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Characterization of a novel rat cytomegalovirus (RCMV) infecting placenta-uterus of *Rattus rattus diardii*

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Summary. A new rat cytomegalovirus (RCMV) isolated from the placenta/uterus of a house rat (*Rattus rattus diardii*) was found to productively infect rat embryo fibroblast (REF) cells. The virus produced typical herpesvirus-like cytopathic effects characterized by a lytic infection. The well-known herpesvirus morphology was confirmed by electron microscopy. Its slow growth in cell culture indicated that the virus is belonging to subfamily *Betaherpesvirinae*. Electron microscopy techniques and immunohistochemistry confirmed the presence of herpesviral inclusion bodies and virus related particles in the cytoplasm and nucleus of infected cells. Hyperimmune serum against the Maastricht strain of RCMV revealed the virus identity in neutralization test, immunoperoxidase and immunofluorescence techniques. Despite typical characteristics of CMV, the viral genome is significantly different from that of Maastricht, English, UPM/Sg and UPM/Kn strains. The dissimilarities, which have not been reported before, had been confirmed by mean of restriction endonuclease analysis. The new RCMV strain, a virus that infects placenta and uterus of rats, has been named as ALL-03.

Introduction

Cytomegaloviruses (CMVs) are species-specific and belong to subfamily *Betaherpesvirinae* of the *Herpesviridae* family. They cause acute, latent, and persisting infections in human and animals. Infection in immunocompromised patients can be very severe and sometimes fatal. Congenital CMV infection is a leading cause of mental retardation and other birth defects in human.

Various nonhuman CMVs have been discovered [7]; most studies have been carried out using the Mouse cytomegaloviruses (MCMV) or Guinea pig Cytomegaloviruses (GPCMV) [2, 8]. The association of a CMV-like agent and

CMV inclusion disease with the rat has been known since the 1930s [9, 28]. Judging from the isolation of the virus from wild rats in China [9], Panama [21], The Netherlands [3], and the United Kingdom [20], the virus is probably distributed worldwide. Rabson et al. [21] isolated a CMV-like virus from wild roof rats (*Rattus rattus*) in Panama. In 1982, Maastricht rat cytomegalovirus (RCMV) (Netherlands) and English RCMV were isolated from wild rats (*Rattus norvegicus*) and extensively studied in molecular level [3, 20]. In Malaysia, two novel strains of RCMV UPM/Kn and UPM/Sg, were isolated from kidneys and salivary glands respectively, of rice-field rats (*Rattus argentiventer*). The results of random amplified polymorphic DNA (RAPD) analysis showed that these two isolates were closely related to the English strain but different from the Maastricht strain [10]. Approximately 50 per cent of serum samples of rice-field rats collected throughout the Malaysia peninsular were positive for RCMV tested in enzyme-linked immunosorbent assay (ELISA) [11]. The prevalence and persistency rates of RCMV infection within the rice-field rat populations are thus considered high.

This paper describes *in vitro* properties of CMV-like virus isolated from a house rat (*Rattus rattus diardii*). There always has been a demand for an appropriate animal model to facilitate studies on lethal CMV disease, protection immunity and the condition for establishing persistent and latent infection in the natural host. Apart from Mouse cytomegalovirus (MCMV) and Guinea pig cytomegalovirus (GPCMV), the RCMV-rat system could be an alternative model for CMV infection and disease in human. This system is expected to be highly suitable, since the pathogenesis of infection in CMV-infected humans is predicted to be similar to that in RCMV-infected rats [26]. An animal model developed by the Maastricht strain of RCMV has been used to elucidate the pathogenesis of CMV infections in immunocompromised rat and evaluate the effect of therapeutic intervention studies [26]. Experimental model studies using RCMV also provide the evidences to link CMV infection with grafts rejection after allogeneic lung [27] and renal transplantation in the rat [12]. However, none of those demonstrated a clear involvement of uterus and placenta in vertical transmission of CMV in rat model. The agent was isolated from placenta-uterus, indicating that it has the ability to cross the placenta and infect the fetus, possibly providing an animal model for the study of congenital CMV infection in human. Additionally, molecular characterization of this or other RCMV strains might lead to generation of rat-specific expression vectors driven by the RCMV major immediate-early gene promoter-enhancer [25], and candidate "viral-vectored immunocontraception" for rat control [16].

Materials and methods

Cell culture

Rat embryo fibroblast (REF) cell cultures were grown in Eagle's minimum essential medium (MEM) supplemented with 10 per cent fetal bovine serum (FBS) and maintained in MEM with 1 per cent FBS. The cells were grown at 37 °C in a humidified CO_2 incubator.

Virus isolation and inoculation

The virus was isolated from placenta and uterus of house rat (*Rattus rattus diardii*). The organs were homogenized in MEM. The homogenate was sonicated and centrifuged at 3000 rpm for 10 min. The resultant supernatant was used for inoculation of confluent REF monolayers in 25 cm^2 flasks (Costar). The cells were examined daily for the appearance of a cytopathic effects (CPEs).

Titration of virus by tissue culture infective dose 50 ($TCID_{50}$)

Ten-fold serial dilution of virus sample starting with 10^{-1} to 10^{-9} was made in serum-free MEM. 100 µl of each dilution were inoculated in quadruplicate onto 24-well plates (Costar) containing confluent monolayers. After 45 min incubation at 37 °C, 900 µl of maintenance MEM was added to each well. Once CPE had stopped progressing, the virus titer was estimated by the Reed-Muench formula.

Virus growth curve

Confluent REF monolayers in 25 cm^2 flasks containing approximately 10^6 cells per flask were inoculated with 10^4 $TCID_{50}$/ml virus suspensions and incubated for 1 hr at 37 °C. The excess inoculum was washed in phosphate-buffered saline (PBS) before adding 10 ml maintenance medium and beginning incubation at 37 °C. Each day, 1 ml supernatant of one flask was removed (extracellular infectious virus). The cells were washed and 10 ml of MEM was added. The whole flask was frozen and thawed twice before taking 1 ml of supernatant (cell-associated virus). The infectivity was assayed by $TCID_{50}$ titration.

Electron microscopy

Extracellular virus suspensions were spun at 25,000 rpm for 2 hrs at 4 °C. The resultant pellets were suspended in 1 ml of TNE buffer (10 mM Tris, 200 mM NaCl, 1 mM EDTA, pH 7.4). The suspension was gently layered on 10–60 per cent linear sucrose density gradient (in TNE buffer), and spun at 25,000 rpm for 2.5 hrs at 4 °C. The white opalescent band was carefully aspirated and diluted 1:4 in TNE buffer and then pelleted. The virus pellet was resuspended in 5 µl of TNE buffer and placed onto formvar carbon-coated grids. After 10 min of adsorption, excess fluid was blotted with filter paper. One per cent phosphotungstic acid (PTA), neutralized with NaOH or KOH (pH 6 to 7.4) was added for 5 min. The grid was dried at room temperature in a desiccator.

Following 4 to 7 days post-inoculation, mock- and infected cells were trypsinized with antibiotic-trypsin-Versene (ATV) solution. The cell pellets were fixed in 4 per cent buffered glutaraldehyde and washed thrice with 0.1 M sodium cacodylate buffer. The pellets were trimmed into 1 mm^3 small blocks and then post-fixed in 1 per cent buffered osmium tetroxide. The blocks were dehydrated in a series of acetone solution. The blocks were embedded into beam capsules and then polymerized. The solidified blocks was trimmed with ultramicrotome and mounted on an EM copper grid, being subjected to uranyl acetate and lead citrate staining. The processed grids were examined under Hitachi, H7100 transmission electron microscope (TEM).

Histopathology and immunochemistry

The mock-infected and 2 to 6 days post-infected REF cells grown in chamber slides (Lab-Tek) were washed twice with PBS and fixed in Carnoy's fixative (ethanol:chloroform:acetic acid; 6:3:1) for 20 min.

Hematoxylin and eosin (H&E) staining

The slides were hydrated and stained with Harris's hematoxylin solution for 5 min. Differentiation was carried out by dipping the slides in acid alcohol solution for 3–5 sec. The slides were washed and counterstained with eosin for 1 min. The slides were rinsed twice in distilled water, followed by dehydration and then cleared in two changes of xylene. The processed slides were mounted and examined under light microscope.

Acridine-orange (AO) staining

The slides were hydrated and then rinsed in 2 mM $MgSO_4$, followed by 1 per cent acetic acid for 1 min. The slides were rinsed in 2 mM $MgSO_4$ and then phosphate buffer (67 mM Na_2HPO_4 and KH_2PO_4, pH 6). The slides were stained with acridine orange solution (0.01 per cent) for 3 min and washed with phosphate buffer for 5 min for two changes. Differentiation was carried out by dipping the slides in 0.1 M $CaCl_2$ and 2 mM $MgSO_4$. The slides were washed and then mounted with buffered glycerol (glycerol:PBS; 9:1). The slides were examined under fluorescence microscope.

Indirect immunofluorescence (IIF) test

The slides were incubated with hyperimmune serum (HIS) against the Maastricht RCMV at dilution 1:100 (HIS:PBS) for 1 hr at 37 °C in a dark and humidified atmosphere. The slides were washed with PBS. Goat anti-rat immunoglobulin conjugated with FITC (Sigma) at dilution 1:200 (conjugate:PBS; v/v) was added. After an hr incubation period, the slides were washed, mounted in buffered glycerol and then observed under fluorescence microscope.

Indirect immunoperoxidase (IIP) test

After fixation, endogenous peroxidase and non-specific binding were eliminated by treating the slides with 0.3 per cent H_2O_2 in absolute methanol; followed by blocking buffer (5 per cent bovine serum albumin in PBS; v/v), for 30 min each. HIS against the Maastricht RCMV at dilution 1:100 (HIS:blocking buffer; v/v) was added and incubated for 1 hr at 37 °C. After washing thrice with PBS, goat anti-rat immunoglobulin peroxidase-conjugated (Sigma) at dilution 1:250 (conjugate:PBS; v/v) was added to the slides and incubated for 1 hr at 37 °C. After washing, 3–3′-diamino benzidine hydrochloride substrate solution (KPL) was added and incubated for 10 min. The stain development was stopped by distilled water. The slides were mounted in buffered glycerol and examined under light microscope.

Neutralization test

Heat-inactivated (56 °C for 30 min) HIS against the Maastricht RCMV was used in the assay. Equal volume of constant virus dilution containing approximately 100 $TCID_{50}$ per 0.1 ml was added to each diluted HIS. The virus serum mixtures were mixed well and incubated at 37 °C for 30 min and then inoculated into 24 wells plates (0.1 ml/well) containing monolayers of REF cells. After 1 hr adsorption, the plates were added 0.9 ml/well of maintenance medium. Serum and cell controls were also prepared. The number of doses of virus was evaluated by using the back-titration assay. The plates were checked daily for progression of CPE for 6 to 10 days.

Restriction endonuclease digestion of viral DNA and gel electrophoresis

Extracellular virus was collected from REF cell culture fluid 5 to 10 days after RCMV infection. Viral DNA was extracted as described by Lai et al. [10]. DNA samples were digested by *Hin*d III and *Eco*RI (New England Biolabs Inc.). Fragments were sized on 0.7, 0.8 or

0.9% agarose gels. Standard markers used were lambda mix 19 and GeneRuler^{TM} 1 kb (MBI Fermentas). Polaroid pictures of the gel or enlarged prints were used to estimate fragment sizes by measuring the electrophoretic mobility. A semi-logarithmic plot of molecular size versus the distance of migration of each fragment of the markers was made. Consequently, the molecular size of each fragment of the digested DNA was estimated from the graph.

Results and discussions

Cytopathogenicity

The virus isolated from placenta-uterus, produced typical herpesvirus-like CPEs beginning from 3 days p.i. CPEs developed are distinguishable from the normal REF cells. CPEs consisted of small round foci of enlarged rounded cells, which then gradually increased in diameter to affect the entire cell monolayer, leaving an empty space in the centre. After 6 days p.i., many cells degenerated and started to detach from the flasks (Fig. 1). Infected cells had more cytoplasmic extensions as compared to mock-infected cells. REF cell cultures inoculated with the virus showed CPEs within several days depending on the amount of virus present in inoculum. For inoculation titers of 10^3 to 10^5 $TCID_{50}$/ml, CPEs started to appear in REF monolayer beginning from 3 days p.i. The finding was similar to those of the previous observations whereby the CPEs started to develop at day 2 [20], 66 hrs [15] and day 4 p.i. [4]. The virus spreads from cell to cell causing infection foci manifesting as clear spots in the monolayer. CPEs produced include changes

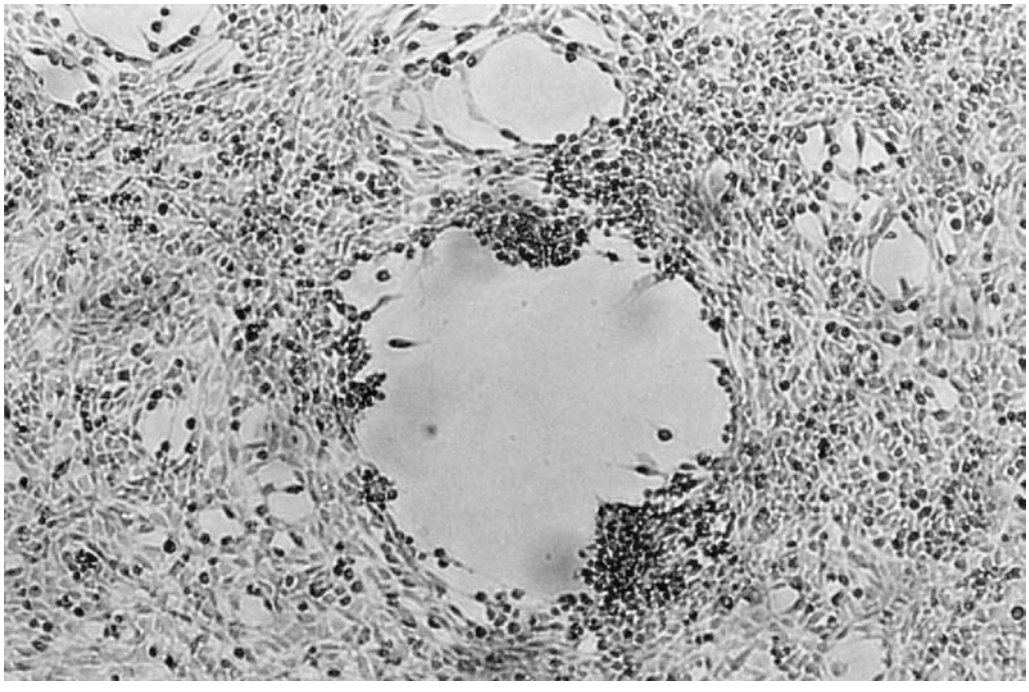


Fig. 1. Focal cytopathic effect of rat agent growing in rat embryo fibroblast (REF) cells, 5 days after inoculation (×400)

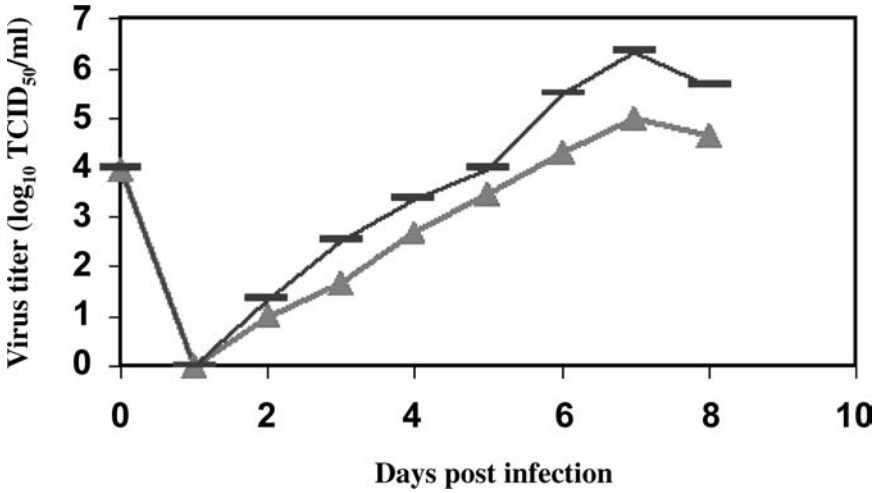


Fig. 2. Growth curve of the rat agent in REF cells. Approximately 10^6 cells per flask were inoculated with 10^4 $TCID_{50}$/ml virus suspensions. The supernatant and freeze-thawed cells were assayed at selected intervals post-infection by $TCID_{50}$ titration. (▲) released virus; (▬) cell associated virus

of shape, size and morphology of the cells, followed by a "halo" production or called plaque before monolayer detached.

Virus growth curve

In REF, maximum titers of intra- and extracellular viruses were at day 7 p.i., at $10^{6.3}$ and 10^5 $TCID_{50}$ per ml; respectively (Fig. 2). Therefore, it is evident that most of the viruses were cell-associated. No virus particles obtained at day 1 p.i. represented the eclipse phase of the virus replication. This virus may have an eclipse period of 24 hr, which was reflected by delayed formation of CPEs. Low virus concentrations prohibit initial infection of all the cells in a culture. Thus, it is difficult to determine the one-step growth curve of the virus; one is generally looking at a series of successive infections. New infectious virions started to be released at day 2 p.i. Titer reached maximum at day 7 p.i. On average, each infected cells produced virus particles of 1.9 $TCID_{50}$. These results showed that the virus isolate exhibited the classic properties of cytomegalovirus, as it caused delayed cytopathology, remained cell-associated, and replicated to comparatively low maximum titers. These observations suggested that the agent recovered was of a herpesvirus under subfamily *Betaherpesvirinae*, the CMV group.

Electron microscopy

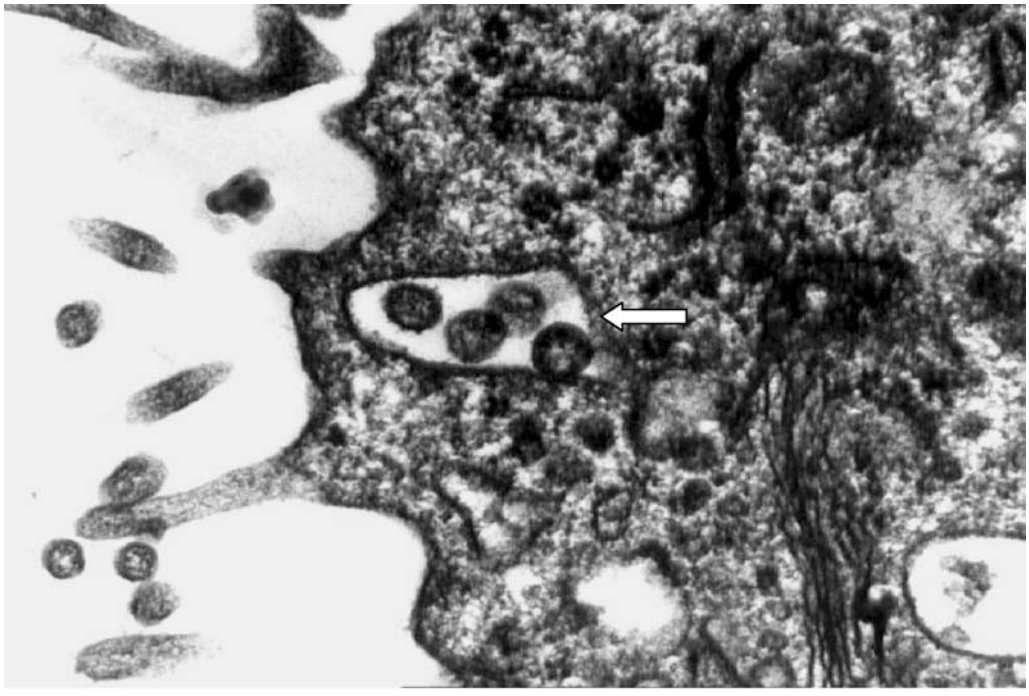
The virions exhibit typical herpesvirus morphology, as they possess an icosahedra capsid of 80 to 94 nm in diameter, which appeared as a hexagonal ring and surrounded by a poorly defined area, the tegument. The structure enclosed by

a lipid bilayer envelope with a variable shape and size to give a final diameter of 138 to 158 nm for the virion.

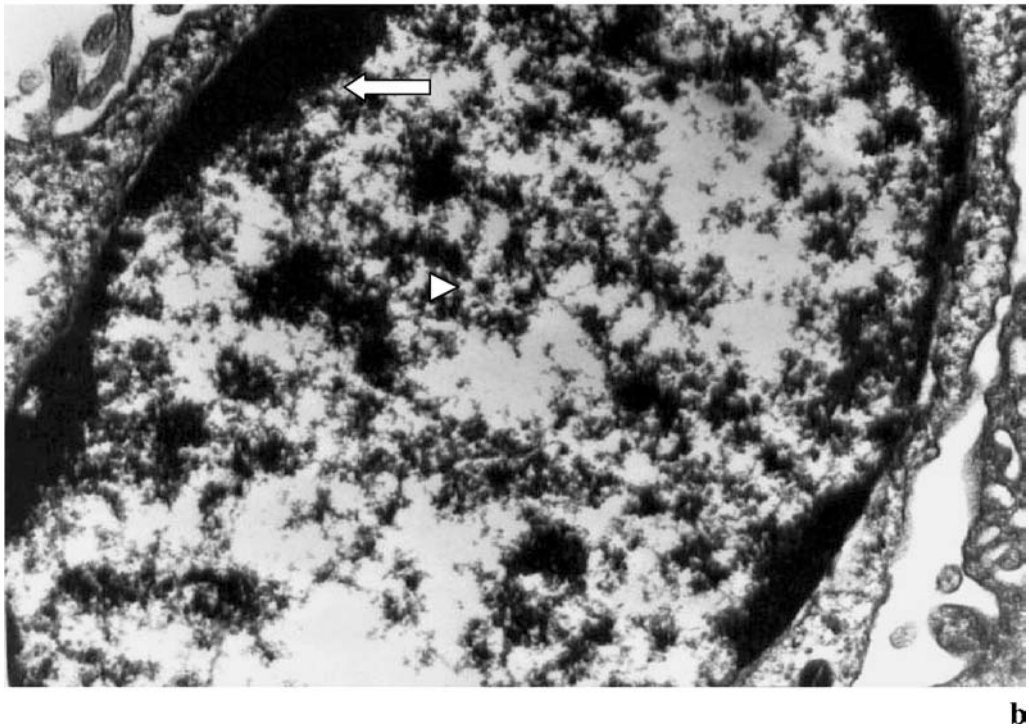
In ultrathin sections, two types of viral particles namely nucleocapsids with electron lucent nucleiods and nucleocapsid with ovoid-shaped electron-dense nucleiods were detected. Majority of the viral particles was found inside the nucleus in 4-day post-infected cells. However, in 7-day post-infected cells, they were mostly present in the cytoplasm. It contained naked particles (nucleocapsids) with diameters ranged from 72 to 94 nm and enveloped particles of 137 nm to 164 nm in range. The nuclear membrane of infected cell is shown with associated virions and nucleocapsids in various stages of envelopment. Egress of virus particle taken place in cytoplasm was also seen. Occasionally infected cells contained intranuclear and large intracytoplasmic inclusion bodies. Besides, chromatin condensation, disintegration of nucleolus, intracytoplasmic tubular aggregates, cytoplasmic vacuoles, spiralled membranous labyrinth network, dense bodies of varying shape and size were evident (Figs. 3a, and 3b). Transmission electron microscopy (TEM) revealed homogenous virus particles, the characteristic of herpesvirus, either in extracellular virus suspension or inside infected cells. Its morphological aspects are similar to that of mouse cytomegalovirus and with rat cytomegalovirus described by Berezesky et al. [1] and Bruggeman et al. [3]. The naked nucleocapsid was from 72 to 94 nm in diameter; slight smaller than that measured by Berezesky et al. [1] but fairly close to the size of RCMV (92 nm) and MCMV (90 nm) as mentioned by Bruggeman et al. [3]. Two types of virus related particles were present: the capsids that have a central core while the other type appeared to be empty or devoid of a core. Similar particles were also observed in other studies [1, 3, 20]. The empty nucleocapsids were immature particles [1]. They were thought to represent non-infectious virions [20]. Maturing by budding through the inner nuclear membrane is a typical feature of herpesviruses [29] although rarely observed in RCMV infections [1], but it was seen in the current preparations.

Ultrastructural examination of infected cells demonstrated that new virions developed in the nucleus. In the nucleus, we found only nucleocapsids. However, both nucleocapsids and virions were evident in the cytoplasm. Envelopment and egress of the virus particles occurred in the cytoplasm. There is aligned the mechanism of virus replication as proposed by Roizman et al. [22, 23]. The nucleocapsids become enveloped while budding through the nuclear membrane, and were transported across the cytoplasm and its organelles before egress through the cell plasma membrane into the extracellular space.

An inclusion body-like structure developed, and its presence in RCMV infected cells has not been reported. Spiralled membranous labyrinth networks associated with virus infection were also observed. Occasionally, inclusion bodies present in the cytoplasmic vacuoles, whereby some virus particles could be seen. These large cytoplasmic inclusion bodies could be precisely confirmed by immunofluorescence staining. The other common feature of cells infected with RCMV was the presence of dense bodies and tubular aggregates. It is impossible to determine these structures by other preparations. The tubular structures have been



a



b

Fig. 3. Electron micrograph of REF cells infected with the rat agent, showing **(a)** numerous nucleocapsids surrounded in a vacuole present in the cytoplasm (arrow) (×60 k) **(b)** margination of chromatin (arrow) and disintegration of nucleolus (arrowhead) (×20 k)

described in guinea pig CMV [6] and the Rabson's isolate of RCMV [1] infected cells. Human CMV however does not produce such structure [18]. The existence of intracellular dense bodies was typical in CMV infected cells. These dense materials may represent lysosome-like structures resulting from the host reaction against the virus. This may explain the phenomenon of attenuation of MCMV derived from tissue cultures [19]. On the other hand the dense bodies contained some virus structural proteins that normally involved in the virus envelopment process [17].

Besides examining the distribution of virus particles, the morphological changes in infected cells were also determined. Similar to previous findings [1], there was no correlation between specific ultrastructural alterations and duration of cellular infection as a result of asynchronous development of RCMV *in vitro* and the enlargement of infected foci progressing over a period of many days.

Histopathology

In hematoxylin and eosin (H&E) stained infected cells, characteristic enlarged, swollen cells were evident with eosinophilic and basophilic cytoplasmic and intranuclear inclusion bodies. Besides, intracytoplasmic vacuole, multinucleated cells with intranuclear inclusions, cytopathic lesions, irregular shape of nuclear

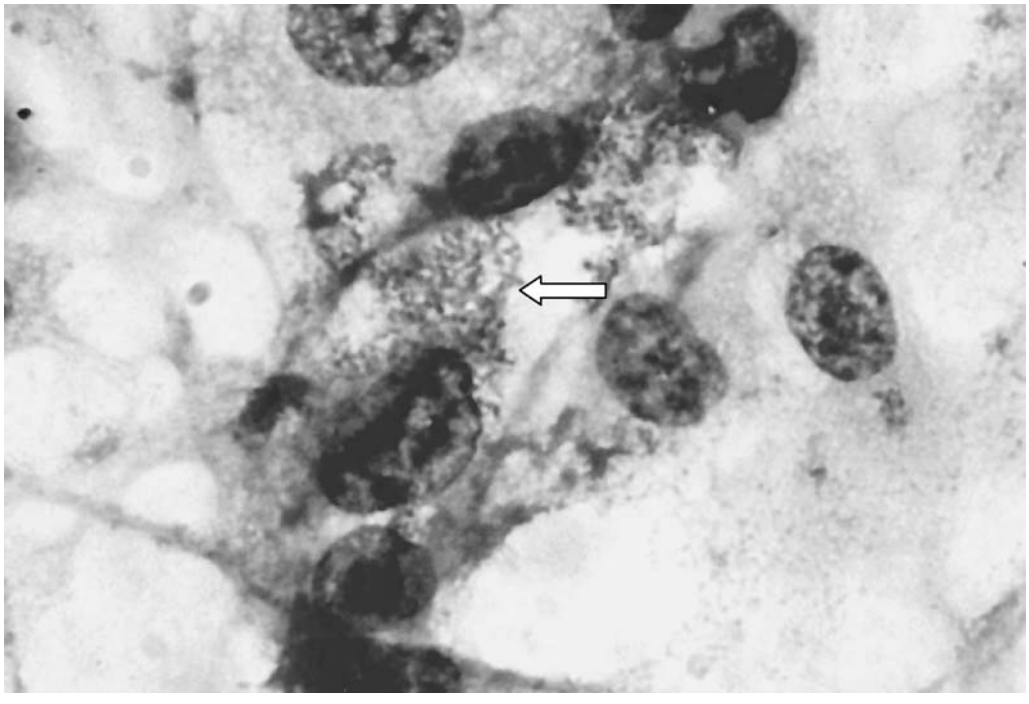


Fig. 4. REF cells stained in hematoxylin and eosin after 3 days post-infection with rat agent showing multiple tiny eosinophilic inclusion bodies present in the nuclei and cytoplasm (arrow) (×1 k)

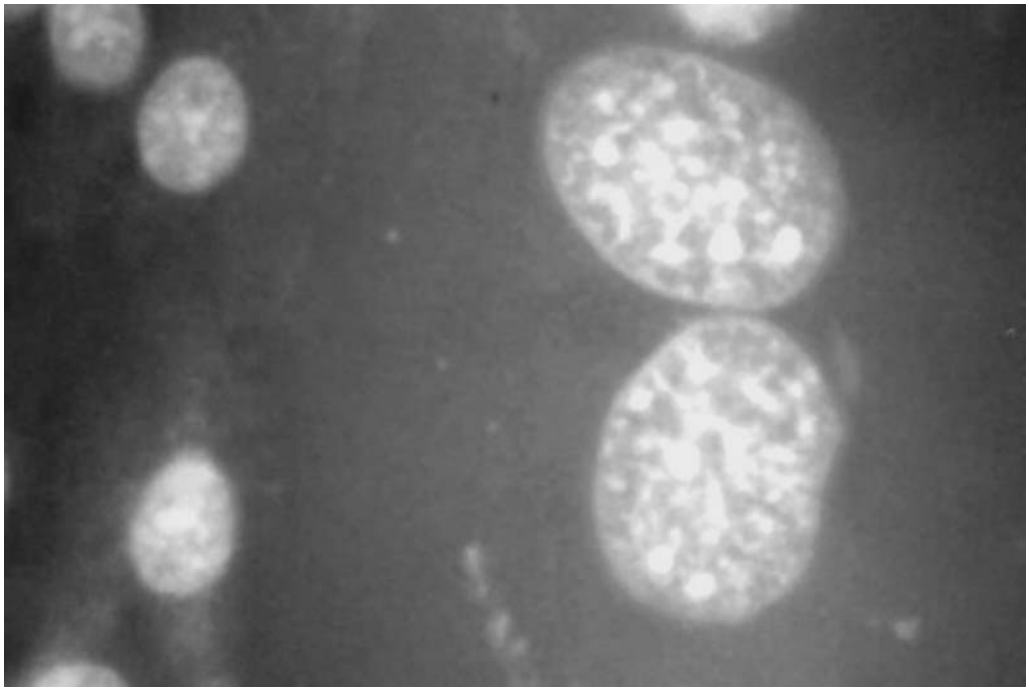


Fig. 5. REF cells stained in acridine-orange after 4 days post-infection with rat agent showing enlarged and swollen nuclei (× 1 k)

membrane, and chromatin margination along the nuclear membrane were also observed as opposed to the mock-infected cells (Fig. 4). Upon acridine-orange (AO) staining, chromatin in infected cells stained as fluorescence greenish yellow marginated along the nuclear membrane and some are condensed. In contrast, the chromatin in mock-infected cells was randomly arranged. There was a clear evidence of enlarged and swollen nuclei present in infected REF cells (Fig. 5). The infected cells showed a strong orange-red fluorescence cytoplasm, in contrast to mock-infected cell cytoplasm, which showed a slightly yellow-orange fluorescence. Conforming to the ultrastructural studies, the formation of cytoplasmic vacuoles, inclusion bodies and the margination of chromatin-like material were also evident in the H&E- and AO-stained cultures, as opposed to the mock-infected cells. As the infection progressed, the infected cells became rounded and enlarged with increases in both nuclear and cytoplasmic volume. This feature was better demonstrated in H&E and AO preparations than in EM samples. In H&E stained infected cell cultures, the cytoplasm were stained deep red and could contain an inclusion-like mass. The nucleus was also enlarged and contained one or more irregular dark purple-red stained inclusion bodies, which contained the virus. The nucleus may be displaced laterally by a pale cytoplasmic zone. Sometimes, a typical 'owl eye' inclusion could be seen. AO staining may stain the intranuclear and intracytoplasmic inclusion bodies but it is difficult to differentiate between them and the chromatin.

Immunofluorescence, immunoperoxidase and virus neutralization

Indirect immunofluorescence and indirect immunoperoxidase as well as virus neutralization test were performed in this study to further confirm the isolated agent. Immunofluorescence pattern obtained with hyperimmune serum is illustrated in Fig. 6. The presence of RCMV virus was indicated by the appearance of greenish yellow fluorescence located in the nucleus and cytoplasm of the REF cells. Multiple inclusion bodies accumulating in cytoplasm were clearly demonstrated. In the advance stage of infection, inclusion bodies increased in size, allowing the whole nucleus to be stained greenish yellow fluorescence. Similarly, immunoperoxidase-stained infected cells showed brown discoloration of inclusion bodies located in both nucleus and cytoplasm of the REF cells, reflecting the presence of RCMV.

At 100 $TCID_{50}$ of virus inoculation, a complete inhibition of CPEs was obtained at the hyperimmune serum dilution of 1:100. No neutralising activity was found at dilutions beyond 1:500. The tests confirmed the new RCMV as the virus specifically reacted with antiserum against the Maastricht RCMV infecting *Rattus norvegicus*. It means they shared some common antigenic proteins at certain extent. Immunoperoxidase staining show a higher sensitivity to locate intranuclear inclusion bodies compared to H&E staining. Inclusion bodies mostly found in the nucleus of infected cells at early stage of infection compared to the advanced stage,

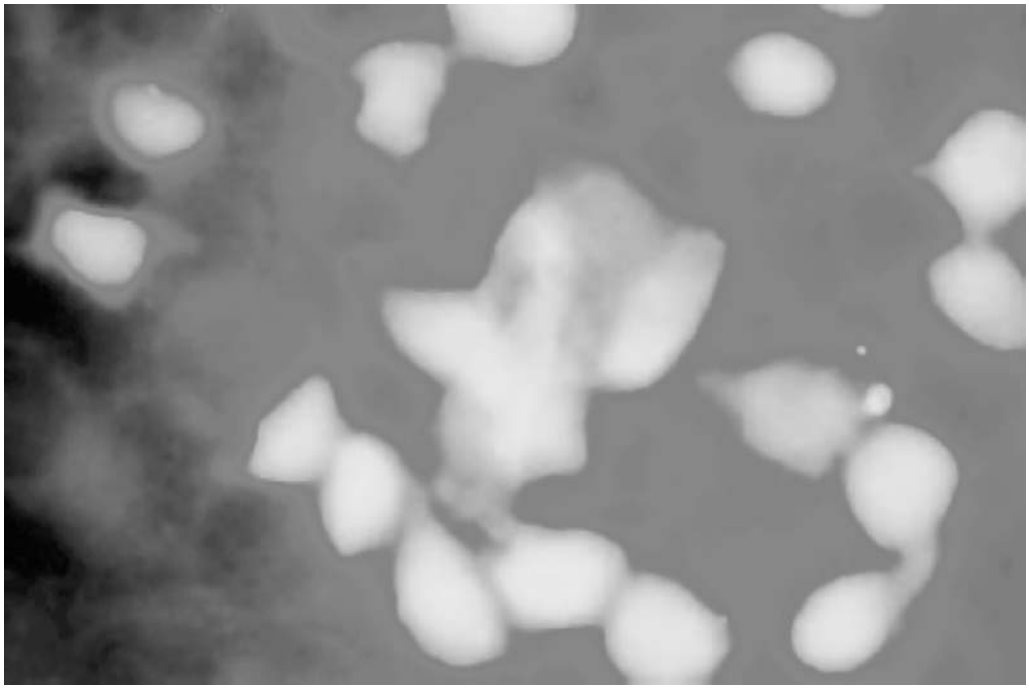


Fig. 6. Indirect immunofluorescence staining of REF cells infected with rat agent, 4 days post-infection (p.i.) using hyperimmune serum (HIS) against the Maastricht RCMV (×1 k)

whereby they were usually found in cytoplasm and left the nucleus unstained. This finding conformed to the TEM examinations.

Restriction endonuclease digestion of viral DNA

The present study was conducted to differentiate the new RCMV to the UPM/Kn, UPM/Sg, English, and Maastricht strains at the genomic level by mean of restriction endonuclease enzyme analysis. *Hin*dIII and *Eco*RI restriction patterns of the new RCMV, UPM/Kn and UPM/Sg are illustrated in Fig. 7. The differences in their restriction patterns are obvious. The new RCMV also differs significantly in its

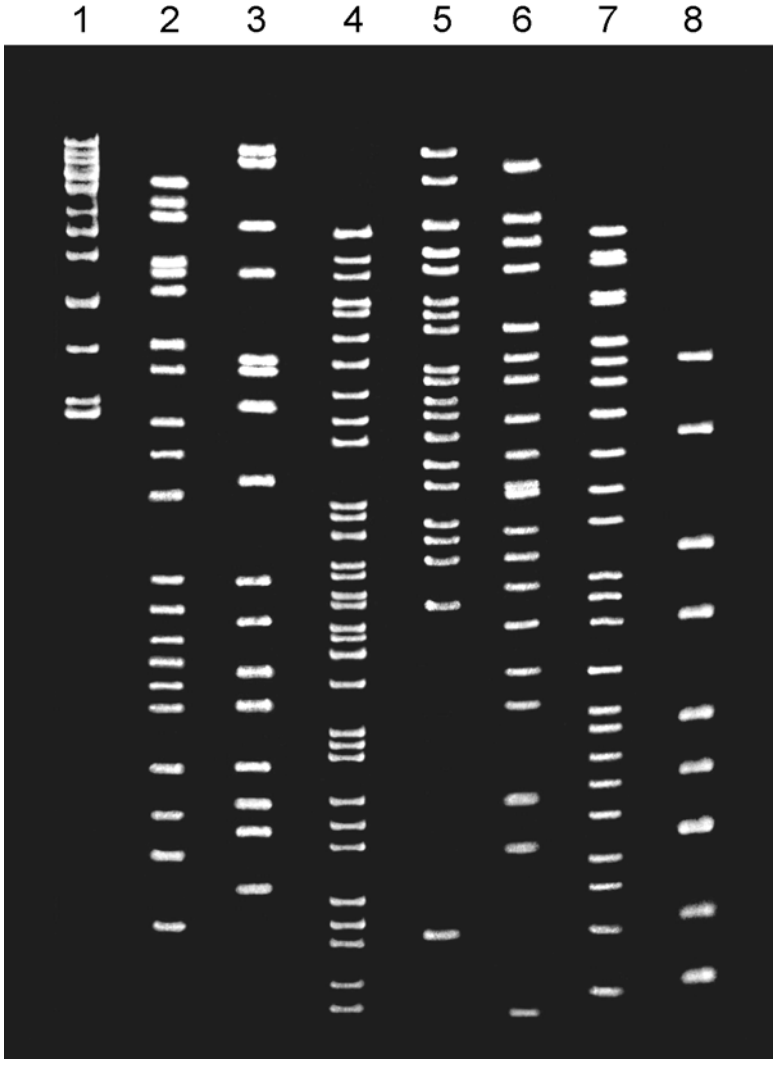


Fig. 7. The restriction endonuclease patterns of the RCMV DNA. RCMV virion DNA from the UPM/Kn strain (*2* and *7*), UPM/Sg strain (*3* and *6*) and new strain (*4* and *5*) were digested with *Hin*dIII (*2*, *3* and *4*) or *Eco*RI (*5*, *6*, *7*) and electrophoresed in 0.7% agarose gels at 1.5 V/cm. *1* and *8* are lambda mix 19 kb and GeneRuler^{TM} 1 kb markers, respectively

restriction patterns from those of Maastricht strain [14, 15] and English strain [5] of RCMV. Meijer et al. [14, 15] reported that *Hin*dIII cleaved the genome of the Maastricht strain to yield only nine fragments, three of which were over 50 kbp in size. Meanwhile twenty-four fragments were produced upon digestion with *Eco*RI, the largest fragment was over 50 kbp in size [15]. Burns et al. [5] reported that *Hin*dIII cleaved the genome of the English strain to produce twenty-five fragments with the largest fragment 32.5 kbp, whereas digestion with *Eco*RI produced more than forty fragments. Interestingly, the new RCMV genome was cut into over 32 fragments upon digestion with *Hin*dIII. Their molecular size was ranging from 1.9 to 19.5 kbp. Upon digestion with *Eco*RI, more than twenty fragments produced with molecular size ranging from 2.4 to 36.6 kbp. The UPM/Kn strain cleaved with *Hin*dIII yielded twenty-one fragments, with molecular size ranging from 2.4 to 27.9 kbp. Digestion with *Eco*RI yielded twenty-five fragments with the molecular size ranging from 2.0 to 18.9 kbp. Upon digestion with *Hin*dIII, the UPM/Sg strain produced sixteen fragments with molecular size ranging from 2.7 to 40.8 kbp. The genome cleaved into twenty fragments with molecular size ranging from 1.9 to 32.3 kbp when digested with *Eco*RI. The results obtained clearly demonstrated the major differences between the viruses. Generally, based on fragment sizes and their distribution, the new RCMV is very distinct from other RCMV viruses studied or published.

Conclusion

The distinct and slow progressing CPE, the herpesvirus morphology, specific antigen-antibody reaction, genetic differences and the origin of the virus which was first time isolated from placenta/uterus are all in line to support that the virus is indeed a new RCMV. The name ALL-03 is proposed for the new RCMV strain.

Further experiments used to be done to analyse the molecular characteristics and pathogenesis of infection of the new strain of RCMV. In particular the determination of the vertical transmission route or transplacental ability in the host and molecular characterization of the major immediate-early gene promoter-enhancer region will be of great interest. Since this is the first CMV virus ever isolated from placenta/uterus, we believe that the virus could be used to develop a suitable animal infection model that mimics CMV infection in human.

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