MOLECULAR CHARACTERISTICS AND PATHOGENICITY OF A NOVEL TRANSPLACENTAL RAT CYTOMEGALOVIRUS

By

LOH HWEI SAN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

January 2005
Dedicated with love and gratitude to:

Father
Loh Swee Fatt

Mother
Chong Hoong Mooi

Brother and Sisters
Kian Loke, Hwei Wen and Hwei Lee

Fiancé
Liew Pit Kang
Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Doctor of Philosophy

MOLECULAR CHARACTERISTICS AND PATHOGENICITY OF A NOVEL TRANSPLACENTAL RAT CYTOMEGALOVIRUS

By

LOH HWEI SAN

January 2005

Chairman: Professor Mohd Azmi Mohd Lila, Ph.D.

Faculty: Veterinary Medicine

Cytomegalovirus (CMV) is a species-specific betaherpesvirus which causes acute, persistent and latent infections in both humans and animals. CMV is the most frequent congenital infection in humans. RCMV strain ALL-03 was the first CMV ever isolated from the placenta and uterus of the house rat (Rattus rattus diardii). As such, hypothetically, this RCMV should be a distinct strain from the existing isolates that is capable to cross placenta and infect the fetus. The objectives of the study were (i) to identify the novelty of the RCMV strain ALL-03, (ii) to characterize its immediate-early (IE) genes, and (iii) to determine its pathogenicity by developing the in utero transmission and neonatal infection models in rats. Overall, the present study signifies the virological and molecular detection of the RCMV antigen, DNA and mRNA in addition to the serological demonstration of the RCMV-specific immune response. Other than the traditional diagnostic methods, the study had also used advanced techniques, for examples, double antibody sandwich enzyme-linked immunosorbent
assay (DAS-ELISA), quantitative real-time reverse transcription-polymerase chain reaction (RT-PCR) and real-time PCR. The study was commenced by characterizing the strain ALL-03. Upon infection, the virus showed delayed cytopathology, cell-association, low maximum titres, the presence of herpesviral inclusion bodies and herpesvirus related particles in infected rat embryonic fibroblast (REF) cells; specific antigen-antibody reaction with RCMV strain Maastricht; and rat-specific are all in accord with a RCMV. The genetic difference at the genome level with that of Maastricht, English, UPM/Sg and UPM/Kn strains had confirmed its novelty. The first recognized genes expressed during CMV infection, the IE genes were studied by analyzing the mRNA transcripts of infected-REF cells. The cDNA libraries were cloned into plasmids for sequencing. Each sequence was then probed towards the databanks for an identity search. Following the PCR and hybridization techniques, two distinct transcripts of unknown identities within the databanks were confirmed to be of the strain ALL-03 origin. These two IE transcripts were found considerably different to the IE genes of RCMV strains Maastricht and English. Meanwhile, a real-time RT-PCR assay was developed specifically to quantify the in vitro transcription levels of the two RCMV IE mRNAs. The kinetic transcription profiles and the bioinformatics analyses suggested them as exon 4 or IE1 and exon 5 or IE2. An in utero infection model demonstrated the clinical signs, pathological changes and anatomical virus distribution to the uterus, placenta, embryo, fetus, lung, kidney, spleen, liver and salivary gland of rats. The placenta was observed to be involved in the maternofetal RCMV infection. The maternal viremia leading to uterine infection which subsequently transmitting to the fetus through the placenta is the most likely phenomenon of congenital CMV
infection in the model. The study has established a useful rat model that mimics the neonatal CMV infection in humans especially for the virus dissemination in different organs, viremia and immune response. The kinetic quantitation of the viral antigen, DNA and antibody was assessed by DAS-ELISA, real-time PCR and ELISA respectively. This neonatal rat model demonstrated a characteristic splenomegaly and acute virus dissemination in blood, spleen, liver, lung and kidney. The salivary gland infection is suggested to augment the antibody response that may be responsible for a reduction of viremia. The study has provided important new insights of CMV disease particularly for a congenital infection in humans. The exploitation of the major IE regions has permitted greatest advances as a candidate of viral-vectored immunocontraception for rat control and generation of eukaryotic expression vectors.
CIRI-CIRI MOLEKUL DAN PENGAJIAN PATOLOGI KE ATAS SEJENIS SITOMEGALOVIRUS TIKUS RUMAH BAHARU YANG BERUPAYA MENERUSI PLASENTA

 Oleh

 LOH HWEI SAN

 Januari 2005

Pengerusi:  Profesor Mohd Azmi Mohd Lila, Ph.D.
Fakulti:  Perubatan Veterinar

Sitomegalovirus (CMV) merupakan betaherpesvirus yang menyebabkan jangkitan-jangkitan akut, berkekalan dan terpendam ke atas kedua-dua manusia dan haiwan. CMV ialah jangkitan kongenital pada manusia yang paling kerap. Strain ALL-03 RCMV merupakan CMV pertama yang dipencilkan dari rahim dan plasenta tikus rumah (Rattus rattus diardii). Justeru itu, RCMV ini dihipotesiskan sebagai satu strain yang sepatutnya berlainan daripada pencilan-pencilan tersedia ada di mana ia berupaya menembusi plasenta untuk menjangkiti fetus. Matlamat-matlamat pengajian ini ialah (i) mengkenalpastikan kebaharuan strain ALL-03 RCMV, (ii) mencirikan gen-gen ‘immediate-early’ (IE)nya, (iii) mengkaji bidang patologinya dengan menubuhkan model-model jangkitan in utero dan neonatal pada tikus-tikus. Secara keseluruhaninya, pengajian ini mementingkan penemuan secara virologik dan molekular ke atas antigen, DNA dan mRNA RCMV ini di samping menunjukkan secara serologi gerakbalas keimunan yang spesifik kepada RCMV. Selain daripada kaedah-kaedah diagnostik yang
ACKNOWLEDGEMENTS

My utmost appreciation and gratitude are conveyed to my supervisor Prof. Dr. Mohd Azmi Mohd Lila for his invaluable guidance; constructive advice, comments and suggestions; patience and encouragement throughout the study. I would like to express my heartfelt thanks and appreciation to my co-supervisors, Prof. Dato’ Dr. Hj. Sheikh-Omar Abdul Rahman and Prof. Dr. Mohd Zamri Saad for their invaluable advices, suggestions and discussions; constructive criticisms; patience and supports which were really helpful towards the completion of my study. Additionally, their efforts spent to improve the quality of the thesis are very much appreciated.

My sincere thanks and gratitude are extended to Associate Prof. Dr. Abdul Rahman Omar, Associate Prof. Dr. Mohd Hair Bejo and Prof. Abdul Rani Bahaman for granting permission to use the equipments and facilities in their laboratories and their precious supports. I am grateful to the staff members of the Biologic Laboratory and Virology Laboratory especially Madam Rodiah Husin and Mr. Mohd Kamarudin Awang Isa for their valuable technical assistances.

My greatest gratitude and thanks are dedicated to Kit Yee, May Ling, Sok Fang, Lee Shun, Tam, Chan, Kim Sing, Su Fun, Zeenat, Zuridah, Zunita, Lih Ling, Sheaw Wei, Wan, Lee Kim, Narumon, Do Yew, Elham, Hossein, John, Yatie, Suria, Elysha, Hanisa, Faizal, Kelvin, Louis, Farah and the other members in Faculty of Veterinary Medicine for their friendship, assistance and encouragement throughout the course of the study.
Last but not least, I am indebted to my beloved parents, brother, sisters, brother-in-law and Pit Kang for their endless encouragement, moral support, patience, understanding and unconditional love all the time. I thank my late pets, BoBo and Popeye for their companionships of days past and the cherished memories that they had gifted to me.
I certify that an Examination Committee met on 26th January 2005 to conduct the final examination of Loh Hwei San on her Doctor of Philosophy thesis entitled "Molecular Characteristics and Pathogenicity of a Novel Transplacental Rat Cytomegalovirus " in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

**Abdul Aziz Saharee, Ph.D.**
Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Chairman)

**Abdul Rani Bahaman, Ph.D.**
Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

**Dato’ Mohamed Shariff Mohamed Din, Ph.D.**
Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

**Hugh John Field, Sc.D., F.R.C. Path., Ph.D.**
Senior Lecturer
Centre for Veterinary Science
University of Cambridge
(Independent Examiner)

---

**GULAM RUSUL RAHMAT ALI, Ph.D.**
Professor/Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:
This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirements for the degree of Doctor of Philosophy. The members of the Supervisory Committee are as follows:

**Mohd Azmi Mohd Lila, Ph.D.**  
Professor  
Faculty of Veterinary Medicine  
Universiti Putra Malaysia  
(Chairman)

**Dato’ Sheikh Omar Abdul Rahman**  
Professor  
Faculty of Veterinary Medicine  
Universiti Putra Malaysia  
(Member)

**Mohd Zamri Saad, Ph.D.**  
Professor  
Faculty of Veterinary Medicine  
Universiti Putra Malaysia  
(Member)

**AINI IDERIS, Ph.D.**  
Professor/Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date:
DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

LOH HWEI SAN

Date: 31/01/2005
TABLE OF CONTENTS

DEDICATION ii
ABSTRACT iii
ABSTRAK vi
ACKNOWLEDGEMENTS ix
APPROVAL xi
DECLARATION xiii
LIST OF TABLES xviii
LIST OF FIGURES xx
LIST OF ABBREVIATIONS xxvi

CHAPTER

1 INTRODUCTION 1.1

2 LITERATURE REVIEW 2.1
2.1 Herpesviruses 2.1
2.1.1 Definition 2.1
2.1.2 Classification 2.1
2.2 Cytomegalovirus 2.3
2.2.1 Virus Structure 2.4
2.2.2 Virus Genome 2.6
2.2.3 Virus Growth Cycle and Viral Gene Expression 2.6
2.3 Epidemiology and Infection Routes of HCMV Infection 2.15
2.4 Pathogenesis and Pathology 2.17
2.5 Host Defenses 2.20
2.5.1 Cell-Mediated Immunity 2.20
2.5.2 Humoral Immunity 2.21
2.5.3 Immune Evasion by CMV 2.22
2.5.4 Latency, Recurrence and Persistency 2.23
2.6 Clinical Features Associated with HCMV Infection 2.24
2.6.1 Congenital Infection 2.24
2.6.2 Infection in the Immunocompromised Host 2.25
2.7 Diagnosis 2.26
2.7.1 Virus Detection 2.26
2.7.2 Detection of the Immune Response 2.29
2.8 Prevention of HCMV Infection and Disease 2.30
2.8.1 HCMV Vaccines 2.30
2.8.2 Current Anti-CMV Treatment 2.31
2.9 Animal Models for CMV Infection 2.32
2.9.1 Rat Cytomegalovirus 2.33
2.9.2 Mouse Cytomegalovirus 2.38
3 ISOLATION AND IDENTIFICATION OF A NOVEL CYTOMEGALOVIRUS OF THE HOUSE RAT, RATTUS RATTUS DIARDII

3.1 Introduction
3.2 Materials and Methods
  3.2.1 Cell Culture
  3.2.2 Isolation of Virus
  3.2.3 Titration of Virus
  3.2.4 CPE Monitoring
  3.2.5 Virus Growth Curve
  3.2.6 Purification of Virus
  3.2.7 Transmission Electron Microscopy
  3.2.8 Histocytochemical Stainings
  3.2.9 Immunocytochemical Assays
  3.2.10 Serum Neutralization Test
  3.2.11 Host Range Specificity Determination
  3.2.12 Preparation of Viral DNA
  3.2.13 Measurement of DNA Concentration and Purity
  3.2.14 RE Analysis of Viral DNA
  3.2.15 Agarose Gel Electrophoresis and Photography
  3.2.16 Molecular Size Estimation of Digested DNA Fragments

3.3 Results
  3.3.1 Cytopathogenicity
  3.3.2 Virus Growth Curve
  3.3.3 Transmission Electron Microscopy
  3.3.4 Histocytochemical Stainings
  3.3.5 Immunocytochemical Assays
  3.3.6 Serum Neutralization Test
  3.3.7 Host Range Specificity Determination
  3.3.8 RE Analysis of Viral DNA

3.4 Discussion
3.5 Conclusion

4 MOLECULAR CHARACTERIZATION AND IDENTIFICATION OF THE IMMEDIATE–EARLY GENES OF RAT CYTOMEGALOVIRUS

4.1 Introduction
4.2 Materials and Methods
5.3.1 Clinical Observation 5.19  
5.3.2 Gross Pathology 5.20  
5.3.3 Histological and Immunohistological Pathology 5.20  
5.3.4 Protein Slot Blotting 5.28  
5.3.5 PCR Detection of IE Gene 5.29  
5.3.6 TEM Examination 5.29  
5.3.7 ELISA for Antibody Detection 5.30  
5.3.8 Fluorescent-Antibody Technique on Buffy Coat Cells 5.32  
5.4 Discussion 5.33  
5.5 Conclusion 5.46  

<table>
<thead>
<tr>
<th>6 PATHOGENICITY OF CYTOMEGALOVIRUS IN NEONATAL RATS</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.1 Introduction 6.1</td>
</tr>
<tr>
<td>6.2 Materials and Methods 6.5</td>
</tr>
<tr>
<td>6.2.1 Neonatal Study of RCMV Infection 6.5</td>
</tr>
<tr>
<td>6.2.2 Measurement of Body Weight and Spleen to Body Weight Ratio 6.6</td>
</tr>
<tr>
<td>6.2.3 Serum Collection and Antibody Titration 6.7</td>
</tr>
<tr>
<td>6.2.4 DAS-ELISA for Antigen Detection 6.7</td>
</tr>
<tr>
<td>6.2.5 Quantitative Real-Time PCR 6.11</td>
</tr>
<tr>
<td>6.2.6 Statistical Analysis 6.13</td>
</tr>
<tr>
<td>6.3 Results 6.13</td>
</tr>
<tr>
<td>6.3.1 Clinical Observation 6.13</td>
</tr>
<tr>
<td>6.3.2 Gross Pathology 6.14</td>
</tr>
<tr>
<td>6.3.3 Measurement of Body Weight and Spleen to Body Weight Ratio 6.14</td>
</tr>
<tr>
<td>6.3.4 Indirect ELISA for Antibody Detection 6.14</td>
</tr>
<tr>
<td>6.3.5 DAS-ELISA for Antigen Detection 6.15</td>
</tr>
<tr>
<td>6.3.6 Quantitative Real-Time PCR 6.18</td>
</tr>
<tr>
<td>6.3.7 Statistical Correlation Assessment 6.19</td>
</tr>
<tr>
<td>6.4 Discussion 6.20</td>
</tr>
<tr>
<td>6.5 Conclusion 6.26</td>
</tr>
</tbody>
</table>

7 GENERAL DISCUSSION AND CONCLUSION 7.1 |
7.1 General discussion 7.1 |
7.2 Conclusion 7.12 |
7.3 Future Prospects and Recommendations 7.13 |

BIBLIOGRAPHY R.1  
APPENDICES A.1  
BIODATA OF THE AUTHOR B.1
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1</td>
<td>Susceptibility of different cell types to new RCMV replication determined by CPE monitoring and IIP test.</td>
</tr>
<tr>
<td>3.2</td>
<td>Estimated molecular size of genomic DNA fragments of three RCMV strains cleaved with HindIII and EcoRI.</td>
</tr>
<tr>
<td>4.1</td>
<td>PCR verification of the recombinant plasmids (pCR®2.1-TOPO and pcDNA3.1) by using different pairs of universal primers.</td>
</tr>
<tr>
<td>4.2</td>
<td>List of primers designed for conventional PCR, RT-PCR and real-time RT-PCR analyses.</td>
</tr>
<tr>
<td>4.3</td>
<td>List of identities for 16 nucleotide sequences based on a database search using BLAST program.</td>
</tr>
<tr>
<td>4.4a</td>
<td>DNA sequence comparison between [IE05], [IE10] and MIE region of English and Maastricht RCMVs by using DNA Homology Search of DNAsis software.</td>
</tr>
<tr>
<td>4.4b</td>
<td>DNA sequence comparison between [IE05], [IE10] and MIE region of English and Maastricht RCMVs by using Pairwise Alignment (Optimal Global Alignment) of BioEdit software.</td>
</tr>
<tr>
<td>4.5</td>
<td>Comparison of G+C ratio of the nucleotides between [IE05], [IE10] and MIE region of English and Maastricht RCMVs.</td>
</tr>
<tr>
<td>4.6a</td>
<td>Comparison of standard curves of BIE and DPC sense RNA oligonucleotides generated by TthPlus and QuantiTect systems.</td>
</tr>
<tr>
<td>4.6b</td>
<td>Comparison of standard curve formulations of BIE and DPC sense RNA oligonucleotides generated by TthPlus and QuantiTect systems.</td>
</tr>
<tr>
<td>4.7</td>
<td>Kinetics of transcription levels of RCMV mRNAs, [IE05] and [IE10] determined by real-time RT-PCR in both mock-infected and infected REF cells.</td>
</tr>
<tr>
<td>5.1</td>
<td>Organ samples collected from the four experiments.</td>
</tr>
<tr>
<td>5.2</td>
<td>Immunoreactivity of IIP test on different tissue sections of treatment groups of the four experiments.</td>
</tr>
</tbody>
</table>
5.3 Protein slot blot reactivity on different tissue homogenates of treatment groups of Experiment C and D.  

5.4 PCR amplification of IE1-specific products on viral DNA extracted from different tissues of treatment groups of the four experiments.  

6.1 Body weight and spleen to body weight ratio of 7-day old newborn rats following primary inoculation with $10^6$ TCID$_{50}$ of RCMV at every 4-day interval (geometric mean ± SD; n = 4).  

6.2 The cut-off absorbance values of various organs based on the calculation of mean OD with three SDs at dilution 1:50 of clarified tissue homogenates.  

6.3 The virus antigen levels distributed in various organs following primary RCMV inoculation in 7-day old newborn rats at every 4-day interval (geometric mean ± SD; n = 4).  

6.4 The correlation matrix developed by the non-parametric Spearman rank test using SPSS program.
<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>Schematic diagram of the process of viral-vectored immunocontraception.</td>
<td>A.6</td>
</tr>
<tr>
<td>2.1</td>
<td>Schematic diagram of a HCMV virion structure.</td>
<td>2.60</td>
</tr>
<tr>
<td>2.2</td>
<td>Schematic diagram showing three distinct forms of capsid which are detectable during HCMV replication: A-capsid, B-capsid and C-capsid.</td>
<td>2.60</td>
</tr>
<tr>
<td>2.3</td>
<td>Temporal expression of the CMV genome proceeds by a cascade synthesis of mRNAs and proteins termed immediate-early (IE), early (E) and late (L).</td>
<td>2.61</td>
</tr>
<tr>
<td>2.4</td>
<td>Organization of the MIE coding region of RCMV showing differential splicing involved in determining IE1 and IE2.</td>
<td>2.61</td>
</tr>
<tr>
<td>2.5</td>
<td>Schematic diagram of the four characteristic phases of PCR, evaluated by real-time PCR fluorescence acquisition.</td>
<td>2.62</td>
</tr>
<tr>
<td>3.1</td>
<td>Cytopathogenicity of the isolated viral agent.</td>
<td>3.38</td>
</tr>
<tr>
<td>3.2</td>
<td>Growth curve of the viral agent in REF cells.</td>
<td>3.39</td>
</tr>
<tr>
<td>3.3</td>
<td>Electron micrograph of negatively stained extracellular viral agent particles.</td>
<td>3.40</td>
</tr>
<tr>
<td>3.4</td>
<td>Electron micrographs of REF cells infected with the viral agent.</td>
<td>3.41</td>
</tr>
<tr>
<td>3.5</td>
<td>H&amp;E-stained mock-infected and infected REF cells with the viral agent.</td>
<td>3.43</td>
</tr>
<tr>
<td>3.6</td>
<td>AO-stained mock-infected and infected REF cells with the viral agent.</td>
<td>3.45</td>
</tr>
<tr>
<td>3.7</td>
<td>IIP-stained mock-infected and infected REF cells with the viral agent.</td>
<td>3.46</td>
</tr>
<tr>
<td>3.8</td>
<td>IIF-stained mock-infected and infected REF cells with the viral agent.</td>
<td>3.47</td>
</tr>
<tr>
<td>3.9</td>
<td>The RE profiles of the genomic DNA of three RCMV strains.</td>
<td>3.48</td>
</tr>
</tbody>
</table>
3.10 Plot of molecular size versus the distance of migration of each fragment of the markers (Lambda 19 mix and GeneRuler™ 1 kb DNA ladder; Fermentas).

4.1 Schematic diagram of cloning process of the two individual plasmids, (a) pCR® 2.1-TOPO and (b) pcDNA3.1 for suspected IE genes.

4.2 RT-PCR profiles using PCR1 primer after gel-purification detected on a 1.2% TAE agarose gel stained with EtBr.

4.3 RE profiles of the recombinant plasmid pcDNA3.1 detected on a 1% TAE agarose gel stained with EtBr.

4.4 PCR profiles of the recombinant pcDNA3.1 and negative control pcDNA3.1 using T7 and BGH primers detected on a 1.2% TAE agarose gel stained with EtBr.

4.5 PCR profiles of the recombinant pcDNA3.1 and negative control pcDNA3.1 using PCR1 primer detected on a 1.2% TAE agarose gel stained with EtBr.

4.6 Nucleotide sequence of [IE05] cDNA in 5’ to 3’ direction.

4.7 Nucleotide sequence of [IE10] cDNA in 5’ to 3’ direction.

4.8 RT-PCR and PCR profiles detected on a 1.2% agarose gel stained with EtBr.

4.9 Dot blot hybridization profiles employed biotinylated probes, prepared from gel-purified PCR amplicons of pcDNA3.1-[IE05] and pcDNA3.1-[IE10] using primer sets, BIE and DPC on positive and negative plasmid control as well as genomic DNA blots.

4.10 Dot blot hybridization profiles employed biotinylated probes on genomic DNA blots of different concentrations.

4.11 RT-PCR profiles of an annealing temperature gradient ranged from 50°C to 72°C using primer sets, BIERT or DPCRT in synthetic sense RNA oligonucleotides which detected on a 2.5% TBE agarose gel stained with EtBr.

4.12 The effects of primer-dimers on ten-fold serial dilutions of BIE sense RNA oligonucleotide in real-time RT-PCR assay using primer set BIERT with annealing temperature of 50°C which detected on a 2.5% TBE agarose gel stained with EtBr.
4.13 Melting curve analysis on the effects of primer-dimers on ten-fold serial dilutions of BIE sense RNA oligonucleotide using primer set BIERT with annealing temperature of 50°C in TthPlus system.

4.14 Real-time RT-PCR assay with modified cycling conditions took place in amplification and quantitation steps which detected on a 2.5% TBE agarose gel stained with EtBr.

4.15 Fluorescence graph showing different patterns of real-time RT-PCR amplification generated by using different concentrations of RNA template in TthPlus system.

4.16 Data graphs of real-time RT-PCR assay generated by using primer set BIERT over six log\(_{10}\) dilutions of BIE sense RNA oligonucleotide in TthPlus system.

4.17 Data graphs of real-time RT-PCR assay generated by using primer set DPCRT over six log\(_{10}\) dilutions of DPC sense RNA oligonucleotide in TthPlus system.

4.18 Data graphs of real-time RT-PCR assay generated by using primer set BIERT over five log\(_{10}\) dilutions of BIE sense RNA oligonucleotide in QuantiTect kit.

4.19 Data graphs of real-time RT-PCR assay generated by using primer set DPCRT over five log\(_{10}\) dilutions of DPC sense RNA oligonucleotide in QuantiTect kit.

4.20 Standard curves showing mean C(T) values plotted versus amount of RNA input for comparison between TthPlus system and QuantiTect kit.

4.21 Melting curve analysis of BIERT/[IE05] real-time RT-PCR products generated from BIE sense RNA oligonucleotide in TthPlus system.


4.23 Melting curve analysis of BIERT/[IE05] real-time RT-PCR products generated from BIE sense RNA oligonucleotide in QuantiTect kit.


4.25 Kinetic quantification of \textit{in vitro} transcription levels of RCMV mRNAs, [IE05] and [IE10] in REF cells based on log\(_{10}\) concentration.
5.1 Gross pathology on infected immunosuppressed rats. 5.51
5.2 Immunopathological changes in salivary glands (IIP staining). 5.52
5.3 Histopathological changes in sublingual gland (H&E staining). 5.53
5.4 Histopathological changes in submandibular gland (H&E staining). 5.54
5.5 Immunopathological changes in lung (IIP staining). 5.55
5.6 Histopathological changes in lung (H&E staining). 5.56
5.7 Immunopathological changes in spleen (IIP staining). 5.58
5.8 Histopathological changes in spleen (H&E staining). 5.60
5.9 Immunopathological changes in liver (IIP staining). 5.62
5.10 Histopathological changes in liver (H&E staining). 5.63
5.11 Immunopathological changes in kidney (IIP staining). 5.64
5.12 Histopathological changes in kidney (H&E staining). 5.65
5.13 Immunopathological changes in uterus (IIP staining). 5.67
5.14 Immunopathological changes in placenta of Experiment D (day 21 p.i.; IIP staining). 5.69
5.15 Immunopathological changes in fetal and neonatal kidneys (IIP staining). 5.72
5.16 Immunopathological changes in fetal and neonatal livers (IIP staining). 5.73
5.17 Immunoreactivity of HIS rose against RCMV towards test strips blotted with different tissue homogenates. 5.74
5.18 PCR profiles using BIE primer set on genomic DNA extracted from different tissues detected on a 1.2% agarose gel stained with EtBr. 5.75
5.19 Electron micrographs demonstrate negatively stained intracellular RCMV particles isolated from placenta sample of an infected immunosuppressed rat of about 17-day pregnancy (day 21 p.i.). 5.76
5.20 Electron micrographs show herpesvirus-like particles present in the ultrathin sectioned-placenta of an infected immunosuppressed rat of about 17-day pregnancy (day 21 p.i.). 5.77

5.21 Determination of BSA concentration. A.9

5.22 Optimization of virus antigen for indirect ELISA. 5.78

5.23 Optimization of conjugate for indirect ELISA. 5.79

5.24 Determination of end-point titration of mean reference serum for indirect ELISA (n = 3). 5.80

5.25 Generation of standard curve based on the serial dilution of reference serum with antibody titre gained from the regression equation in Figure 5.24. 5.81

5.26a The mean absorbance values of control and treatment groups of the four experiments. 5.82

5.26b The mean antibody titres of control and treatment groups of the four experiments. 5.83

6.1 Body weight of 7-day old newborn rats mock-infected and infected with RCMV at every 4-day interval. 6.32

6.2 Spleen to body weight ratio of 7-day old newborn rats mock-infected and infected with RCMV at every 4-day interval. 6.33

6.3 The absorbance values of 7-day old newborn rats mock-infected and infected with RCMV obtained by indirect ELISA procedure at every 4-day interval. 6.34

6.4 The mean antibody titres of infected newborn rats obtained by indirect ELISA procedure at every 4-day interval. 6.35

6.5 Optimization of capture antibody for DAS-ELISA. 6.36

6.6 Optimization of detector antibody for DAS-ELISA. 6.37

6.7 Generation of standard curve for virus antigen quantitation in DAS-ELISA procedure. 6.38

6.8 The virus antigen absorbance values of infected newborn rats obtained from DAS-ELISA procedure. 6.39
6.9 The mean virus antigen levels distributed in various organs of infected newborn rats at every 4-day interval.

6.10 Data graphs of real-time PCR assay generated by using primer set BIERT over six $\log_{10}$ dilutions of pure RCMV DNA in DyNAmo™ SYBR® green qPCR kit.

6.11 Standard curve for RCMV DNA quantitation generated by a plot of mean C(T) values versus amounts of RCMV DNA input.

6.12 Melting curve analysis of real-time PCR assay generated by using primer set BIERT on pure RCMV DNA in DyNAmo™ SYBR® green qPCR kit.

6.13 Real-time PCR profiles of DNA samples extracted from pure RCMV and buffy coat cells which detected on a 2.5% TBE agarose gel stained with EtBr.

6.14 Kinetics of RCMV DNA load based on log$_{10}$ concentration quantitated by real-time PCR assay in buffy coat cells.
LIST OF ABBREVIATIONS

AIDS  Acquired Immunodeficiency Syndrome
AP   Assembly Protein
BCIP  5-Bromo-4-Chloro-3-Indolyl-Phosphate
BHK  Baby Hamster Kidney
BMT  Bone Marrow Transplant
bp   Base Pair
BSA  Bovine Serum Albumin
C(T) Threshold Cycle
cDNA Complementary DNA
CDV  Cidofovir
CHPMPC Cyclic Derivative of HPMPC
CMI  Cell-Mediated Immunity
CNS  Central Nervous System
CpA  Cytosine-Phosphate-Adenosine
CPE  Cytopathic Effect
CpG  Cytosine-Phosphate-Guanodine
CRFK Crandal Reese Feline Kidney
CTL  Cytotoxic T Lymphocyte
DAB  3-3’-Diamino Benzidine Hydrochloride
DAS-ELISA Double Antibody Sandwich ELISA
DEPC Diethyl Pyrocarbonate
dH₂O Distilled Water
DHPG 9-(1, 3-Dihydroxy-2-Propoxymethyl) Guanine
DMEM Dulbecco Minimum Essential Medium
DMSO Dimethyl Sulfoxide
DNA Deoxyribonucleic Acid
DNase Deoxyribonuclease
dNTP Deoxyribonucleotide Triphosphate
DTT Dithiothreitol
E  Early
EBV Epstein Barr Virus
EDTA Ethylenediaminetetraacetic Acid
ELISA Enzyme-Linked Immunosorbent Assays
EMBL European Molecular Biology Laboratory
FBS Fetal Bovine Serum
FITC Fluorescence Isothiocyanate
FOS Pyrophosphate Analogue Foscarnet
g  Gravity
gB Glycoprotein B
GCV Ganciclovir (same compound with DHPG)
GPCMV Guinea Pig Cytomegalovirus
GPCR G-Protein-Coupled Receptor
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>RK</td>
<td>Rabbit Kidney</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic Acid</td>
</tr>
<tr>
<td>RNase</td>
<td>Ribonuclease</td>
</tr>
<tr>
<td>rpm</td>
<td>Revolutions per Minute</td>
</tr>
<tr>
<td>RT-PCR</td>
<td>Reverse Transcription-Polymerase Chain Reaction</td>
</tr>
<tr>
<td>s</td>
<td>Second</td>
</tr>
<tr>
<td>s.c.</td>
<td>Subcutaneous</td>
</tr>
<tr>
<td>SCID</td>
<td>Severe Combined Immunodeficient</td>
</tr>
<tr>
<td>SD</td>
<td>Standard Deviation</td>
</tr>
<tr>
<td>SDS</td>
<td>Sodium Dodecyl Sulphate</td>
</tr>
<tr>
<td>SEM</td>
<td>Standard Error of Mean</td>
</tr>
<tr>
<td>SNT</td>
<td>Serum Neutralization Test</td>
</tr>
<tr>
<td>SPF</td>
<td>Specific Pathogen Free</td>
</tr>
<tr>
<td>SPSS</td>
<td>Statistical Program for Social Science</td>
</tr>
<tr>
<td>TAE</td>
<td>Tris-Acetate-EDTA</td>
</tr>
<tr>
<td>TE</td>
<td>Tris-EDTA</td>
</tr>
<tr>
<td>TEM</td>
<td>Transmission Electron Microscopy</td>
</tr>
<tr>
<td>TMB</td>
<td>Tetra Methyl Benzidine</td>
</tr>
<tr>
<td>TNE</td>
<td>Tris-NaCl-EDTA</td>
</tr>
<tr>
<td>TpG</td>
<td>Thymine-Phosphate-Guanodine</td>
</tr>
<tr>
<td>UL</td>
<td>Unique Long</td>
</tr>
<tr>
<td>UPM</td>
<td>Universiti Putra Malaysia</td>
</tr>
<tr>
<td>US</td>
<td>Unique Short</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet</td>
</tr>
<tr>
<td>V</td>
<td>Volt</td>
</tr>
<tr>
<td>v/v</td>
<td>Volume per Volume</td>
</tr>
<tr>
<td>Vero</td>
<td>Cell Line Derived from Green African Monkey Kidney</td>
</tr>
<tr>
<td>VP</td>
<td>Virion Polypeptide</td>
</tr>
<tr>
<td>vs</td>
<td>Versus</td>
</tr>
<tr>
<td>VZV</td>
<td>Varicella-Zoster Virus</td>
</tr>
<tr>
<td>w/v</td>
<td>Weight per Volume</td>
</tr>
<tr>
<td>w/w</td>
<td>Weight per Weight</td>
</tr>
<tr>
<td>X-gal</td>
<td>5-Bromo-4-Chloro-3-Indolyl-B-D-Galactopyranoside</td>
</tr>
</tbody>
</table>