### viii

### TABLE OF CONTENTS

	PAGE
ACKNOWLEDGEMENTS	iii
ABSTRACT	iv
TABLE OF CONTENTS	viii
LIST OF TABLES	xii
LIST OF ILLUSTRATIONS	xiii
ABBREVIATIONS	XV
CHAPTER I: INTRODUCTION	1
Objectives of this study	3
Education/Application advantages of this study	4
CHAPTER II: LITERATURE REVIEW	5
1. Biology of Human cytomegalovirus (HCMV)	5
1.1. Taxonomy	5
1.2. Viral structure and viral genome	5
1.3. Viral multiplication	8
1.3.1. Virus binding and penetration	8
1.3.2. Viral replication	8
1.3.3. Regulation of viral gene expression	9
1.3.4. Viral assembly, maturation, and release	9
1.4. Target cells of HCMV infection	9
1.5. Pathogenesis of HCMV infection	10
1.6. Latency and reactivation	11/
1.6.1. Sites of latency	11
1.6.2. The latent state	11
1.6.3. Reactivation from latency	12
1.7. HCMV transmission	12

2. Host responses to HCMV infection	13	
2.1. Cell-mediated immunity	13	
2.2. Humoral immunity	13	
2.3 Immune evasion by human cytomegalovirus	14	
3. HCMV infection in non-HIV infected population		
3.1. HCMV infection in general population	15	
3.2. HCMV infection in pregnant women and infants	15	
4. HCMV infection in HIV infected population		
4.1. HCMV infection in HIV infected adults	16	
4.2. HCMV infection in HIV infected infants	18	
and children		
5. Interaction between HCMV and HIV-1	19	
5.1. Role of co-infection	19	
5.2. Receptor	20	
5.2.1. Up-regulation of CD4 co-receptor expression	20	
5.2.2. Induction of alternative HIV receptors	20	
5.2.3. Pseudotype formation	20	
5.3. Cytokine release		
6. Laboratory diagnosis for HCMV infection		
6.1. Virological techniques	21	
6.1.1. Non-molecular methods	22	
6.1.1.1. HCMV culture assay	22	
6.1.1.2. Spin-amplification shell vial assay	22	
6.1.1.3. Antigenemia assay	23	
6.1.2. Molecular methods	23	
6.1.2.1 PCR conventional method	23	
6.1.2.2. Real time PCR assay	24	
6.1.2.3 Others Nucleic acid amplification	26	
method		

6.2. Serological technique		
CHAPTER III: RESEARCH DESIGN, MATERIALS AND METHODS		
1. Study population		
2. The study design		
3. Sample collection	31	
4. Determination of HCMV Infection	33	
4.1. Detection of IgG antibodies by ELISA technique	33	
4.2. Detection of IgM antibodies by ELISA technique	33	
4.3. CMV DNA qualitation by real time PCR	34	
4.3.1. DNA extraction from white blood cell pellets	34	
4.3.2. Real time PCR probe and primer	35	
4.3.3. Preparation of HCMV positive control	38	
4.3.3.1. Transformation of the recombinant	38	
plasmid DNA		
4.3.3.2. Screening for the recombinant	39	
plasmid DNA in the transformed		
bacteria		
4.3.3.3. Purification of HCMV recombinant	39	
plasmid		
4.3.3.4. Quantitation of the purified HCMV	40	
plasmid DNA by spectrophotometry		
4.3.3.5. Quantitation of extracted DNA by	41	
the fluorescence assay		
5. Categorization of HCMV infection	42	
6. Determination of HIV disease progression	43	
7. Statistical analysis	43	
CHAPTER IV: RESULTS		
1. Preparation of standard HCMV gene fragment control	45	
by PCR cloning method		

2. HCMV infection in children born to HIV-1 infected mothers					
2.1. Characteristics of the population					
<ul><li>2.2. The rate of HCMV infection within 18 months</li><li>2.3. Congenital HCMV infection in infants</li><li>2.4. HCMV infection in twins</li></ul>					
			2.5. Evaluation of risk factors associated with		
			HCMV perinatal transmission in infants		
born to HIV-1 infected mothers					
	2.5.1. Maternal risk factors	54			
	2.5.2. Infant's risk factors	59			
	2.6. Time of first diagnosis of HCMV infection	61			
	2.7. HCMV infection and HIV-1 disease progression	62			
	within the first 18 months of life				
CHAP	PTER V: DISCUSSION AND CONCLUSION	64			
REFE	RENCES	67			
APPENDIX					
CIRRICULUM VITAE		85			

# 

#### LIST OF TABLES

TABI	NATING.	PAGE
1.	The members of Herpesviridae Family.	7
2.	HCMV PCR primers and Taqman® probe information.	38
3.	Mother's characteristic descriptions.	49
4.	Infant's characteristic descriptions.	50
5.	Rates of HCMV infection in HIV-1 infected and HIV uninfected	51
	infants within 18 months of age.	
6.	Comparison of HCMV infection in HIV-1 infected and uninfected	52
	infants within 18 months of age.	
7.	Comparison of congenital HCMV infection in HIV-1 infected and	53
	HIV-1 uninfected infants.	
8.	Maternal risk factors associated with HCMV transmission in	55
	HIV-1 infected and HIV-1 uninfected infants.	
9.	Infant's risk factors associated with HCMV transmission in	59
	HIV-1 infected and HIV-1 uninfected infants.	

# 

### LIST OF ILLUSTRATIONS

FIGURE 9381336		
1.	Estimated number of adults and children living with HIV in 2005.	1
2.	Pathways leading to opportunistic versus cofactor relationships	2
	between HCMV and HIV.	
3.	Structure of human cytomegalovirus virion and human cytomegalovirus	6
	genome.	
4.	Amplicon detection by 5' nuclease oligoprobes.	25
5.	PHPT hospital networks in Thailand.	29
6. 7	The schematic diagram of the research design in this study.	32
7.	The location of HCMV DNA target for amplification.	37
8.	Screening of successfully transformed bacteria.	46
9.	The confirmation of the presence of HCMV IE gene fragment in purified	47
	plasmid DNA after large scale production by conventional PCR.	
10.	Maternal risk factors associated with HCMV transmission in	57
	HIV infected infants.	
11.	Maternal risk factors associated with HCMV transmission in	58
	HIV uninfected infants.	
12.	Infant's factors associated with HCMV infection in HIV-1 infected and	60
	HIV-1 uninfected infants.	
13.	Evaluation of survival free of diagnosed HCMV in HIV-1 infected infants	61
	and HIV-1 uninfected infants.	
14.	Accumulated rate of infants died or met criteria for HIV disease	62
	progression among HIV-1/HCMV coinfected infants and infant with	

63

15. Kaplan-Meier estimates curve showing the probability of HIV disease progression among HIV-1/HCMV coinfected infants and infants with



## ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่ Copyright © by Chiang Mai University All rights reserved

### **ABBREVIATIONS**

95% CI	95 percent confidence interval
% 9/3/17	Percent
α	Alpha
β	Beta
γ	Gamma
μ	Micro
μg	Microgram
μ	Microliter
°C 3	Degree Celsius
A	Adenine
C	Cytosine
G	Guanine
T	Thymine
AIDS	Acquired immunodeficiency syndrome
bp	Basepair
bp CD	Cluster of differentiation
CCR-5	Receptor for CC-chemokine
CXCR-4	Receptor for CXC-chemokine
DBS	Dried blood spot
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphate
dATP	Deoxyriboadenosine triphosphate
dCTP	Deoxyribocytosine triphosphate
dGTP	Deoxyriboguanine triphosphate
dTTP	Deoxyribothymine triphosphate
dUTP	Deoxyribouracil triphosphate
dsDNA	Double stranded deoxyribonucleotide triphosphate

EDTA Ethylenediaminetetraacetic acid

ELISA Enzyme liked immunosorbent assay

g Gram

gB Glycoprotein B

HCMV Human cytomegalovirus

HIV-1 Human immunodeficiency virus type-1

IE gene Immediate-early gene

IgG Immunoglobulin G
IgM Immunoglobulin M

IL-1β Interleukin-1 beta

IL-6 Interleukin-6 IL-8 Interleukin-8

IQR Interquartile range

Kbp Kilobasepair

LB medium Luria-Bertani medium

LTR gene Long terminal repeated gene

M Molarity
mg Miligram

min Minute

MHC Major histocompatibility complex

mL Milliliter

mM Millimolar

mRNA Messenger ribonucleic acid

MW Molecular weight

ng Nanogram

nm Nanometer

no. Number

O.D. Optical density

Phosphoprotein 24, typical protein of lentiviruses

#### xvii

PBL Peripheral blood leukocyte

PCR Polymerase chain reaction

pmol Picomole

RNA Ribonucleic acid

rpm Rounds per minute

RT-PCR Reverse transcription polymerase chain reaction

TGF-β Tumors growth factor – beta

TNF-α Tumors necrosis factor – alpha

UV Ultraviolet light

vs. Versus

w/v Weight by volume

ZDV Zidovudine

## ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่ Copyright © by Chiang Mai University All rights reserved