0.48 M-phosphate buffer at 65°C.
‡ Percentage of virus DNA was obtained by subtracting the amount of self-reaction of the labelled virus DNA when incubated with normal mouse DNA at an equivalent concentration from the extent of the reactions for each tissue. In these experiments the virus DNA showed 99% reassociation under the same incubation conditions while the number of virus copies present in the DNA solution was determined by hybridization kinetics and the number of cellular DNA equivalents represented as measured by E₂₆₀.
§ Co-culture of tissues with allogeneic (BALB/c) embryo cells. Ratio represents the number of cultures from which MCMV was recovered over total number of cultures observed.

Identification of the MCMV genome in tissues

Groups of C3H/St and BALB/cSt mice infected in utero or at birth with MCMV were sacrificed at either 7 weeks or 12 weeks of age. Various tissues were harvested and assayed for presence or absence of MCMV DNA. Table 2 shows that virus DNA was detected only in the spleens and salivary glands but not in the brains, thymuses, livers or kidneys. In both experimental groups 8 to 10% of the labelled MCMV DNA hybridized with host cell DNA extracted from the spleen pool. In contrast in only one group did the DNA extracted from the salivary gland pool hybridize to MCMV DNA (4% hybridization). In control experiments to check the reactivity of the MCMV probe, over 50% (51 to 53%) of the labelled MCMV DNA hybridized with DNA extracted from infected mouse embryo cultured cells by a Cot of 2.9. The number of virus genomes per 100 host cells present in tissues from mice infected with MCMV amounted to 3 to 4 for the spleen, 2 for the salivary gland, and 10⁵ for MCMV-infected cultured embryo cells. These figures are calculated by determining the second-order rate constants for the reactions and comparing the determined Cot with the Cot of the mouse single copy nucleotide sequences as described by Britten & Kohne (Kohne, 1968). In no instance was MCMV DNA found in tissues from uninfected mice. In parallel studies to recover infectious virus from tissues of mice used for DNA...
Pathogenesis of MCMV infection

Fig. 6. Renal glomerulus from a 6-week-old C3H/St mouse infected with MCMV at birth. Preparation was stained with fluorescein-conjugated rabbit antiserum to mouse IgG. IgG is deposited predominantly in the mesangial areas and to a lesser part along the peripheral walls of glomerular capillaries (see Tables 3 and 4).

hybridization study, we were able in all instances to activate and recover MCMV from spleen cells following allogeneic co-cultivation with mouse embryo cells but not from salivary gland, kidney, liver, thymus or brain tissues. MCMV was not recovered from spleen cells following co-cultivation with syngeneic feeder cells.

Immune complex deposits in MCMV infected mice

Mice infected with MCMV in utero or at birth showed significant deposits of host IgG and C3 restricted to the renal glomeruli. These deposits were usually of moderate amounts, occurred in a discontinuous granular pattern predominantly in the mesangial area and to a lesser degree along glomerular capillary basement walls (Fig. 6). Deposits of MCMV antigens were also found in similar loci. There were no deposits of fibrinogen or albumin in the renal glomeruli indicating that the Ig deposited was not due to non-specific trapping of serum proteins.

Table 3 shows the incidence of glomerular deposits of IgG and C3 in different murine strains and at different ages. As can be seen, few mice had IgG deposits during the first 2 weeks of age. By 1 to 3 months of age, nearly all C3H/St, C3H/HeJ, C57Br/cdJ and Ha/ICR infected mice had deposits, while most uninfected mice of the same age failed to show deposits. From 3 to 6 µg of IgG per kidney could be eluted from the renal glomeruli of all 1- to 3-month-old infected mice.

To determine the specificity and concentration of the IgG deposited in renal glomeruli of infected mice, we eluted and tested the recovered IgG for immunospecific binding to MCMV, LCMV and polyoma virus targets. As shown in Table 4, 33 % of the total IgG
Table 3. Renal glomerular deposits of host IgG and C3 in mice infected with MCMV in utero or at birth

<table>
<thead>
<tr>
<th>Strain</th>
<th>Infection</th>
<th>2 weeks</th>
<th>6 to 8 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>C3H/St</td>
<td>MCMV infected</td>
<td>0/14*</td>
<td>14/14</td>
</tr>
<tr>
<td>C3H/St</td>
<td>Not infected</td>
<td>ND†</td>
<td>0/10</td>
</tr>
<tr>
<td>C3H/HeJ</td>
<td>MCMV infected</td>
<td>4/6</td>
<td>11/11</td>
</tr>
<tr>
<td>C3H/HeJ</td>
<td>Not infected</td>
<td>0/5</td>
<td>1/20</td>
</tr>
<tr>
<td>C57 Br/cdJ</td>
<td>MCMV infected</td>
<td>ND</td>
<td>9/13</td>
</tr>
<tr>
<td>C57 Br/cdJ</td>
<td>Not infected</td>
<td>0/14</td>
<td>15/16</td>
</tr>
<tr>
<td>Ha/ICR</td>
<td>MCMV infected</td>
<td>0/6</td>
<td>2/12</td>
</tr>
<tr>
<td>Ha/ICR</td>
<td>Not infected</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

* Number of mice having IgG and C3 deposits over total number of mice studied.
† Not done.

Table 4. Specificity of IgG eluted from glomeruli of adult mice showing immune complex deposits*

<table>
<thead>
<tr>
<th>Ig eluted from mice infected with</th>
<th>Viable embryo culture cells†</th>
<th>Disrupted embryo culture cells†</th>
<th>Virus preparations</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCMV</td>
<td>MCMV infected 33‡, 7</td>
<td>MCMV infected 60, 14</td>
<td>MCMV 43, ND 5, ND</td>
</tr>
<tr>
<td>LCMV</td>
<td>5, ND§</td>
<td>12, ND</td>
<td></td>
</tr>
</tbody>
</table>

* Kidneys from mice 1 to 3 months old having moderate to heavy deposits of Ig along the glomerular basement membrane and in the mesangia were pooled, IgG eluted by treatment with 0.02 M-glycine-HCl, pH 2.8, buffer and analysed by radioimmune agar analysis. Mice were infected in utero or at birth with MCMV or at birth with LCMV.
† BALB/c/St mouse embryo cells used for absorption. Cells were disrupted by freezing and thawing 3 times.
‡ Percentage of the total IgG that was removed by absorption.
§ ND: not done.

recovered was absorbed with viable MCMV-infected mouse embryo cells, but in contrast only 7% was absorbed with uninfected mouse embryo cells, a specific absorption of 26%. Further specificity for MCMV was shown since purified MCMV absorbed 43% of the total IgG eluted from the glomeruli of infected mice but purified polyoma virus did not absorb significant amounts of IgG. In addition, IgG eluted from mice persistently infected with LCMV was not absorbed by MCMV infected cells. When IgG purified from the sera of adult mice which had been infected in utero or at birth was absorbed with MCMV-infected cells, absorption was 8% compared to 26% with IgG from the renal eluate of such infected mice, indicating a concentration of virus-specific antibody in the renal glomeruli. Similar results occurred when MCMV infected cells were disrupted and used as an immunoabsorbant.

Autoimmune manifestations in MCMV infected mice

We assayed several strains of mice infected with MCMV in utero and at birth for the presence of ANA and anti red blood cell antibody. ANA titres were significantly increased only in the C57Br/cdJ mice. C3H/St, C57Bl/6, BALB/c/St, NZW or Ha/ICR mice had no such increases when compared to control uninfected mice. To categorize these antibody responses plasma from 18 C57Br/cdJ mice having the highest ANA titres (mean of 1:256
Pathogenesis of MCMV infection

Table 5. Autoimmune responses in 6- to 9-month adult C57 Br/cdJ mice infected in utero or at birth with MCMV*

<table>
<thead>
<tr>
<th>Group</th>
<th>ANA†</th>
<th>DNA‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% positive</td>
<td>titre</td>
</tr>
<tr>
<td>MCMV infected</td>
<td>70</td>
<td>1:256</td>
</tr>
<tr>
<td>Uninfected</td>
<td>20</td>
<td>&lt; 1:5</td>
</tr>
</tbody>
</table>

* Plasma from 18 C57Br/cdJ-infected mice were assayed as were the plasma from 15 age and sex matched controls. Presence of antibodies to red blood cells was assayed by both direct and indirect Coombs test.
† ANA: antinuclear antibody titre number represents the last dilution of plasma showing positivity.
‡ Values are given in μg DNA bound/ml of plasma. DS, double-stranded DNA; SS, single-stranded DNA; Neg, negative.
§ sNP value was done at a 1:10 dilution of plasma. After the addition of DNA, the sNP binding was unchanged when compared to binding in the absence of DNA. Numbers represent the % of radiolabelled counts of sNP precipitated.

with a range of 1:128 to 1:1280) were pooled and assayed for antibodies to DNA, sNP, SM and RNP. We found evidence of antibodies to single-stranded DNA and sNP, but no evidence of antibody(s) to double-stranded DNA or RNP (Table 5). We were unable to find enhanced anti red blood cell antibody responses in any of the mouse strains infected with MCMV.

DISCUSSION

Infecting mice of several strains with MCMV in utero or at birth produced three different models. In the first model, mice died within 1 month after virus challenge and showed evidence of cytomegalovirus inclusions, cellular injury and necrosis in multiple tissues of the body including thymus, lymph nodes, heart, liver, lung, brain and kidney. Strain variations were most clearly seen in mice infected at birth; over 50% of C3H/St, BALB/cSt and Ha/ICR survived, while in contrast less than 12% of the C57Br/cdJ and C3H/HeJ mice lived longer than 4 weeks. The most susceptible animals were the nude mice which uniformly died within the first 2 to 3 weeks following in utero or at birth infection. At necropsy, tissue injury seen under the light microscope was similar to that reported by others (McCordock & Smith, 1936; Ruebner et al. 1966; Schwartz, Daniels & Klintworth, 1975). Recently Selgrade & Osborn (1974) noted different susceptibilities among strains of adult mice infected with mouse-passed MCMV virus Other studies to be reported later showed that adult nude mice (nu/nu) were significantly more susceptible to i.p. challenge with mouse tissue passed MCMV than their nu/+ litter mates but in general died several days later than did the nu/+ mice.

The second model consisted of approx. 25% of those mice which survived infection in utero or at birth. This group was characterized by chronic infection with virus and an apparent continuous shedding of MCMV from the salivary gland. The third model comprised the other 75% of survivors, and was characterized by latent virus infection. In the animals of these latter two groups, MCMV infection was associated with immune complex deposits of MCMV and antibody to MCMV in all strains, and enhanced ANA responses in one strain.

The group of mice with latent infections was studied in depth. Infectious virus was not recovered by co-cultivation of numerous fluids or tissues with syngeneic feeder cells. In
contrast, virus was readily recovered from spleen cells of MCMV infected mice co-cultivated with allogeneic feeder cells. The recovery of virus from actively dividing spleen cells following allogeneic stimulation is similar to our previous recovery of latent MCMV from spleen cells, principally the B cell enriched population. Latent virus was activated by allogeneic stimulation or incubation with a B cell mitogen, lipopolysaccharide (Olding et al. 1975).

Hybridization studies with an MCMV DNA probe showed that the MCMV virus genome was present in spleen cells, and to a lesser extent in salivary gland tissue of the latently infected, surviving mice. Recently, virus genome was detected in testicular tissue (D. J. Kingsbury & M. B. A. Oldstone, unpublished observations). However, virus gene copies were not detected in thymuses, brains, livers or kidneys of the same animals. The inability to find the MCMV genome in these sites by using hybridization complements our inability to detect infectious virus in the same tissues by syngeneic or allogeneic co-cultivations. Hence, in this model system infectious virus could be activated from virus products found in spleen or salivary gland cells and not in thymus, liver, kidney or brain. Similarly, no histopathological evidence of cytomegalovirus infection was found in these tissues. Why virus is restricted to certain tissues after an apparent initial generalized infection is not clear, nor is the mechanism by which tissues such as thymus, liver, kidney and brain can clearly limit MCMV's occupancy. Among the possibilities are that the MCMV genome was present in these tissues and not detected at the sensitivity of our assay (2 virus DNA copies per 100 cells), that in animals surviving infection instituted in utero or at birth, thymus, liver, kidney and brain tissues were never infected, or that infections initiated in these tissues were effectively cleared and eliminated by the host. These possibilities are now being evaluated.

Despite our inability to detect the virus genome in kidneys, virus antigen was found complexed to host Ig and C3 as a virus-antibody immune complex in the renal glomeruli. Presumably virus is activated or shed in minimal amounts from infected tissues and initiates a continual anti-MCMV humoral response. Antibody is then able to complex with virus or virus antigens in the circulation and deposit in the renal glomeruli. The evidence for immune complex deposits in the MCMV model is (1) the immunopathological picture of granular deposits of host Ig, C3 and virus antigen in the mesangia and along the glomerular basement membranes which is pathognomonic of immune complex deposits, (2) elution of specific antibody to MCMV from the renal glomeruli, and (3) concentrated, highly specific anti MCMV antibody activity found (by absorption studies) in the renal glomeruli as compared to that found in plasma. However, the amount of Ig deposited was small, 3 to 6 μg/kidney, and in the range found in kidneys of mice persistently infected with LDV but 10-fold less than seen in mice with LCMV persistent infection and approx. 50-fold less than in (New Zealand Black × New Zealand White) F1 mice with autoimmune disease (Oldstone & Dixon, 1971 b). Like mice persistently infected with LDV, MCMV infected mice showed minimal evidence of glomerulonephritis by light microscopy. Other persistent virus infections in both man and animals have been associated with immune complex deposits. Although most of these contain RNA budding viruses, non-budding RNA viruses and DNA viruses also participate in immune complex formation (reviewed Oldstone, 1975).

Cytomegalovirus infection in man has been associated with enhanced ANA responses (Kantor, Goldbey & Johnson, 1970). We noted that one of five mouse strains studied had a significantly enhanced ANA response. These (C57 Br/cdJ) animals also had enhanced responses to single stranded DNA and to sNP. This observation, along with accumulated evidence from other systems (Tonietti, Oldstone & Dixon, 1970; Cannat & Varet, 1972; Croker et al. 1974), suggests that multiple agents may be capable of triggering autoimmune responses in the genetically appropriate host. In contrast to enhanced ANA responses
associated with MCMV infection and in agreement with past studies, MCMV infection did not alter the anti red blood cell response (Tonietti et al. 1970). The present studies demonstrate that MCMV is a useful model for investigating not only analogous human infection but also general problems of latency and tissue tropism in virus infections. In addition, the variations in susceptibility among mouse strains may reflect the different genetic abilities of various hosts in responding to virus infection.

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