

**ANTIGENIC EPITOPE ANALYSIS OF THE p101 NUCLEOCAPSID
PROTEIN OF HUMAN HERPES VIRUS 6 (VAR. B)**

by

**KATHERINE ANNE FRANCIS
B. Sc. (Hons.)**

A DISSERTATION SUBMITTED TO THE INSTITUTE OF POSTGRADUATE
STUDIES AND RESEARCH IN FULFILMENT FOR THE DEGREE OF
MASTER OF PHILOSOPHY

FEBRUARY 2000

INSTITUTE OF POSTGRADUATE STUDIES AND RESEARCH
UNIVERSITY OF MALAYA
KUALA LUMPUR
MALAYSIA

Perpustakaan Universiti Malaya



A508764681

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Dimikrofilmkan pada... 04. 12. 2001
No Mikrofis... 15130
Jumlah Mikrofis... 3

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UNIT REPROGRAFI
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Abstract

Human herpes virus 6 (HHV6) is an ubiquitous, lymphoproliferative β -herpesvirus, which infects almost all individuals during infancy and persists lifelong. Two variants of HHV6 (A and B) have been described. The present study is on HHV6B, which causes exanthem subitum, also known as roseola infantum, a common febrile childhood illness.

Two methods of detection for anti-HHV6B in body fluids were used, being indirect immunofluorescence assay (IFA) and peptidylated pin-ELISA. For IFA, the target cells were HHV6B-infected human cord blood mononuclear cells (HCBMC). Ninety eight infant sera were tested by IFA and all were found to be IgG-HHV6 positive. Seventeen breast milk samples were also analyzed and 9 were IgA-HHV6 positive against HHV6-infected HCBMC. Eight saliva samples tested were all IgA-HHV6 negative in IFA. Serum, breast milk and saliva samples were collected from different individuals. No two sample was from the same individual.

Anti-HHV6 IFA was carried out on 10 cord blood samples and all were found IgG positive but IgA negative, thus eliminating the possibility that IgA is transferred through cord blood. Age prevalence study was carried out on 240 subjects between 0-20 years old. Distinctly different serum IgA and IgG profiles were obtained. However, a correlation study carried out showed that there is correlation between prevalence of IgG and IgA. For the IgG anti-HHV6, the proportion with antibody was high at birth, about 80% and the antibody levels remained high till about 2 years. It then decreases slowly and increases again before reaching a plateau at adulthood. As for HHV6 anti-IgA

antibodies were low during childhood till about 4 years of age and then increased gradually. However, the increase did not follow a consistent pattern with age.

For the peptidylated pin-ELISA, HHV6 peptides were synthesized onto polyethylene pins and served as target antigens in ELISA. A 101kDa (p101) protein of the HHV6 variant B strain Z29 (858 amino acids) has been found to be highly immunoreactive (Pellet *et al*, 1993). In the present study, 334 amino acids of the carboxyl terminal was synthesized as a set of 46, 14 mer peptides, overlapping by 7-mers, since antigenicity of a protein is frequently localized at the carboxyl terminal (Neipel *et al*, 1992). The sequence selected was also based on antigenic predictions using hydrophilicity based on Hopp and Woods (1982), accessibility and secondary structure based on the NNPREDICT method. All three methods were obtained through Protscale Analysis from SWISSPROT (<http://expasy.hcuge.ch/cgi-bin/protscale.pl>.)

The antigenic profiles for IgG for 25 HHV6 IFA positive sera to HHV6 peptides were studied and 6 regions were identified. Of the 6, peptide 44 (IRQDGETDEETVP) showed the highest reactivity, recognized by IgG in 24 of the 25 sera tested. A percentage reactivity of 96% was obtained.

In the saliva samples tested, 3 samples were IgA positive when reacted with 5 major HHV6 peptides. The reason for the HHV6 IFA-negative saliva showing reactivity in ELISA may be because the peptide regions recognized by salivary IgA were not exposed in the virus- infected HCBMC. This may be due to the conformational structure of the protein. When synthesized the protein structure is linearized and thus may have been possible to recognized. But as a whole antigen in IFA, it exists in its native conformation and could not be detected.

Six of the HHV6 IFA-positive and 3 HHV6 IFA-negative breast milk samples were tested against the HHV6 peptides. The IFA-positive and IFA-negative both reacted with the similar HHV6 peptides in ELISA for IgA, except for peptide 13 (KGNSRDLYSGGNAE). This peptide was predominantly recognized by IgA in HHV6 IFA-positive breast milk. This suggests the presence of a shared IgA antigenic epitope of IgA expressed in both the virally-infected HCBMC and the HHV6 peptide. Since serum IgG but not IgA to HHV6 was detected by IFA in cord blood, the presence of IgA anti-HHV6 in breast milk may serve to provide another means of passive immunity besides the transplacental IgG.

The HHV6 antigenic regions from the ELISA studies were also analyzed for their predicted hydrophilicity, accessibility and secondary structure by methods from Protscale, Swissprot. Besides these prediction methods, mobility as well as coil structured parameters were used to confirm our findings. The major immunodominant regions for IgG and IgA, being peptides 44 and peptide 13 respectively were found to be hydrophilic as well as surface accessible. They were also highly mobile and showed to be made up of coiled structures. The secondary structure of both these peptides were made up of mostly coiled structures. Peptide 44 had a helix-coil-helix structure and peptide 13 was a coil-helix-coil structure. As coils frequently exist as protrusions from the main body, they are preferentially recognized by antibodies.

Abstrak

Human herpes virus 6 merupakan beta-herpesvirus yang menginfeksi kebanyakan individu semasa dalam peringkat bayi dan kekal seumur hidup. Terdapat dua varian HHV6 iaitu A dan B. Kajian ini adalah mengenai HHV6B yang menyebabkan exanthem subitum, yang juga dikenali sebagai roseola infantum.

Dua cara pengesanan anti-HHV6B dalam secair badan telah digunakan, iaitu kaedah immunopendafluoran dan pin-ELISA peptida. Bagi kaedah immunopendafluoran, sel sasaran adalah sel mononuklear darah tali pusat (HCBMC). 98 sera bayi telah dikaji dengan kaedah ini dan didapati kesemuanya mempunyai antibodi IgG terhadap HHV6B. Daripada 17 sampel susu ibu yang dikaji, 9 daripadanya adalah positif bagi antibodi IgA terhadap HHV6B. Lapan sampel air liur dikaji dan didapati tiada yang mempunyai antibodi IgA terhadap HHV6B. Kesemua sampel didapati dari individu berlainan.

Sepuluh sampel darah tali pusat telah dikaji dan kesemuanya didapati mempunyai antibodi IgG tetapi antibodi IgA tidak dapat dikesan. Ini menolak jangkaan bahawa IgA dipindah melalui tali pusat. Apabila kajian seroprevalen mengikut umur dijalankan ke atas 240 sampel berumur 0-20 tahun, profil IgA dan IgG yang berlainan diperolehi. Tetapi kiraan korelasi menunjukkan bahawa kedua-dua IgA dan IgG mempunyai korelasi antara mereka. Bagi antibodi IgG, takat yang tinggi diperolehi bagi subjek berumur 0-1 tahun, iaitu 80%, menunjukkan adanya antibodi ibu yang dipindahkan kepada bayi. Takat ini kekal ke umur 2 tahun dan seterusnya menurun ke takat 55% sebelum meningkat ke takat dewasa. Takat antibodi IgA terhadap HHV6B adalah rendah pada masa baru lahir hingga ke 4 tahun dan seterusnya meningkat ke takat dewasa. Walau bagaimanapun

peningkatan ini tidaklah berkadar dengan umur. Takat antibodi IgA adalah rendah secara relatif berbanding antibodi IgG.

Bagi kajian menggunakan pin-ELISA, peptida p101 yang ditemui antigenik oleh Pellett *et al.*(1993) telah di sintesis atas pin polyethylene. Sebanyak 334 asid amino dalam set 46, peptida 14 mer dengan pertindihan 7 mer. Peptida dipilih dari hujung karboksil. Pilihan peptida antigenik dilakukan berdasarkan analisa hidrofilik, aksesibiliti permukaan serta struktur sekunder. Ketiga-tiga analisa dilakukan melalui program Protscale (<http://expasy.hcuge.ch/cgi-bin/protscale.pl>). Di samping itu, analisa mobiliti serta struktur koil juga dilakukan merujuk kepada metoda Protscale. Enam kawasan antigenik dikenali. Daripadanya, peptida 44 menunjukkan reaktiviti tertinggi dengan antibodi IgG dengan mengenali 24 daripada 25 sampel sera yang dikaji. Peratus reaktiviti sebanyak 96% diperolehi.

Dalam sampel air liur yang dikaji, 3 didapati IgA positif dan bertindakbalas dengan 5 peptida utama HHV6B. sampel air liur didapati negatif dalam immunopendafluoran tetapi kesan positif bila ditindakkan dengan peptida. Ini mungkin segmen peptida yang di kenali oleh IgA semasa ELISA peptida tidak berada di permukaan sel yang diinfeksi. Kemungkinan ini disebabkan struktur konformasi protein. Bila dalam keadaan asal, permukaan protein berada dalam keadaan tersembunyi tetapi bila dilinearkan, dapat dikesan.

Enam daripada sampel susu yang positif terhadap IgG dan 3 negatif terhadap IgA di dalam kaedah immunopendafluoran telah dikaji dengan kaedah pin-ELISA. Kedua-dua set sampel itu telah bertindakbalas dengan segmen peptida yang sama kecuali untuk

peptida 13. Hanya sampel susu yang positif terhadap IgA yang bertindak dengan peptida 13.

Memandangkan hanya antibodi IgG dan bukan IgA yang dikenali dalam darah tali pusat, kehadiran IgA dalam susu ibu mungkin menyumbangkan terhadap imuniti pasif.

Kawasan antigenik utama bagi IgG dan IgA didapati adalah peptida 44 dan 13 masing-masing. Didapati kedua-dua peptida tersebut adalah hidrofilik dan mempunyai aksesibiliti permukaan. Ia juga mempunyai mobiliti yang tinggi dan terdiri daripada struktur koil. Peptida 44 mempunyai struktur heliks-koil-heliks dan peptida 13 terdiri daripada struktur koil-heliks-koil.

Kesimpulannya, kajian ini menunjukkan bahawa kedua-dua peptida 44 dan 13 merupakan segmen antigenik berpotensi.

ACKNOWLEDGEMENTS

I would like to extend my heartfelt thanks and gratitude to my two supervisors Assoc. Prof. Dr. Sam Choon Kook and Prof. Dr. Cheng Hwee Ming for their constant assistance and support. This thesis would never have reached its completion if not because of their encouragement and guidance.

Many thanks also to Dr. Chua Kaw Bing for supplying the HHV6B stock used in the present study. I am particularly indebted to Dr. Kula and his staff of the Maternity Ward, University Hospital for their co-operation in making this study a success.

I am truly grateful also to the staff and students of the NPC lab especially Chandrika, Padmaja, Siew Choo, Uncle Chong, Kien Hui and Shabana for their friendly support and encouragement. This acknowledgement would be incomplete without thanking my dearest friends Shamini, Renu and Vinder for their companionship, encouragement and faith in me.

To the staff of IPSP, especially Cik Zubaidah and the staff of IRPA, a very big thank you. Thank you also to the University of Malaya for the financial assistance received.

Finally, to my most beloved family, I love you and thank you for your love, encouragement and understanding. Without you all, I would never have made it through this project. This thesis is a dedication to them.

Lastly, to the Lord Almighty for His blessings and for watching over me at all times.

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ABBREVIATIONS

ACIF	:	anti-complement immunofluorescence assay
AIDS	:	Acquired Immunodeficiency syndrome
CNS	:	central nervous system
CPE	:	cytopathic effect
CSF	:	cerebrospinal fluid
DIC	:	diisopropylcarboimide
DMF	:	dimethylformamide
ELISA	:	enzyme-linked immunosorbent assay
ES	:	exanthem subitum
FCS	:	fetal calf serum
HCBMC	:	human cord blood mononuclear cells
HHV6A	:	human herpes virus 6A
HHV6B	:	human herpes virus 6B
HBLV	:	human B-lymphotrophic virus
hCMV	:	human cytomegalovirus
HHV7	:	human herpes virus 7
HHV8	:	human herpes virus 8
hCG	:	human chorionic gonadotrophin
HOBt	:	1-hydroxybenzotriazole
IE	:	immediate early
IFA	:	immunofluorescence assay

IFN	:	interferon
Ig	:	immunoglobulin
kDa	:	kiloDalton
MCP	:	major capsid protein
MS	:	multiple sclerosis
nm	:	nanometer
OPD	:	orthophenylenediamine
PBMC	:	peripheral blood mononuclear cells
PBL	:	peripheral blood lymphocytes
PCR	:	polymerase chain reaction
rpm	:	rotation per minute
TNF	:	tumour necrosis factor
IU	:	international units
g	:	grams
L	:	litres
ml	:	millilitres
mg	:	milligrams
μl	:	microlitres
°C	:	degrees Celcius
μg	:	micrograms

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