

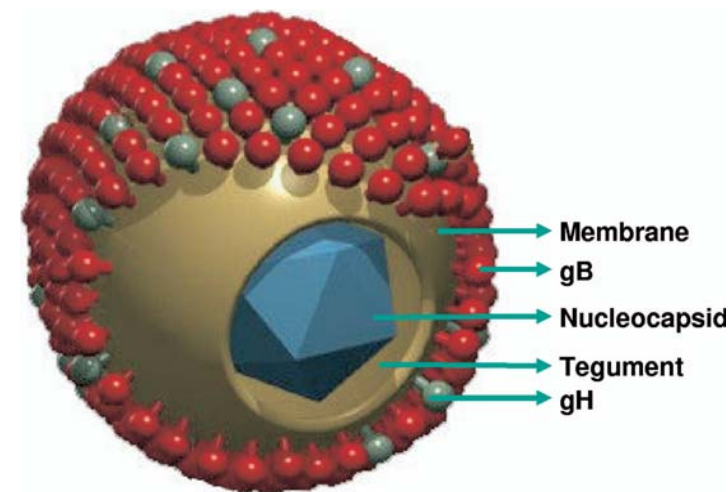


Genetic Polymorphism of US28 of Human Cytomegalovirus infection in HIV infected and non HIV-infected children

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□ Human cytomegalovirus (HCMV)

- linear double stranded DNA virus
- member of
 - Cytomegalovirus genus
 - *Herpesviridae* family
 - *Betaherpesvirinae* subfamily



(CLINICAL MICROBIOLOGY REVIEWS, Jan. 2009, p. 76–98)

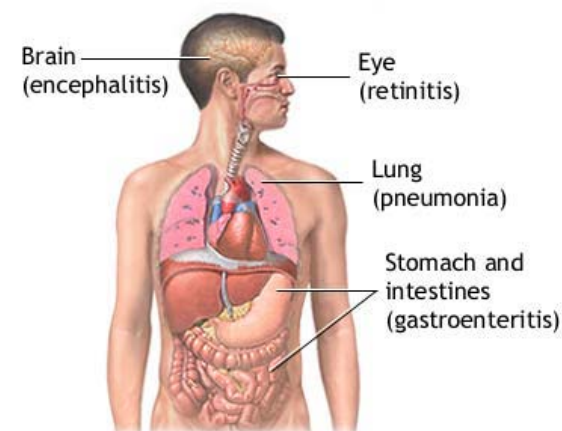


□ Epidemiology

- endemic throughout the world
- child care center which serve as a major reservoir (Gandhi, M. and R. Khanna (2004)).
- The prevalence rate of common infections in adults by HCMV is seropositive 60 to 99% globally (Cheng, J., Q. Ke, et al. (2009)).
- In Thailand, the seroprevalence rate is 70.7 to 90 % in pregnant women and adult blood donors (Bhattarakosol, P. and S. Chantaraarphonkun (2007)).
- For the group of children who was infected with HCMV and born to HIV-1 infected woman shows the circulation of HCMV up to 89% (Likitnukul, S., P. Bhattarakosol, et al. (2003)).

□ People who are at risk of HCMV infection

- Immature fetus with HCMV congenital infection :
mental retardation sensorineuronal deafness
- The immunocompromised host
 - organ transplant recipients
 - patients undergoing immune suppressive drug treatment
 - HIV-infected patients



ADAM.



□ Virulence of HCMV

- **Host factors:** immune status, age, genetic background of the host
- **Viral factors:** viral strain, dose of infection, special genes

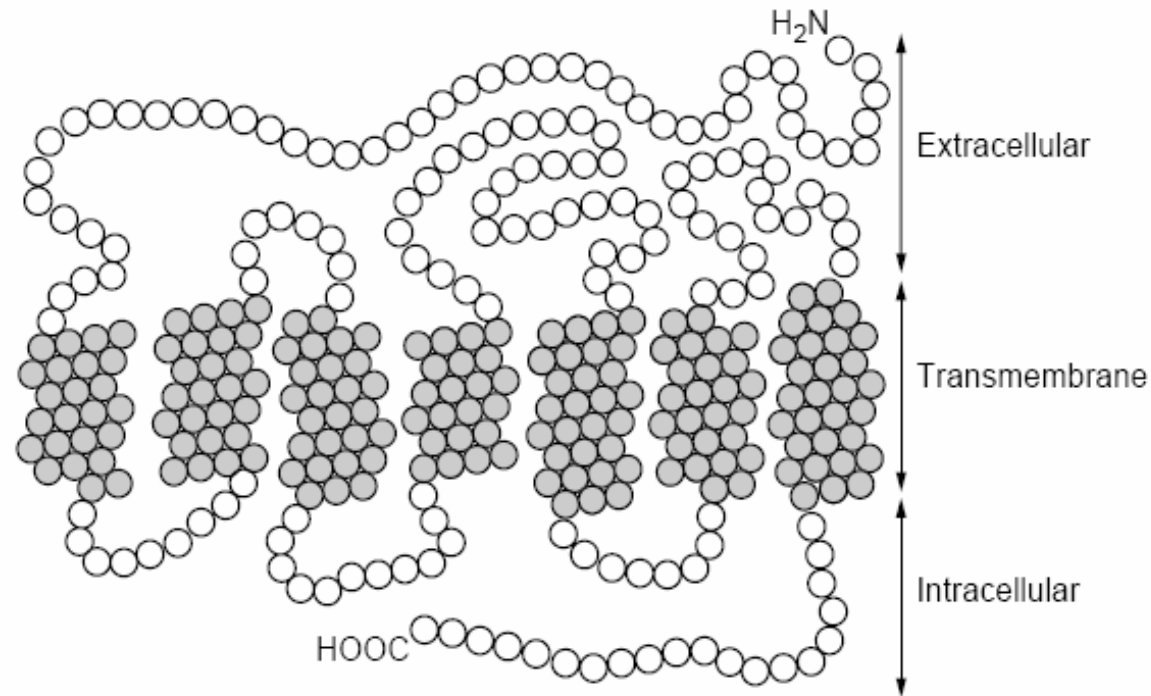


What is G protein-coupled receptors (GPCRs)?

□ GPCRs is

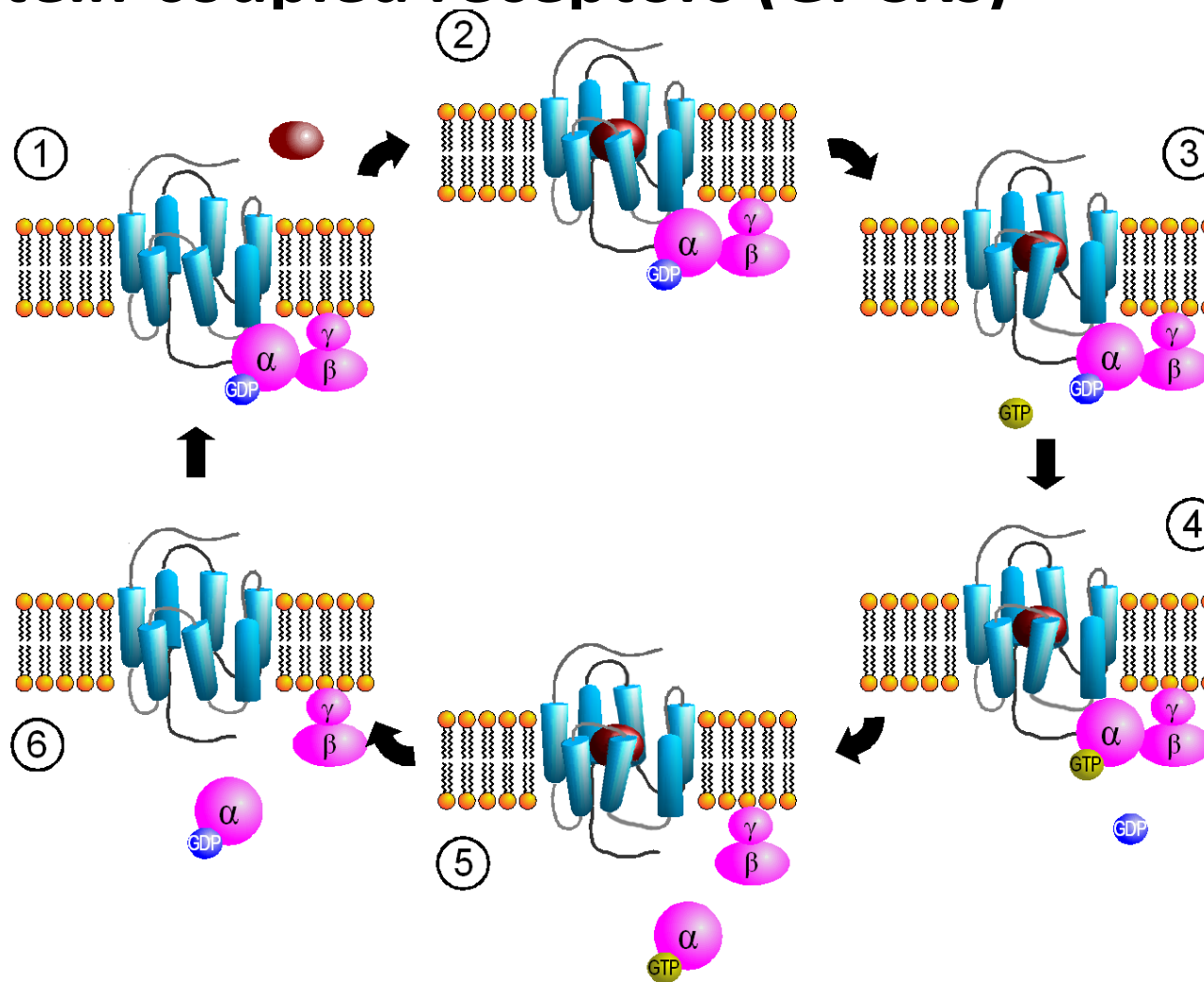
- The greatest family of membrane associated proteins
- Function in sensory receptor and receptor for neurotransmitters, peptide hormones and chemokines receptors.

□ G protein-coupled receptors (GPCRs)



- Seven hydrophobic transmembrane helices: consist of ~25-38 amino acid residues and linked by intra or extracellular loop, intracellular C-terminus as well as extracellular N-terminus

□ G protein-coupled receptors (GPCRs)



http://xray.bmc.uu.se/lars/Practicals/Signal/trans_app.html



□ Viral-encoded GPCRs

Virus	CXCR	CCR	UL33	other
KSHV/HHV-8	ORF74/KSHV GPCR			
HCMV		US27/US28	UL33	UL78
MCMV			M33	M78
RCMV			R33	
HHV-6			U12	U51 (induces CCR7)
HHV-7			U12	U51 (induces CCR7)
HVS	ORF74/ ECRF3			
EHV-2	ORF74			E6
EBV				induces CCR7
RRV	ORF74			
Swine pox virus				K2R
Capri-pox virus				Q2/3L

Pharmaceutica Acta Helvetiae 74 (2000) 299-304



□ G protein-coupled receptors (GPCRs) homologues

- HCMV encodes GPCRs genes that have the sequences or characters that resemble to those of the human.
- Couty *et al.* suggested that HCMV uses the GPCRs homologue strategy to **escape the immune system** and redirect the cellular networks then cause the pathogenesis and chronic disease



□ HCMV encoded GPCRs gene

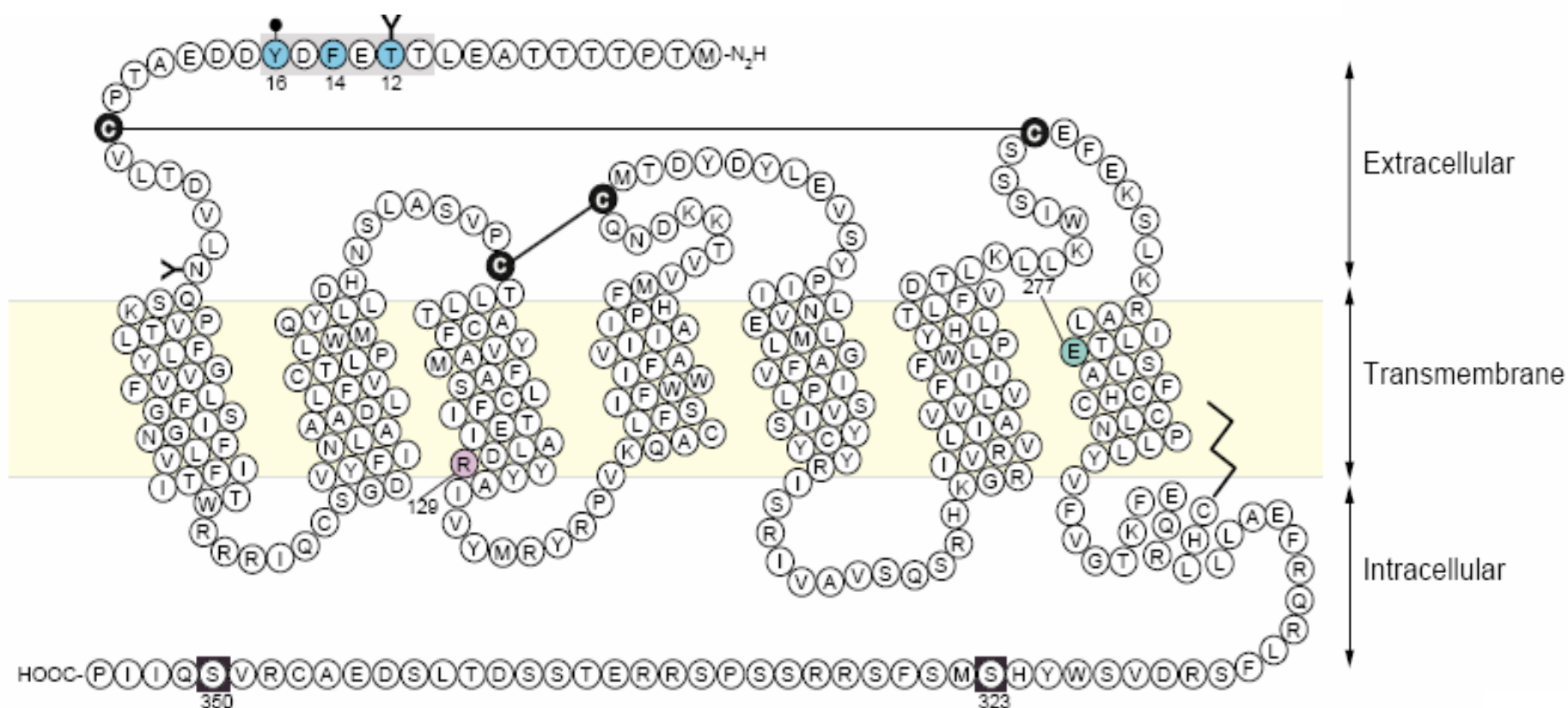
- **US 27** : homologue of CCR1, CCR2 and CX3CR1
- **US 28** : homologue of CCR1, CCR2 and CX3CR1
- **UL33** : homolog of CCR10
- **UL78** : limit sequence identical to chemokine receptors or any other GPCRs but shared some character to the conserve region



□ US28 encoded G protein coupled receptors gene

- The **pUS28 protein** can sequestrate CC, CKs in the infected cells leads to the modulation of immune response
- In the year 2000, Ohagen A. *et al.*, reported that US28 gene encodes the homolog chemokine receptor function as co-receptor for entry of HIV-1

US28 encoded G protein coupled receptors gene



TRENDS in Pharmacological Sciences



Polymorphism of US28 genes



□ Urine samples

- 11 cases were HIV infected children
- 32 cases were non HIV-infected children
- Children from Phayathai Babies's Home children with age less than 5 years old were used in this study

□ Samples preparation

Viral DNA from urine sample was extracted and purified by Axygen[®] AxyPrep body fluid viral DNA/RNA miniprep kit (Axygen[®] Biosciences, USA)

□ Amplification of the US28 gene of HCMV by PCR method

- US28 gene : Hemi-nested PCR primers (Ravit Arav-Boger, 2002)



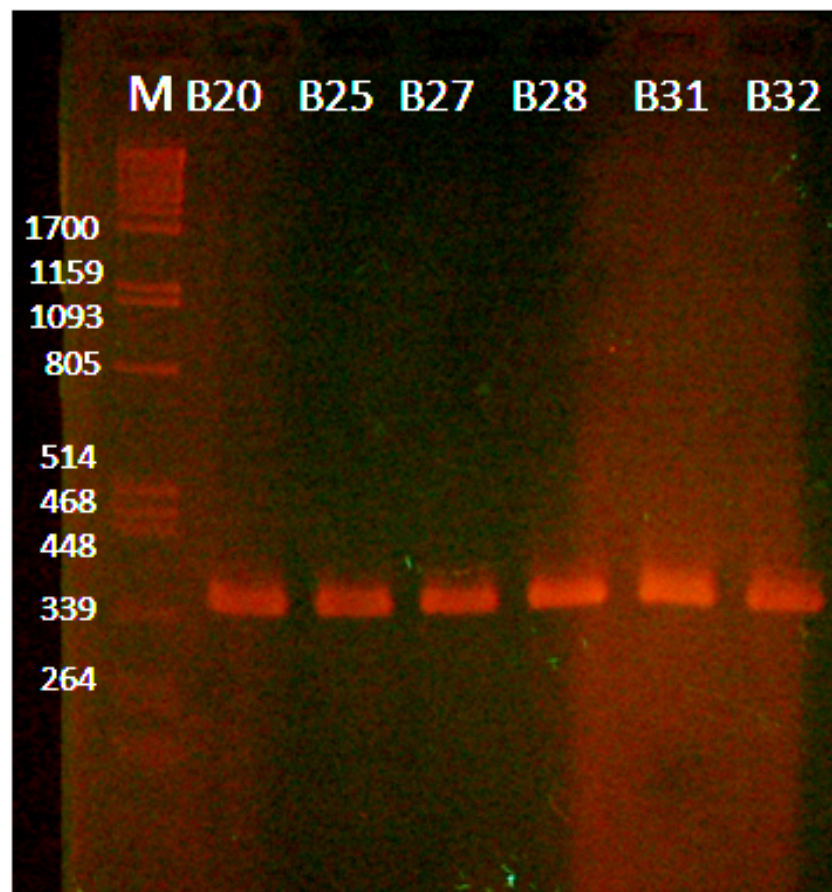
- ▶ Forward primer (28NF) : 5' gtg aac cgc tca tat aga cc 3'
- ▶ Reverse primer (28NR1) : 5' gaa aca ggc agt gag taa cg 3'
- ▶ Hemi-nested reverse primer (28NR2) : 5' cat cca cag agg tag tgt ac 3'



Analysis of GPCRs gene on 2% agarose gel

Size of PCR
fragment

PCR product of
US28: 365 bp





□ DNA sequencing analysis

- analyzed by sequencer software, Molecular Evolutionary Genetics Analysis(MEGA) 4.1 program
- Compared the homology to the reference sequences of US28 gene from GenBank



The variation of amino acid substitutions at the N-terminus domain

Reference strain AD 169	Position of amino acid							
	8 (A)	15 (D)	18 (E)	19 (G)	21 (T)	24 (V)	25 (F)	49 (I)
amino acid substitution	T	E	D L	A D	A	T	L V	V

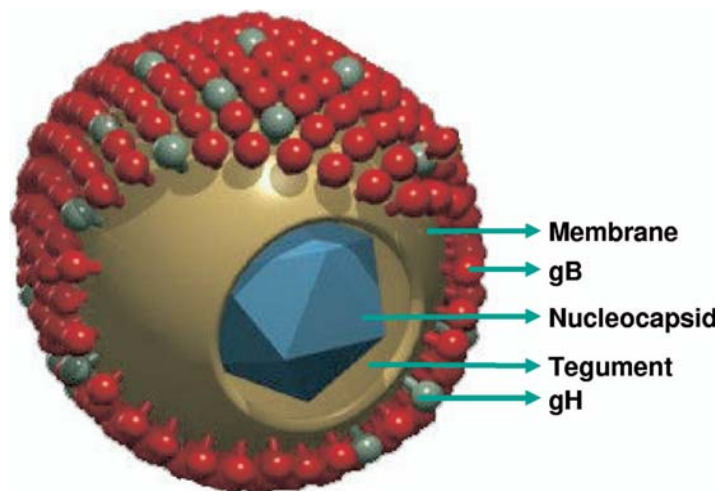
From: 1. The Journal of infectious diseases 2002; 186: 1057-64.
2. Virus Genes 2006 Oct; 33(2): 175-81.



In this study: Amino acid substitutions at the N-terminus domain

Reference strain AD 169 Accession no. NC_001347	Position of amino acid								
	8	15	18	19	21	24	25	45	49
	(A)	(D)	(E)	(D)	(T)	(V)	(F)	(L)	(I)
amino acid substitution	T	E	L	A G	-	T	-	I	-

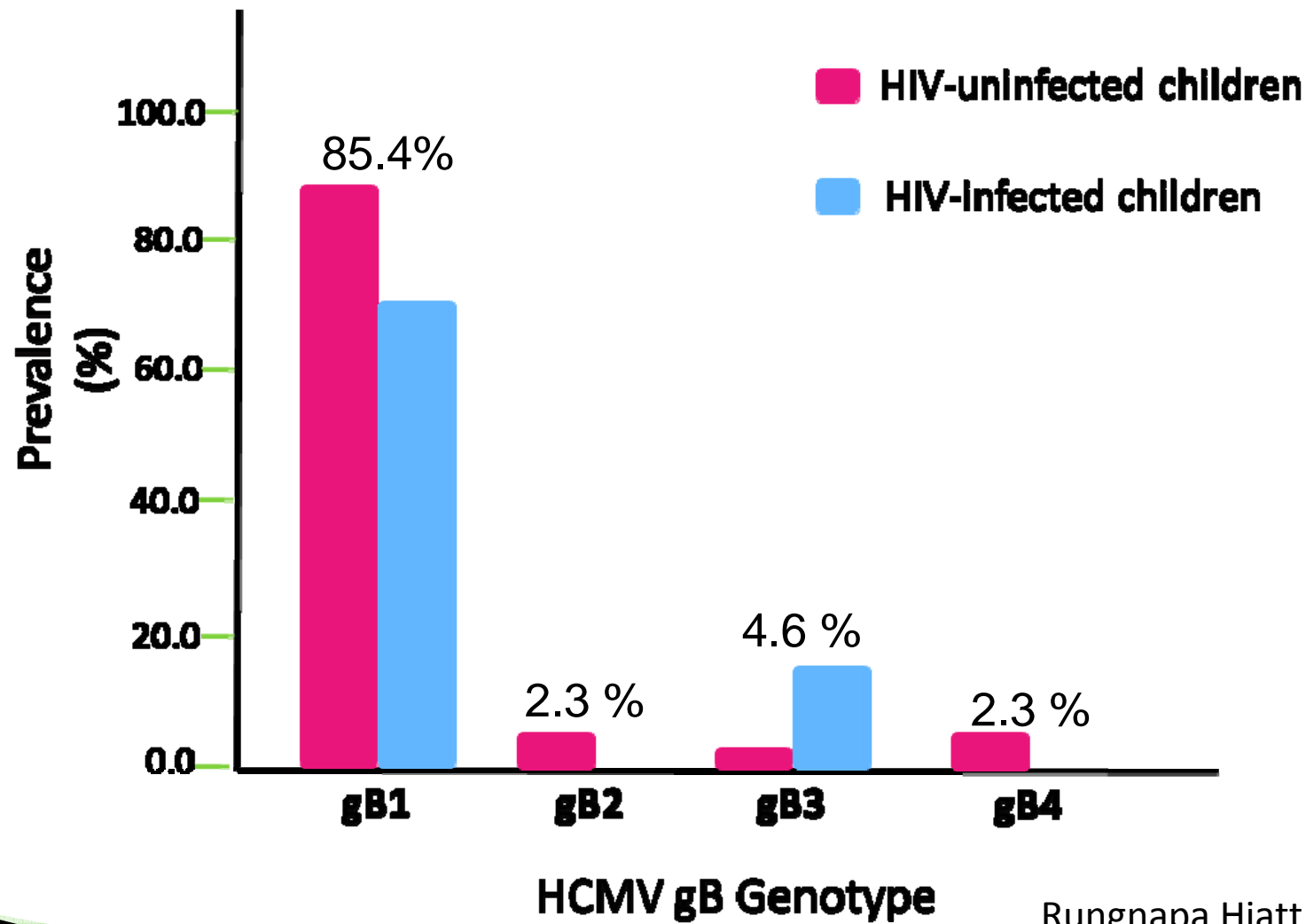
□ HCMV glycoprotein B (UL55)



CLINICAL MICROBIOLOGY REVIEWS,
Jan. 2009, p. 76–98

- Major envelope glycoprotein
- Functions: host cell entry, cell-cell transmission, target for neutralizing antibodies
- Four patterns of HCMV genotypes
 - gB1
 - gB2
 - gB3
 - gB4

HCMV gB genotypes distribution in the group of HIV infected and uninfected children



Rungnapa Hiatt, 2008



► Relation of amino acid substitution and gB genotype

Total	gB1		gB2		gB3		gB4	
	non HIV (28)	HIV (7)	non HIV (1)	HIV (1)	Non HIV (2)	HIV (4)	Non HIV (1)	HIV (0)
AD169	5	4	1	-	1	3	1	-
Toledo	12	1	-	-	1	-	-	-
Mutation:A8T,D15E,E18L,D11G,V24T	1	-	-	-	-	-	-	-
Mutation:D15E,E18L,D19G,V24T	2	-	-	-	-	-	-	-
Mutation:E18D,D19E,F25L	2	-	-	-	-	-	-	-
Mutation:E18L,D19G,V24T	6	1	-	1	-	-	-	-
Mutation:E18L,D19G,V24T,L45I	-	1	-	-	-	-	-	-
Mutation:D19A,L45I	-	-	-	-	-	1	-	-



Conclusion

- ❑ Sequencing analysis of the US28 gene N-terminus domain reveals some amino acid substitutions: **A8T, D15E, E18D/L, D19A/G/E, V24T, F25L, and L45I.**
- ❑ Percent of **amino acid substitution** at the N-terminus domain of the US28 gene **in non HIV-infected** children is appeared to be **higher than** that of **HIV** infected children.



Conclusion

- The AD169 homologous sequence of the US28 gene N-terminus domain was observed more frequent in samples of HIV infected children



Acknowledgement

▶ **Special thanks to :**

❖ **Assistant Professor Dr. Saowakon Paca-Uccaralertkun,
Department of Microbiology, Faculty of Science, Mahidol
University**



Thank you for your attention



**Any questions ?
&
All suggestions are welcome.**