HLA POLYMORPHISM IN MALAY SUB-ETHNIC GROUPS IN PENINSULAR MALAYSIA

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HLA POLYMORPHISM IN MALAY SUB-ETHNIC GROUPS IN PENINSULAR MALAYSIA

by

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LIST OF ABBREVIATIONS

A	Adenine
bp	Base pair
C	Cytosine
CLIP	Class II-associated invariant-chain peptide
CTL	Cytotoxic T-lymphocytes
ddATP	Dideoxyadenosine triphosphate
ddCTP	Dideoxycytidine triphosphate
ddGTP	Dideoxyguanosine triphosphate
ddNTP	Dideoxynucleotide triphosphate
ddTTP	Dideoxythymidine triphosphate
DNA	Deoxyribonucleic Acid
dNTP	Deoxynucleotide triphosphate
dsDNA	Double strand DNA
EDTA	Ethylene diamine tetra acetic
G	Guanine
H_2O	Water
HS	Heavy strand
GVHD	Graft versus host disease
HLA	Human leukocye antigen
HWE	Hardy-Weinberg equilibrium
kb	kilo base
LINES	Long interspersed element sequences

M	Molar
$MgCl_2$	Magnesium chloride
MHC	Major histocompatibility complex
mM	millimolar
mtDNA	Mitochondrial DNA
MRCA	Most recent common ancestor
n	Number of individuals
NaCl	Sodium chloride
Na_2EDTA	Disodium ethylene diamine tetra acetic acid
ng	Nanogram
NaOH	Sodium hydroxide
NJ	Neigbor-Joining
PCR	Polymerase chain reaction
psi	Pound force per square inch
RNA	Ribonucleic acid
rpm	Revolution per minutes
T	Thymine
Taq	*Thermus aquatic*
TBE	Tris-borate-ethylene-diamine tetra acetic acid
Tris HCl	Tris-hydrochloric acid
RFLP	Restriction fragment length polymorphism
SBT	Sequence based typing
SINES	Short interspersed element sequences

SLP	Single locus polymorphism
SNP	Single nucleotide polymorphism
SSOP	Sequence specific oligonucleotide probe
SSP	Sequence specific primer
TAP	Transporter associated with antigen processing
TBE	Tris-borate-ethylene diamine tetra acetic acid
TCRs	T-cell Receptors
u	Unit
µg	microgram
µl	microliter
UV	ultra violet
VNTRs	Variable number of tandem repeats
Y-STRs	Y-chromosome short tandem repeats

LIST OF PUBLICATIONS

Journal

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Poster presentation

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HLA POLIMORFISMA DI KALANGAN KUMPULAN SUB-ETNIK MELAYU DI SEMENANJUNG MALAYSIA

ABSTRAK

Di dalam kajian ini, Human Leukocyte Antigen (HLA) kelas I dan II telah dianalisa dengan menggunakan kaedah pencetus penjujukan khusus (Sequence Specific Primer) di kalangan 176 individu yang tiada pertalian kekeluargaan dari 6 kumpulan sub-etnik Melayu di Semenanjung Malaysia: Kelantan (n=25), Minangkabau (n=34), Jawa (n=30), Bugis (n=31), Banjar (n=33) dan Rawa (n=23). Alel HLA yang biasa ditemui di kalangan semua sub-etnik ini adalah HLA-A*24 (26 – 48%), HLA-B*15 (22% - 41%), -Cw*07 (21% - 32%), DQB1*03 (25% - 55%) dan DRB1*12 (15% - 40%). Walaupun terdapat perbezaan yang spesifik di antara kumpulan sub-etnik Melayu ini, mereka menunjukkan hubungan yang rapat di antara satu sama lain dan juga kepada populasi lain di Asia. Melayu Banjar, Bugis dan Jawa tidak menunjukkan perbezaan yang nyata di antara satu sama dan ini mungkin kerana ketiga-tiga sub-etnik Melayu ini berasal dari kepulauan di sekitar Pulau Jawa. Di samping berkongsi haplotip yang mempunyai kekerapan yang tinggi, analisis filogenetik dan *principal coordinate (PCO)* menunjukkan kesamaan genetik antara Melayu Minangkabau dan Rawa. Ini dipercayai kesan dari asal-usul yang sama, iaitu dari Sumatera. Secara statistiknya, Melayu Kelantan menunjukkan perbezaan yang nyata dengan sub-etnik Melayu yang lain, juga pada kandungan haplotip yang biasa ditemui dan ini berkaitan dengan perbezaan asal-usul dan populasi lain yang mempengaruhi sub-etnik ini sepanjang masa. Analisa ke atas data HLA sub-etnik Melayu

secara statistik juga menemui parameter forensik yang meyakinkan untuk aplikasi forensik. Selain itu, data HLA dari kajian ini juga boleh digunakan untuk pembangunan vaksin, mencari penderma yang sesuai untuk pemindahan organ, kajian hubung kait penyakit dan juga sebagai panduan untuk program pencegahan penyakit di masa hadapan.

HLA POLYMORPHISM IN MALAY SUB-ETHNIC GROUPS IN PENINSULAR MALAYSIA

ABSTRACT

In this study, the Human Leukocyte Antigen (HLA) class I and II were examined through Sequence Specific Primer (SSP) typing in 176 unrelated individuals from 6 Malay sub-ethnic groups of Peninsular Malaysia: Kelantan (n=25), Minangkabau (n=34), Jawa (n=30), Bugis (n=31), Banjar (n=33) and Rawa (n=23). The common HLA alleles in all the sub-ethnic groups were HLA-A*24 (26 – 48%), HLA-B*15 (22% - 41%), -Cw*07 (21% - 32%), DQB1*03 (25% - 55%) and DRB1*12 (15% - 40%). The Malay sub-ethnic groups studied showed close relationship to each other and to Asian populations despite specific differences between them. Banjar, Jawa and Bugis Malays showed no significant differences to each other, which could be a result of their related origin from the islands around the Java Sea. Besides sharing in the most common haplotype found, phylogenetic and principal coordinate (PCO) analysis showed a genetic similarity between Minangkabau and Rawa Malays. This could be a consequence of their common origin from Sumatera. The Kelantan Malays, show statistical significant difference with the other groups and also revealed differences for the most frequent haplotypes which could be related to their different origin and the different populations influence along time. Statistical analysis on the Malay sub-ethnic groups HLA data also revealed credible forensic parameters for forensic applications. In addition, the HLA data obtained from this study can also be applied for vaccine development, searching for

suitable donor for transplantation, disease association studies and as a guideline for infectious disease prevention programs.

CHAPTER 1: INTRODUCTION

1.1 The Malays

Malaysia is situated at the geographic coordinates 1^0 to 7^0 latitude north of the equator and 100^0 to 120^0 of the east longitude within Southeast Asia (SEA). The total landmass of Malaysia is 329,847 km² consisting of thirteen states and three federal territories. West Malaysia which is also known as Peninsular Malaysia comprises of 11 states and 2 federal territories and separated from East Malaysia (Sabah and Sarawak) by the South China Sea (Figure 1.1).

According to the federal constitution of Malaysia, the term "Melayu" (or Malay in English) refers to a person who is practicing Islam and the Malay culture, speaks the Malay language and whose ancestors are Malays. The history and the origin of the present day Malays have been the subject of much speculation among scholars.

The linguistic and archaeological evidences suggested that the Proto-Austronesian speakers (the forerunner of Proto-Malays) were inhabitants of Taiwan around 4,000 to 3,000 B.C. Between 2,500 and 1,500 B.C, the first migration of "the forerunner of Proto-Malays" took place towards Borneo, Sulawesi, Central Java and Eastern Indonesia through the Philippines. The second migration of the "Proto-Malays" took place from central Java to Peninsular Malaysia through the Straits of Malacca between 1,500 and 500 B.C (Andaya, 2001, Hamid, 1991 and Hussien *et al*., 2007). Subsequently, an influx of many other population groups consisting of Arabs, Chinese, Indians and Siamese into

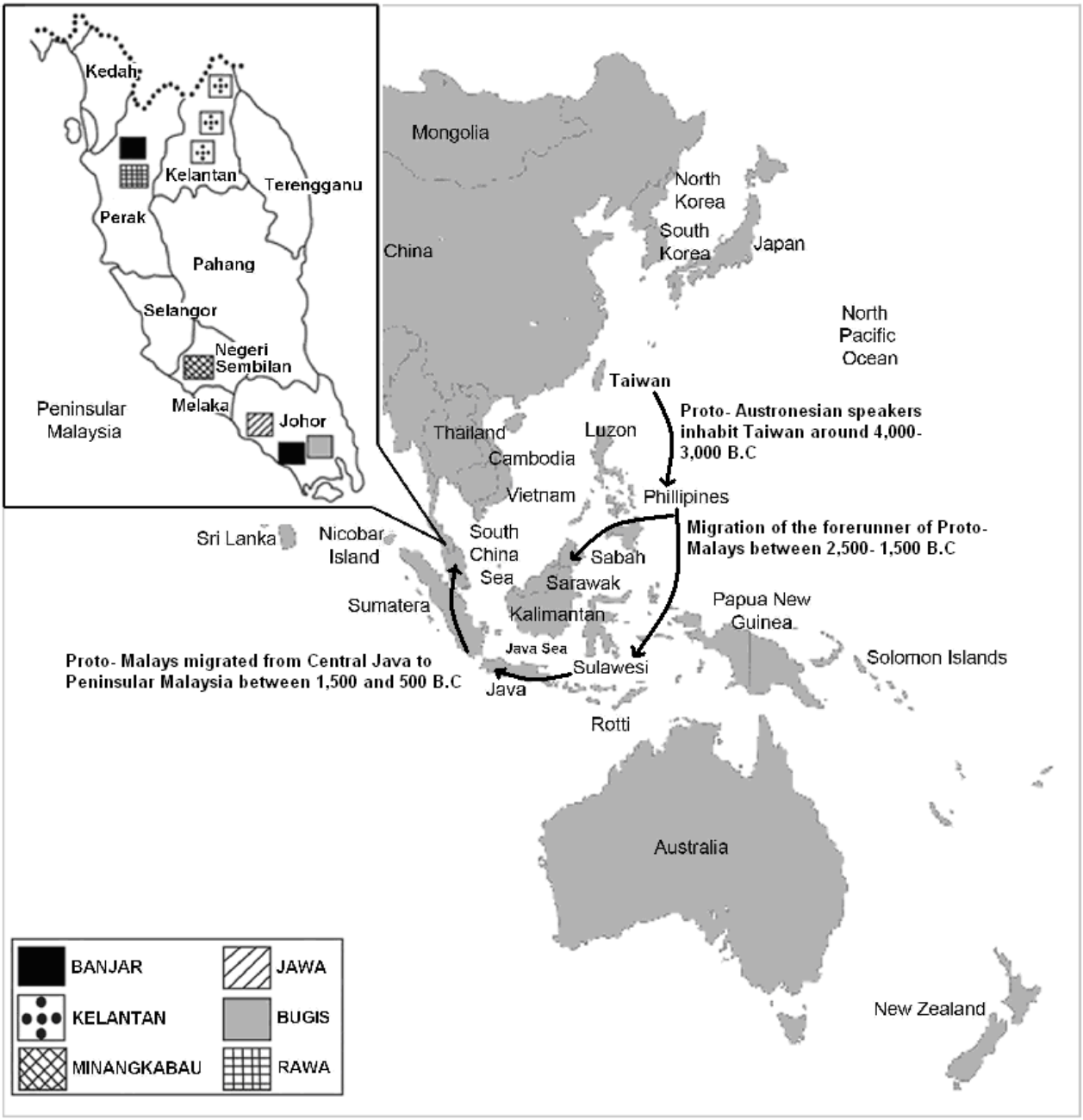

Figure 1.1: Map showing the geographic location of East and West Malaysia within South East Asia, international and state boundaries, where the samples were collected and the Malays migration pattern

Modified from: www.holcimfoundation.org/T536/A09_About_AP.htm and http://www.bioversityinternational.org/publications/Web_version/572/p191b.gif

Peninsular Malaysia occurred. They married the local Proto-Malays, resulting to a more diverse population known as the Deutero-Malays. This modern group inhabits mostly the coastal areas of the Malay Archipelago and had indirectly pushed the more primitive Proto-Malays into the rural and mountainous area (Hussien *et al*., 2007 and Sainuddin, 2003).

In the late nineteenth century, the migration of the Malays from the Indonesian Archipelago to Peninsular Malaysia occured, which was further enhanced during British colonization (Mohd Jali *et al*., 2003). Therefore, the Malays in Peninsular Malaysia are also the descendants of various ethnic groups from Kalimantan (Banjar), Java (Jawa and Bawean), Sulawesi (Bugis and Makasa) and Sumatera (Minangkabau, Batak, Rawa, Riau, Kerinci, Mandailing, Aceh, Siak, Inderagiri, Palembang and Kubu) (Sainuddin, 2003).

The Minangkabau Malays from Sumatera settled in Negeri Sembilan especially in Naning, Sungai Ujong and Rembau in the early 14th century (after the fall of the Sultanate of Malacca) while the Bugis Malays moved to Johor and Selangor in the last quarter of the 17th century after being expelled from Makasar by the Dutch (Hussien *et al*., 2007). The Rawa Malays which originated from Sumatera is commonly found in Gopeng, Perak while Banjar Malays which came from Banjarmasin, Kalimantan are more concentrated in Kerian and Parit Buntar (also in Perak), Sabak Bernam (Selangor) and Batu Pahat (Johor) (Mohd Jali *et al*., 2003). The drastic migration of Jawa Malays from Java occurred after 1884 when the Johor government welcomed immigrants to

open new districts in the state (Sainuddin, 2003). The Jawa Malays not only settled in Johor, but also in Selangor.

The Kelantan Malays, who are indigenous to the State of Kelantan in the northeast of Peninsular Malaysia were chosen as a sample group in this study based on their close relationship with populations to the North of the Peninsula Malaysia (Hussien *et al*., 2007 and Sainuddin, 2003). Moreover, Kelantan Malays seem to possess a higher number of Mongoloid markers compared to Modern Malays (Zafarina and Nurhaslindawaty, 2008).

Genetic markers based on phylogeography and ethnogenesis of the Malay sub-populations is very scanty. Recent studies on mitochondrial DNA (mtDNA) sequences have suggested that the people inhabiting the islands of Southern Asia (Indonesia, Malaysia, Borneo, Singapore and the Philippines) and those in Oceania (Melanesia and Micronesia together referred as Oceanians) were originated from the eastern regions of Austronesia. This refers to any area from Taiwan to the Philippines as well as south to eastern Indonesia (Lum *et al*., 1994, Melton *et al*., 1995 and Melton *et al*., 1998). Mack *et al*., (2000) reported the HLA class II allele frequencies distribution among the various Pacific/Asian populations (Hawaiian, Samoan, Malay, Papua New Guinea, Indonesia) using high resolution PCR-SSOP. They elucidated the similarities and differences that exist in the HLA class II allele distribution between these population groups.

Report on the distribution of allele frequencies (population database) on various genetic markers namely HLA (Bugawan *et al*., 1999, Dhaliwal *et al*., 2003, Dhaliwal *et al*.,

2007 and Koh and Benjamin, 1994), STR (Lim *et al*., 2001, Maruyama *et al*., 2008 and Seah *et al*., 2003) and mtDNA (Bekaert *et al*., 2006 and Zainuddin and Goodwin, 2004) for the Malay population have also been put forward. However, none of the studies focused on the Malay sub-ethnic population groups. Hence, the present preliminary study was undertaken to determine the HLA distribution to elucidate the inter-relationships between the various Malay sub-ethnic groups in Peninsular Malaysia.

1.2 HLA

The major histocompatibility complex (MHC) is located on the short arm of human chromosome 6 and codes for three classes of MHC; class I, II and III (Figure 1.2) (Guillemot, 1988). In human, the MHC is also known as the human leukocyte antigen (HLA) region. HLA class I molecules (HLA-A, HLA-B, HLA-Cw) are present on all nucleated cells while HLA class II molecules (HLA-DQ, HLA-DR and HLA-DP) are usually found on antigen presenting cells (APC) (Davies, 1997). Both, HLA class I and II molecules are synthesized in the endoplasmic reticulum (ER) and are involved in peptide presentation to T cells. HLA class I molecules bind antigenic peptides derived from endogenous antigens while HLA class II molecules bind antigenic peptides derived from exogenous antigens. The HLA class III molecules are not involved in T cell recognition and comprise components of the complement system (Nisonoff, 1987).

HLA class I molecules consist of a nonpolymorphic β_2-microglobulin (coded by the gene on chromosome 15) non-covalently linked to the highly polymorphic α-chain glycoprotein (Schwartz, 1991). The polymorphic α-chain is coded by three different major loci which determine the HLA-A, -B and -Cw types (Brodsky, 1997). HLA class II molecules consist of a heterodimer of two polymorphic transmembrane glycoproteins, α- and β-chains (Davies, 1997). The α- and β-chains for HLA class II are coded by three different major loci which determine the HLA-DP, -DQ and –DR types. The genes encoding the α- and β-chains are designated as HLA-DPA1 and HLA-DPB1 for HLA-

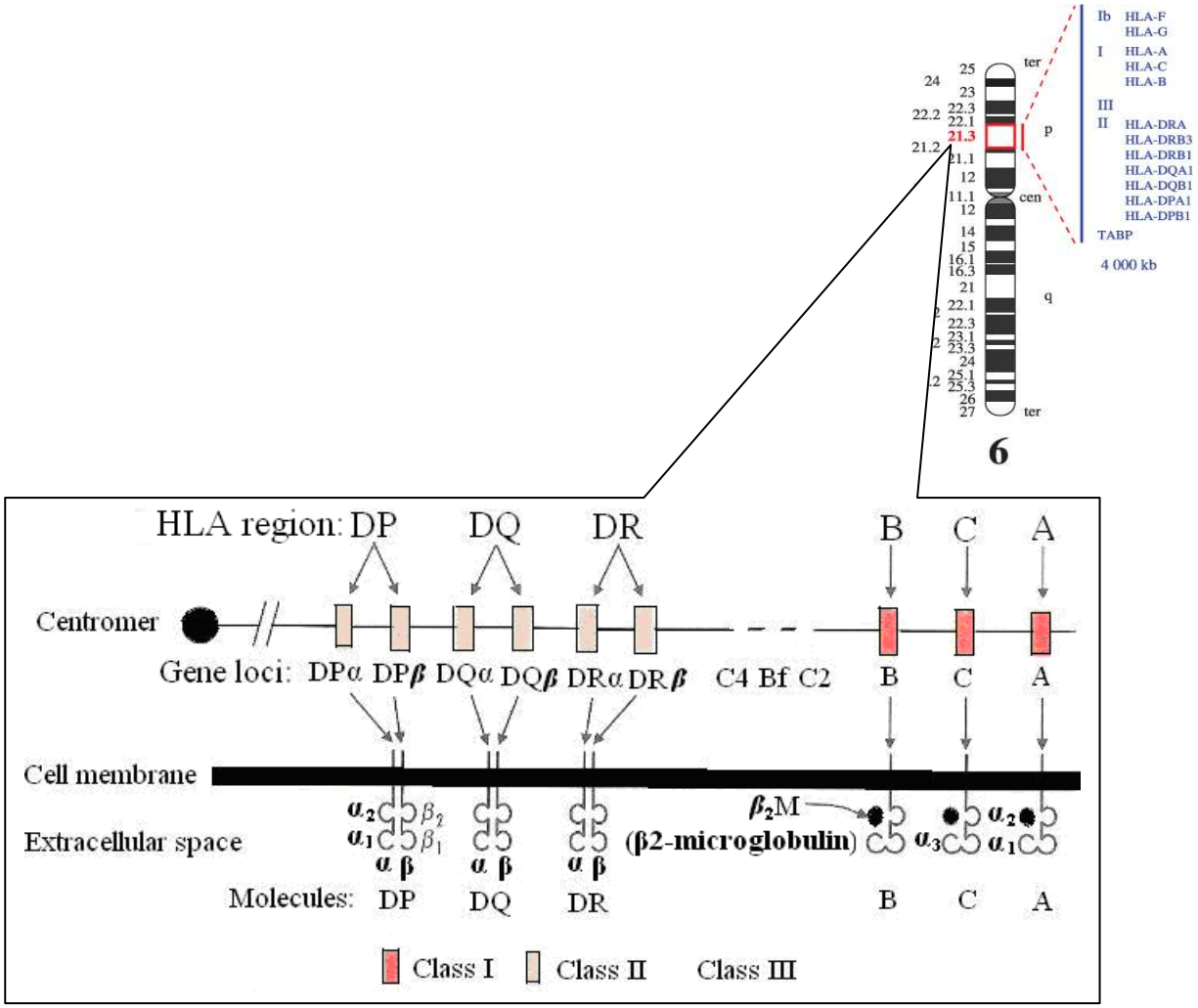

Figure 1.2: HLA region on the short arm of human chromosome 6

Modified from: Benjamini *et al*., 1996 and

http://imgt.cines.fr/textes/IMGTrepertoireMHC/LocusGenes/chromosomes/human/Hu_MHCchrom6.jpg

DP type, HLA- DQA1 and HLA-DQB1 for HLA-DQ type, and HLA-DRA1 and HLA-DRB1 for HLA-DR type. In addition to the common DRA1 and DRB1 genes, most of the haplotypes in the HLA-DR region express additional β-chains coded by either DRB3, DRB4 or DRB5 genes (Ishihara *et al*., 1995). The additional β-chains coded by HLA-DRB3, -DRB4 and -DRB5 genes are associated with corresponding α- and β-chains coded by HLA-DRA1 and HLA-DRB1 genes and no haplotype has more than one of these three genes (Marsh *et al*., 2000). The HLA-DRB3 gene was found to be associated with HLA-DR3 (DRB1*03), -DR5 (DRB1*11 and DRB1*12) and -DR6 (DRB1*13 and DRB1*14) haplotypes, HLA-DRB4 with HLA-DR4 (HLA-DRB1*04), -DR7 (HLA-DRB1*07) and -DR9 (HLA-DRB1*09) haplotypes and HLA-DRB5 with HLA-DR2 (HLA-DRB1*15 and -DRB1*16) haplotypes (Tiercy *et al*., 1992).

The allelic sequence diversity of HLA class I and II molecules are localized on the gene coding for the so-called peptide binding groove (Bona and Bonilla, 1990). The shuffling of these regions by recombinational mechanism generates extensive allelic diversity at these loci (Erlich and Gyllensten, 1991; Erlich *et al*., 2001). Except for the DRB3/4/5 loci, each individual can have up to 6 and 12 HLA class I and II alleles, repectively (Hertz and Yanover, 2006). An extraordinarily high level of allelic diversity in HLA class I and II regions (Appendix 15 to Appendix 20) can produce more than 80,000 different combinations of haplotypes (Bodmer, 1987).

The alphanumeric nomenclature has been developed for designation of HLA alleles in the International Histocompatibility Workshop (IHW) held by the World Health Organization (WHO). In this nomenclature, it starts with the letter denoting the locus,

followed by asterisk and then continues with two numbers to specify the allele group and two other numbers for the allele (Leffell, 2002). Synonymous nucleotide changes and non-coding allelic variations are shown by adding optional numbering (Beck and Trowsdale, 2000). For example, B*0735 is an allele of the locus HLA-B, belonging to the B7 antigen while 35 refers to one of the B*07 alleles.

1.2.1 Organization of HLA class I and II antigens and antigen presentation

1.2.1.1 HLA class I

HLA class I molecules (Figure 1.3 a) consist of α- and β-chains. The HLA class I α-chain is divided into three regions -an extracellular domain consisting α_1, α_2, α_3 domains, a transmembrane hydrophobic region and an intracellular hydrophilic region (Bona and Bonilla, 1990). Each of α_1 and α_2 domains consist of an α-helix and β-strands which associate together forming the peptide binding cleft (Figure 1.3 c) of HLA class I molecules (Guillemot, 1988). The α_3 domain provides structural support for HLA class I molecules through the interaction with β_2-microglobulin and with α_1 and α_2 domains (Kostyu *et al*., 1997). The non-covalent interaction of β_2-microglobulin domain with α-chain is important for facilitating transportation of HLA class I molecules to the cell surface and stabilizing the structure of HLA class I molecules (Brodsky, 1997).

The peptide binding clefts of newly synthesized HLA class I molecules bind to antigenic proteolytic fragments that are being transported into the ER (Figure 1.4) in an ATP dependent way by the transporter associated with antigen processing protein (TAP) (Tong *et al*., 2004). These antigenic proteolytic fragments are degraded by the action of proteasomes, mainly derived from antigenic proteins that have been endogenously synthesized (endogenous antigens) in the cytosol of the cell (Thorsby, 1999). The binding of antigenic peptides to peptide binding clefts of HLA class I molecules are facilitated by the ER protein, Tapasin (Momburg and Tan, 2002: Chaplin, 2003). The antigenic peptides associated with HLA class I molecules are then moved out to the

surface membrane from the Golgi apparatus (Davies, 1997). The antigenic peptides associated with HLA class I molecules on the cell surface form a ligand for T-cell receptors (TCRs) of $CD8^+$ T cytotoxic cell recognition (Schwartz, 1991).

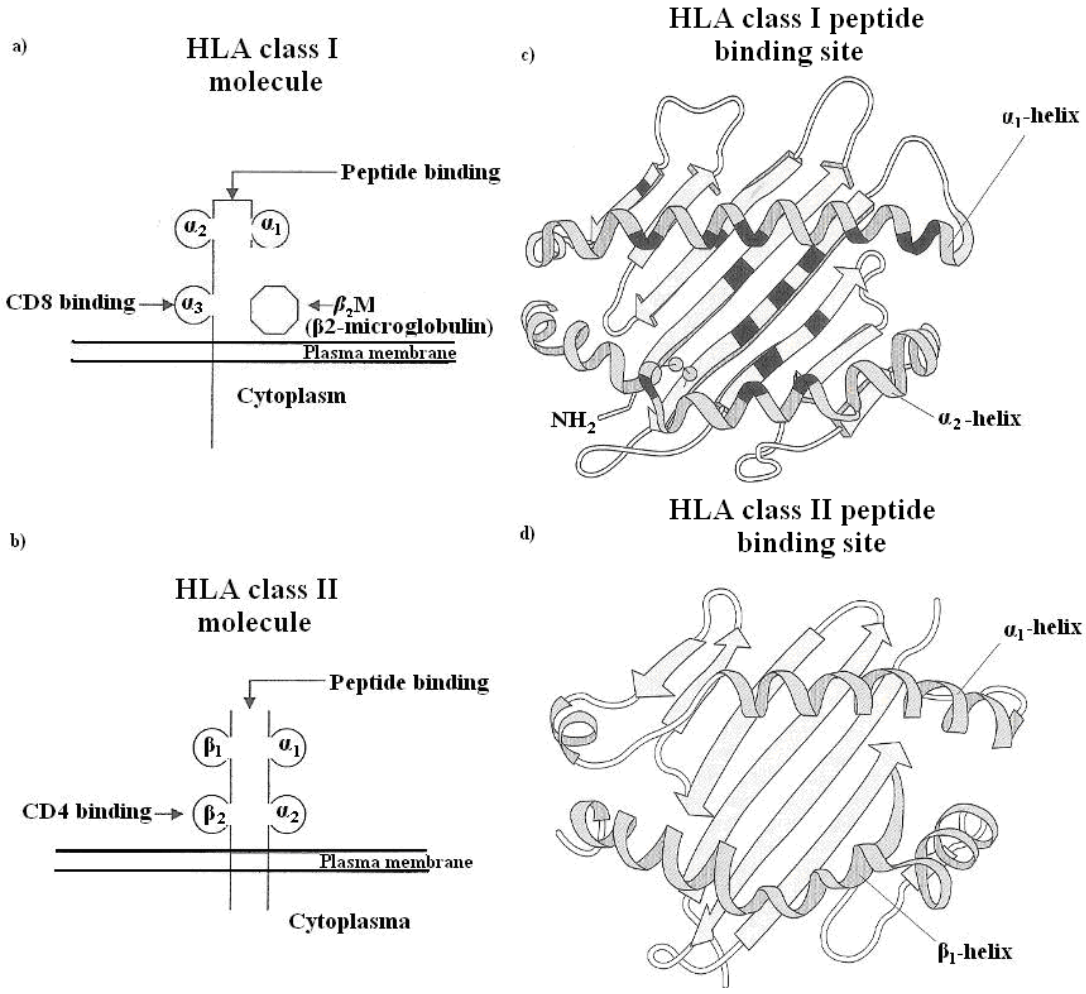

Figure 1.3: Structure of HLA class I (a) and II (b) with their corresponding peptides binding sites (c and d, respectively)

Peptide binding site of HLA class I molecule consisting of α helices (narrow coils), β sheets (broad arrows) and highly polymorphic residues among HLA alleles (black stripes). Structure of HLA class II molecule peptide binding sites consist of α_1-, β_1-helix and β strands.

Modified from: Brodsky, 1997 and Wise and Carter, 2002

1.2.1.2 HLA class II

HLA class II molecules (Figure 1.3 b) are cell surface heterodimers consisting of non-covalently bound α-chain (33 to 34 kd) and β-chain (28 to 29 kd) (Guillemot *et al*., 1988). Each of the HLA class II (HLA-DP, -DQ, -DR) α- and β-chains consists of a short cytoplasmic anchor, a transmembrane domain and two external domains (designated as α_1 and α_2 for α-chain and β_1 and β_2 for β-chain). The peptide binding groove (Figure 1.3 d) is formed by the α_1 and β_1 domains and structurally supported by the α_2 and β_2 domains (Konig *et al*., 1992; Chaplin, 2003).

In the ER (Figure 1.4), the invariant chains will bind to the peptide binding site of newly synthesized class II HLA molecules (Thorsby, 1999). The invariant chains promote correct assembly of α and β-chains of newly synthesized HLA class II molecules and prevent the binding of other peptides transported by TAP molecules in the ER (Davies, 1997). In the endosomal compartment, the invariant chain is digested with cellular proteinases and replaced with class II ligand (Brodsky, 1997). The class II ligands are processed exogenous antigens which are mainly derived from endocytosed plasma membrane proteins and extracellular fluid proteins (Davies, 1997). The HLA molecules-peptide complexes are then transported to the Golgi apparatus and transported to the cell surface by vesicles for $CD4^+$ T helper cell recognition (Benjamini *et al*., 1996).

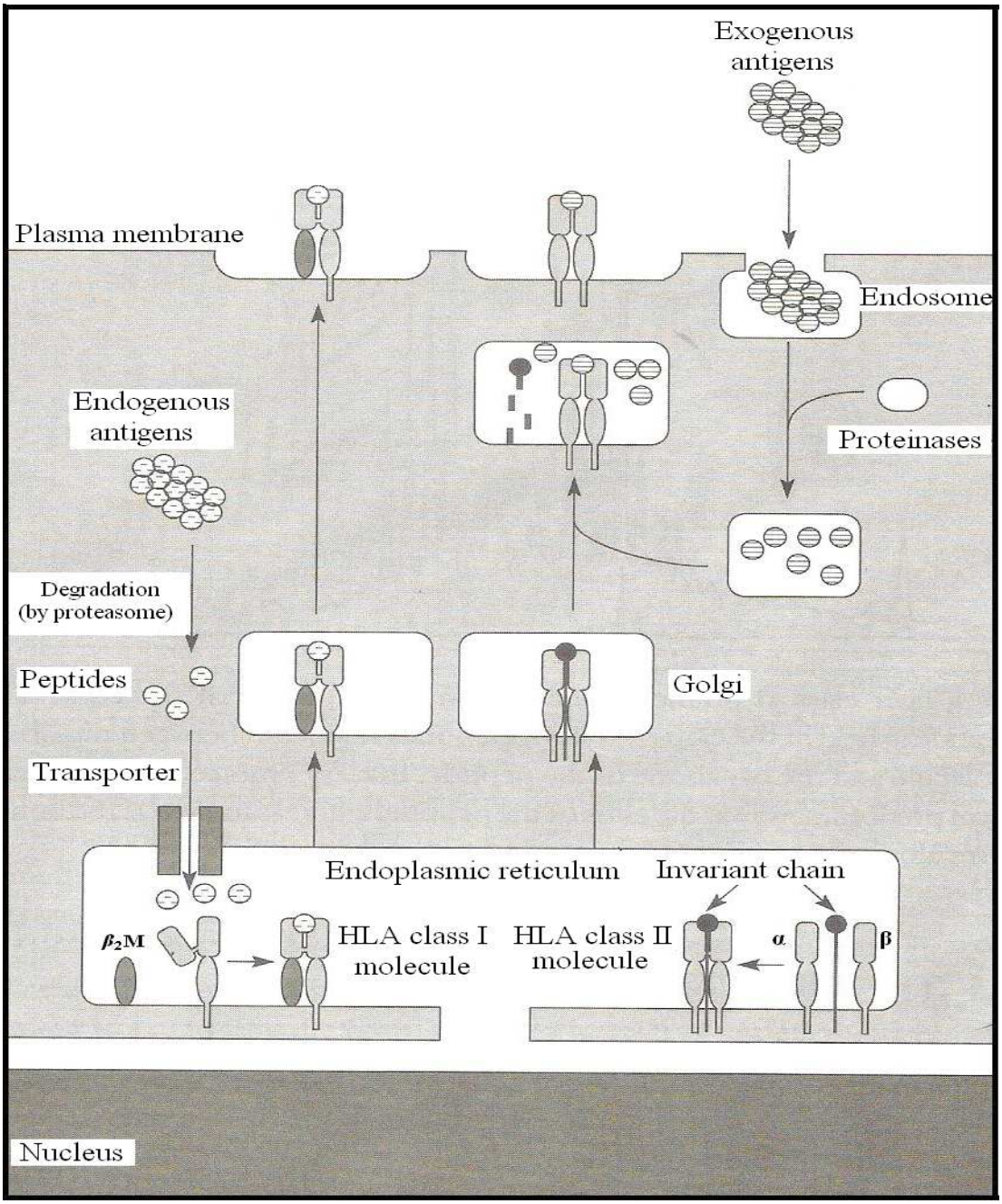

Figure 1.4: The pathway of endogenous and exogenous derived antigenic peptides presented by HLA class I and II to $CD8^+$ and $CD4^+$ T cells, respectively

Modified from: Davies, 1997

1.3 HLA Typing

Complement-dependent microcytotoxity, also known as serological method, is the first typing method used to detect polymorphism in HLA class I loci. This method uses specific HLA alloantisera to detect HLA-A, -B and –Cw in peripheral blood lymphocytes (Pacho *et al*., 2004). Later, the reaction patterns of refined B cells toward specific alloantisera were used to type HLA-DQ and –DR loci (Dyer *et al*, 2000). However, serological HLA typing depends on the adequate expression of HLA antigen on the cell surface, availability of a complete set of antisera, is time consuming and has limited level of resolution (Schaffer and Olerup, 2001).

The advents of molecular methods have provided more accurate and specific HLA characterization. The application of molecular methods for HLA typing started with cloning and characterization of the class I and II genes by recombinant DNA technology in the mid-70s and analysis of the extensive allelic sequence diversity of HLA loci by polymerase chain reaction (PCR) in the mid-80s (Erlich *et al*., 2001). As a result, DNA based HLA typing has increased allograft survival (Ferraz *et al*., 2002) and was found useful for searching suitable donor in the field of unrelated hematopoietic stem cell transplant (Pedron *et al*., 2005). In population studies, HLA databases created by powerful molecular methods provide an accurate interpretation of observed HLA variability and diversity, which can be used to investigate HLA differentiation among populations throughout the world (Sanchez-Mazas, 2001). Currently, restriction fragment length polymorphism (RFLP), sequence specific primers (SSP), sequence

specific oligonucleotide probe (SSOP) and sequence based typing (SBT) are among the molecular methods applied to HLA typing.

1.3.1 Restriction fragment length polymorphism-based HLA typing

Restriction fragment length polymorphism (RFLP)-based HLA typing was the earliest molecular method used for HLA typing. This method involved digestion of genomic DNA with specific restriction endonuclease. The digested fragments were then subjected to *in situ* denaturation after gel electrophoresis. These fragments were then transferred to hybridization membrane by suction or capillary action, where specific HLA alleles were determined by hybridization of radiolabeled genomic fragments or cDNA probes with single-stranded DNA on the membrane (Cann *et al*., 1983). This HLA typing method is relatively cumbersome, requires large amounts of high molecular weight genomic DNA and most of the restriction sites are not in the polymorphic region of HLA (Erlich *et al*., 2001).

1.3.2 Sequence specific primer-based HLA typing

The sequence specific primers (SSP)-based HLA typing requires a series of different PCRs to distinguish various combinations of HLA alleles. The amplified products are fractionated by agarose gel electrophoresis (Olerup and Zetterquist., 1992 and Olerup *et al*., 1993). The presence or absence of specific bands reflects the individual's HLA type. Despite requiring many amplification reactions, time consuming and difficult to be

automated, PCR-SSP typing is an effective method for routine laboratory practice for HLA typing (Casamitjana *et al*., 2005).

1.3.3 Sequence specific oligonucleotide probe-based HLA typing

The sequence specific oligonucleotide probe (SSOP)-based HLA typing makes use of specific primers to amplify polymorphic regions of HLA loci. In SSOP-based HLA typing, specific HLA alleles are determined by hybridization of labeled oligonucleotide probes to the immobilized denatured amplified DNA products (Erlich, 2000). These methods require longer processing time eventhough giving high output (Casamitjana *et al*., 2005).

1.3.4 Sequence-based typing of HLA

In sequence-based typing (SBT), the nucleic acid sequences of the HLA alleles carried by an individual are determined after locus specific PCR amplification and purification (Kotsch *et al*., 1999). With the availability of several different automated DNA sequencers and additional software for sequencing data analysis, SBT is often considered as the most definitive typing method (Leffell, 2002). This method is relatively expensive, especially for routine clinical typing and in large scale analysis.

1.4 Application of HLA

1.4.1 Application of HLA in human health

HLA has important applications in human health. Some of HLA roles related to human health are as follows:

I. Susceptibility and resistance toward infectious disease

Appropriate immune response toward infectious disease is mounted once TCRs of T cells recognize antigenic peptide presented by HLA molecules (Brusic *et al*., 2002). Since HLA molecules bind to specific antigenic peptide epitopes, the resistance and susceptibility toward diseases depend on the efficiency of individual HLA alleles carried by individual. In addition, individuals who are heterozygous at a particular HLA locus may mount vigorous immune response compared to homozygous individuals (Lipsitch *et al*., 2003). This is because, individuals with HLA homozygous at one or more loci may have decreased numbers of HLA alleles in combating infectious disease (Trachtenberg and Erlich, 2001). Therefore, HLA alleles carried by individuals can be used to study and predict their susceptibility and resistance toward infectious disease.

II. Transplantation

Transplantation is a graft of cells, tissues or organs between a donor and a recipient. In order to avoid graft rejection and graft versus host disease (GVHD), the donor and recipient must have a negative T and B cell donor specific cross-match and should be compatible for HLA and ABO blood group antigens (Reilly and Ray, 2006). Careful

ABO and HLA cross-matching can prevent hyperacute rejection of the graft caused by preformed antibodies within a few minutes to hours of taransplantation (Benjamini *et al*., 1996). The pre-existing of antibodies against HLA class I in the recipient can be produced by a previous transplant, blood transfusion and exposure to allogenic lymphocytes during pregnancies (Nairn and Helbert, 2002). The incompatibility of HLA type between the donor and recipient may cause acute and chronic rejection. Following transplantation, acute rejection takes place within a few days to two weeks while chronic rejection occurs in months (Wise and Carter, 2002). Acute and chronic rejection occurs due to direct and indirect allorecognition of circulating T cells, respectively. Direct allorecognition take place when the recipient's $CD4^+$ and $CD8^+$ T cells directly recognize foreign peptide/HLA class I and II complexes expressed in the cell membrane of the transplanted cells (Garavoy *et al*., 1991). The indirect allorecognition has been described in section 1.2.1.1 and 1.2.1.2.

Incompatible HLA between donor and recipient may also cause GVHD which predominantly occur in bone marrow transplantation. GVHD is an immune response initiated by donor T cells against foreign HLA molecules of the recipient (Baker, 2000). However, the occurrence of transplantation rejection and GVHD can be reduced with the application of DNA-based HLA typing between the recipient and donor. In addition, the strategies of searching unrelated donors for transplantation will be greatly enhanced with the identification, characterization and compilation of HLA alleles and haplotypes (HLA databases) from various ethnics and racial groups (Tang *et al*., 2007).

III. Vaccine development

The success of vaccination requires activation of T and B cells which depend on the presentation of antigenic peptides by HLA molecules (Ada and Ramsay, 1997). The peptide binding motif vary between HLA molecules and only peptides containing position-specific amino acids bind to this peptide binding motif (Brusic *et al*., 2002). However, the HLA alleles can be grouped into a set of supertypes (sets of alleles that bind to similar peptides) which are important in the development of epitope-based vaccines for high population coverage (Hertz and Yanover, 2006).

IV. HLA and disease studies

The ability to resist infectious diseases is dependent on the immune responses of individuals which are genetically determined by selected HLA alleles (Brodsky, 1997). In contrast, the expression of particular HLA alleles may also predispose individually towards certain diseases. There are two different approaches to study the relationship between HLA and diseases - population and family studies. Family studies involve relatives of a family who reveal genetic linkage of HLA loci with disease, while population studies involve samples from unrelated individuals which provide information regarding association between HLA loci and disease (Svejgaard and Ryder, 1977). Several studies have been published on the relationship between disease and HLA alleles. The linkage of HLA-B*27 and -DR4 alleles in familial spondyloarthropathy (Said-Nahal *et al*., 2002) and the linkage of HLA-DQB1*0302 and -DQB1*0201 alleles in type I diabetes (Santos *et al*., 2001) are among family studies that have been carried out to relate the linkage of HLA alleles with diseases. In contrast the association of HLA-Cw*0602 allele in patients with psoriatic arthritis (Gladman *et*

al., 1999), HLA-A*02 and -B*61 alleles in Japanese patients with abdominal aortic aneurysm (Sugimoto *et al*., 2003), HLA-Cw*02-B*27 haplotype in South Indian patients with spondyloarthropathies (Thomas *et al*., 2006) and association of the HLA-DR2, -DQB1*0501 and -DQB1*0601 alleles in the Malay patients with systemic lupus erythematosus (Azizah *et al*., 2001) are among population studies that have been carried out to relate the association of HLA alleles/haplotypes with diseases.

1.4.2 Application of HLA in forensics

The detection of a small number of single nucleotide polymorphisms (SNPs) in the HLA-DQA1 gene became the first PCR-based technique applied in forensic DNA test (Hart, 2001). In 1986, Cetus Corporation developed the first commercial and validated PCR-based typing which detected 28 HLA-DQA1 alleles for forensic applications (Walsh *et al*., 1991; Buckingham and Flaw, 2007). The PCR-based HLA-DQA1 typing is a rapid and novel approach for degraded and tiny amount of sample analysis but has a major disadvantage in terms of the low power of discrimination (Rudin and Inman, 2002).

Most of the criminal cases usually encounter degraded and tiny amounts of samples which are impossible for DNA based HLA typing even for a single locus. Some other technical challenges and issues such as proper interpretation of HLA typing results, appropriate population database for probability calculations, method validation, quality control and assurance should also be taken into consideration before DNA based HLA typing can be established for forensic applications (Wu and Csako, 2006). In addition, in some cases, HLA are not informative enough due to linkage disequilibrium and the predominance of certain HLA alleles (Grubic *et al*., 2004).

Currently, STR analysis is the most commonly used for forensic DNA analysis. STRs can be typed using degraded DNA samples, are highly polymorphic, provide sufficient discrimination power for forensic caseworks and up to 16 loci can be typed simultaneously by multiplex PCR (Wu and Csako, 2006). In addition, various countries

have their own STRs databases for probabilities calculation which is used as an intelligent tool for crime prevention and investigation. For example, 'second-generation multiplex' (SGM) plus system and FBI Laboratory's Combined DNA Index System (CODIS) are the STR databases used in United Kingdom and United State of America, respectively.

Eventhough STR analysis is dominating forensic DNA analysis, HLA analysis has the potential to be used in combination with STR analysis due to the several factors below:

I. Super polymorphic markers

The HLA is a superpolymorphic genetic marker. The number of officially known HLA class I and II alleles are 1180 and 732, respectively (Marsh *et al*., 2005). The extreme number of HLA alleles for HLA class I and II produce powerful forensic statistical parameters for criminal identification and kindship analysis (Jiang *et al*., 2006).

II. Analysis of tiny amounts and degraded samples

Traditionally, analysis of HLA relied on serological technique. This technique depends on the availabity of HLA class I and II antigens on the cells, suitable antisera for HLA antigen recognitions and of course, is inappropriate for degraded crime scene samples. Currently, DNA based HLA typing such as RFLP, SSOP, SSP and SBT has dominated most of the field of HLA studies either for forensic or medical applications. Studies done by Ota *et al*., (2006) and Sato *et al*., (2003) (both using SSP-based HLA typing) found that the application of DNA based HLA typing is possible even for a very degraded and tiny amount of DNA samples.

III. Simultaneous typing of multiple polymorphic loci

One of the reasons for STRs to be established in forensic caseworks is the capability of multiplex PCR to analyze multiple polymorphic loci of STRs system. The advances in new platforms of molecular testing such as microsphere-based genotyping and microarray has improved earlier molecular techniques such as RFLP-, SSP-, SSOP- and SBT-based HLA typing. The new platform of molecular testing techniques which are cost effective, time efficient and able to perform simultaneous genotyping for an almost unlimited number of loci are slowly dominating HLA genotyping (Wu and Csako, 2006).

IV. Mode of inheritance and linkage disequilibrium

The parent of a child can be identified utilizing genetic markers. These markers occur in pairs and are passed from each parent to the child. For each marker, one is inherited from the mother (the maternal marker or allele) and the other one is inherited from the father (the paternal marker or allele). The same goes for HLA alleles where HLA alleles in each of the HLA locus of a child should be paternally or maternally inherited. The well established modes of inheritance of HLA alleles are very important in forensic caseworks especially for paternity testing (Hawkins, 1997).

Linkage disequilibrium is predominant among the pairs of HLA loci. Certain combination of HLA class I and II are inherited more frequently than expected and one can predict the presence of other alleles with the presence of specific linkage allele (Ferrer *et al*., 2005). This phenomenon decreases the informativeness of HLA loci for