CHAPTER II

LITERATURE REVIEWS

2.1 Human Leukocyte Antigen

2.1.1 Genomic Organization of the Human MHC

The human major histocompatibility complex (MHC), or human leukocyte antigen (HLA) consists of three major classes of gene called HLA class I, class II and class III. The complex of genes are encoded on the short arm of human chromosome 6 (6p21) as presented in Figure 2.1 (Francke and Pellegrino, 1977). These gene regions span approximately 3,800 kilobases of DNA. The class I region is located at the telomeric end of the complex, the class II region is at the centromeric end, and the class III region is between the other two (Figure 2.2).

The class I region comprises the classical genes of HLA-A, HLA-B, and HLA-C and the non classical genes, or pseudo-genes, of HLA-E, HLA-F, HLA-G, HLA-H, HLA-J, HLA-K, and HLA-L (Bodmer et al., 1997). The HLA-A, HLA-B and HLA-C loci encode the heavy chains of class I molecules. Some of the non-classical class I genes are polymorphic, for example HLA-G and MICA.

The class II region consists of a series of sub-regions, each containing A and/or B genes encoding α and β chains, respectively (Bodmer et al., 1997). The DR, DQ, and DP sub-regions encode the major expressed products of the class II region. The HLA-DR and HLA-DQ genes are so close (recombination frequency < 2%) that they usually inherited haplotypes together (Hansen et al, 1992). In addition to the loci encoding the class II regions, several other genes are involved in antigen processing

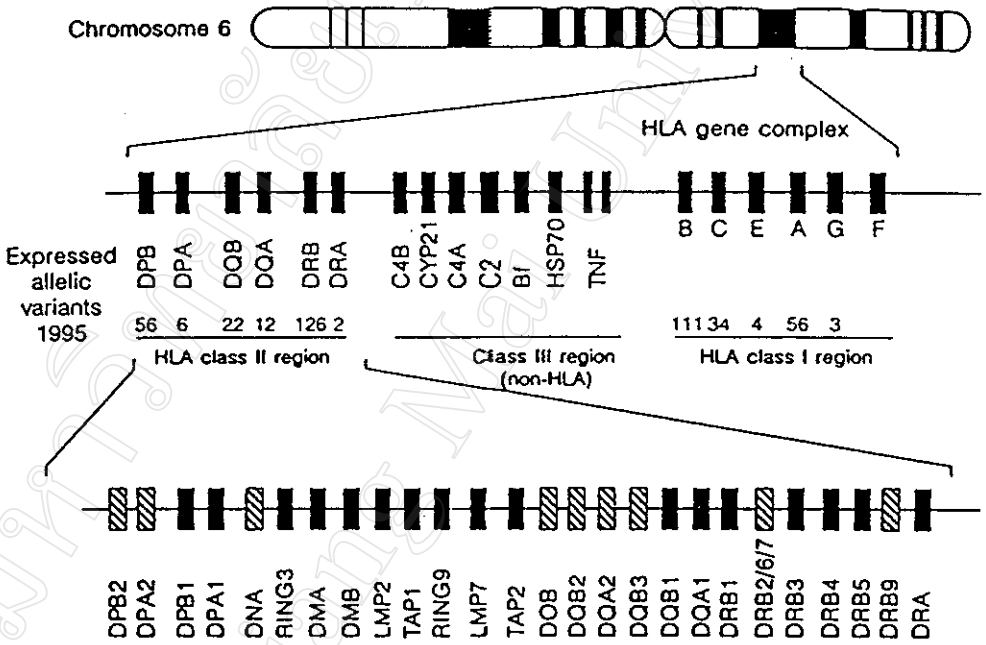

Figure 2.1 The HLA gene complex on the short arm of chromosome 6 (Howell and Navarrete, 1996).

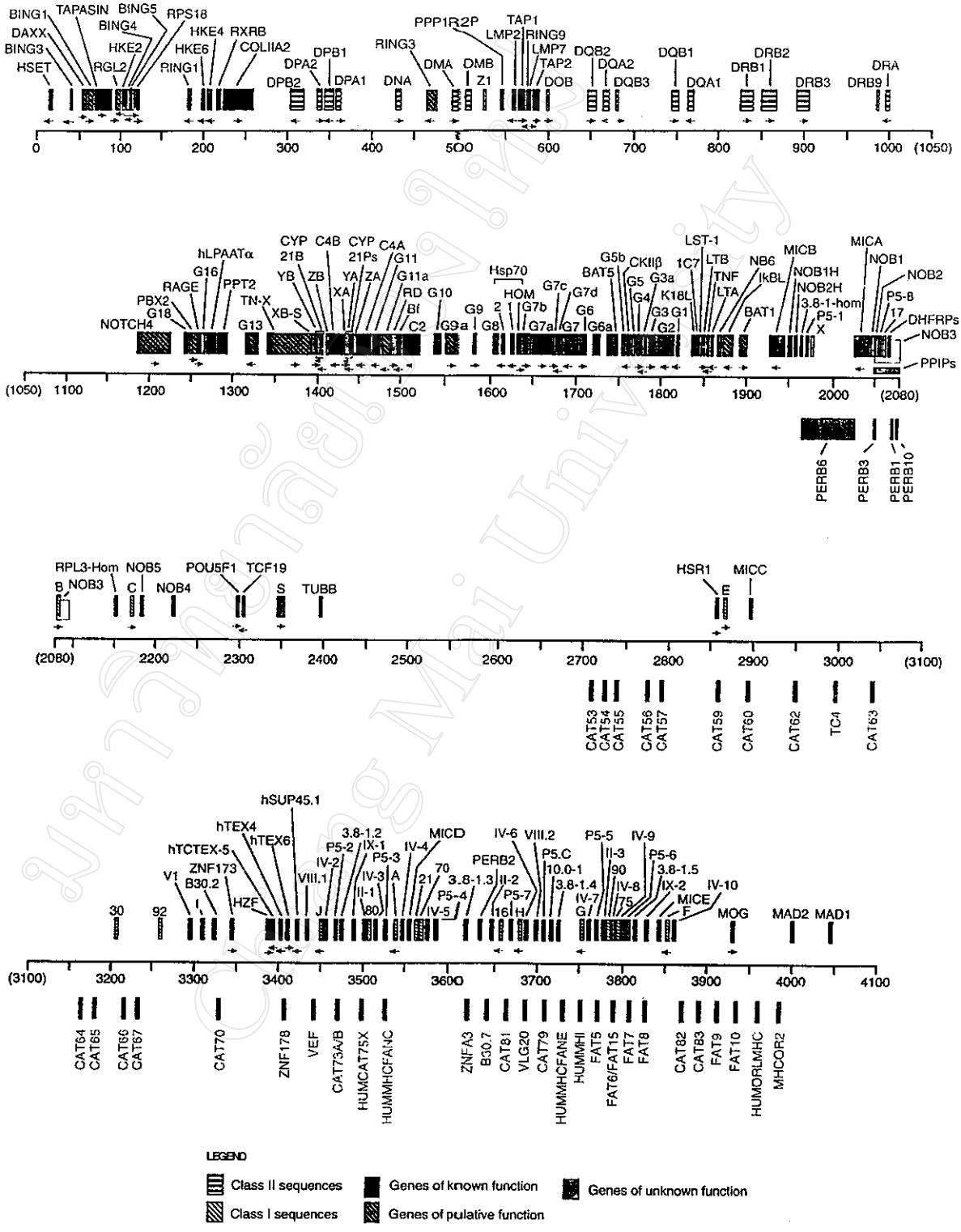

Figure 2.2 Map of the human major histocompatibility complex (Milner et al., 2000).

and peptide loading. These include peptide transporters (TAP1 and TAP2) and sub units of a large cytoplasmic proteosome (LMP2 and LMP7). The DR gene family consists of a single DRα gene and multiple DRβ genes (DRβ1 to DRβ9). Different HLA haplotypes contain particular numbers of DRB loci. The DRB1, DRB3, DRB4, and DRB5 loci are usually expressed, and the other DRB loci are pseudogenes. The DRA locus encodes an invariable α chain and it binds various β chains. DR antigen specificities are determined by the polymorphic DRβ1 chains encoded DRB1 alleles. The DQ and DP families each have one expressed gene for α and β chains and a second pair of genes that may not be functional. The DQA1 and DQB1 gene products associate to form the DQ molecule, and the DPA1 and DPB1 products form DP molecules (Table 2.1).

The HLA class III region does not encode HLA molecules, but contains a rather diverse collection of over 20 genes, including those for complement components (C2, C4, factor B), 21-hydroxylase, tumor necrosis factors (TNFα , TNFβ), and some that are involved in the processing of an antigen (Roitt et al., 1996).

2.1.2 Structure and Polymorphism of HLA Molecules

The human leukocyte antigen is a set of tightly linked genes of cell surface glycoproteins, which is involved in the regulation of the immune response (Sachs, 1984). The HLA system is divided into two different sets of highly polymorphic cell surface structures: HLA class I and HLA class II. The class I molecules consist of glycosylated heavy chains of 44-45 kDa and a noncovalently bound extracellular 12 kDa β2-microglobulin (β2m) (Bjorkman et al., 1987). Human β2m is invarient and encoded by a non-MHC gene from chromosome 15 (Maffei et al., 1997). The HLA class I heavy chain has three extracellular domains (α1, α2 and α3), a transmembrane

Table 2.1 Name for genes in the HLA region (Bodmer et al., 1999).

Name	Previous	Molecular characteristics
HLA-A	-	Class I α-chain
HLA-B	-	Class I α-chain
HLA-C	-	Class I α-chain
HLA-E	E, '6.2'	associated with class I 6.2-kB Hind III fragment
HLA-F	F, '5.4'	associated with class I 5.4-kB Hind III fragment
HLA-G	G, '6.0'	associated with class I 6.0-kB Hind III fragment
HLA-H	H, AR, '12.4'	Class I pseudogene associated with 5.4-kB Hind III fragment
HLA-J	cda12	Class I pseudogene associated with 5.9-kB Hind III fragment
HLA-K	HLA-70	Class I pseudogene associated with 7.0-kB Hind III fragment
HLA-L	HLA-92	Class I pseudogene associated with 9.2-kB Hind III fragment
HLA-DRA	DRα	DR α chain
HLA-DRB1	DRβI, DR1B	DR β1 chain determining specificities DR1, DR2, DR3, DR4, DR5 etc.
HLA-DRB2	DRβII	Pseudogene with DR β–like sequences
HLA-DRB3	DRβIII, DR3B	DR β3 chain determining DR52 and Dw24, Dw25, Dw26 specificities
HLA-DRB4	DR βIV, DR4B	DR β4 chain determining DR53
HLA-DRB5	DRβIII	DR β5 chain determining DR51
HLA-DRB6	DRBX, DRBσ	DRB pseudogene found on DR1, DR2 and DR10 haplotypes
HLA-DRB7	DRBι1	DRB pseudogene found on DR4, DR7 and DR9 haplotypes
HLA-DRB8	DRBι2	DRB pseudogene found on DR4, DR7 and DR9 haplotypes
HLA-DRB9	M4.2 β-exon	DRB pseudogene, isolated fragment
HLA-DQA1	DQα1, DQ1A	DQ α chain as expressed
HLA-DQB1	DQβ1, DQ1B	DQ β chain as expressed
HLA-DQA2	DXα , DQ2A	DQ α-chain-related sequence, not known to be expressed
HLA-DQB2	DXβ , DQ2B	DQ β–chain-related sequence, not known to be expressed

Table 2.1 Name for genes in the HLA r egion (Bodmer et al., 1999). (continued.)

Name	Previous	Molecular characteristics
HLA-DQB3	DVβ , DQB3	DQ β-chain-related sequence, not known to be expressed
HLA-DOA	DNA,DZα ,DOα	DO α chain
HLA-DOB	DOβ	DO β chain
HLA-DMA	RING6	DM α chain
HLA-DMB	RING7	DM β chain
HLA-DPA1	DPα1, DP1A	DP α chain as expressed
HLA-DPB1	DPβ 1, DP1B	DP β chain as expressed
HLA-DPA2	DPα2, DP2A	DP α-chain-related pseudogene
HLA-DPB2	DPβ2, DP2B	DP β–chain-related pseudogene
TAP1	RING4, Y3, PSF1	ABC (ATP Binding Cassette) transporter
TAP2	RING11, Y1, PSF2	ABC (ATP Binding Cassette) transporter
LMP2	RING12	Proteasome-related sequence
LMP7	RING10	Proteasome-related sequence
MICA	MICA, PERB11.1	Class I chain-related gene
MICB	MICB, PERB11.2	Class I chain-related gene
MICC	MICC, PERB11.3	Class I chain-related pseudogene
MICD	MICD, PERB11.4	Class I chain-related pseudogene
MICE	MICE, PERB11.5	Class I chain-related pseudogene

region formed by disulphide binding and an intracyte plasmic domain. Each extracellular domain comprises about 90 amino acids. The α1 and α2 domains contain variable amino acid sequences and they determine the serologic specificities of class I antigens. Three dimensional structures of the extracellular portion of several HLA class I molecules have been revealed by x-ray crystallography, as shown in Fig.2.3. A groove is formed by the two α-helices and the β–pleated floor, and this is the binding site for a processed peptide fragment of around 8 or 9 amino acid residues in length (Bjorkman et al., 1987). HLA class I molecules are expressed on the surface of virtually all cells except for mature erythrocytes and placental trophoblasts; and also found on platelets. Only some class I antigens have been found on red cells (Howell and Navarrete, 1996). HLA class I molecules present foreign antigens to CD8+ T lymphocytes that act mainly as cytotoxic cells (Figure 2.4). Peptides associated with class I molecules derive from endogenously synthesized proteins using the host cell's machinery, such as a virus infected cell or cell expressing foreign alloantigens (Brodsky et al., 1996).

The HLA class II molecules are the heterodimers of the two non-covalently associated glycosylated polypeptide acidic α chain of 30 to 34 kDa and the β chain of 26 to 29 kDa. The α and β chains are transmembranes, which are composed of two extracellular domains: α1, α2 and β1, β2. The α1 and β1 domains form antigen-binding grooves between 15 to 20 amino acid residues, which consist of a long groove formed by the floor of the beta-pleated segment and the surrounding of the alpha helical segment (Rudensky et al., 1991). The three dimentional structures of the HLA class II molecule are similar to those of the HLA class I molecule, as shown in Fig. 2.5. The peptide binding region from different individuals exhibits a structural polymorphism that enables them to bind to structurally distinct selective peptides. The α2 and β2 domains are similar to immunoglobulin constant domains and they are

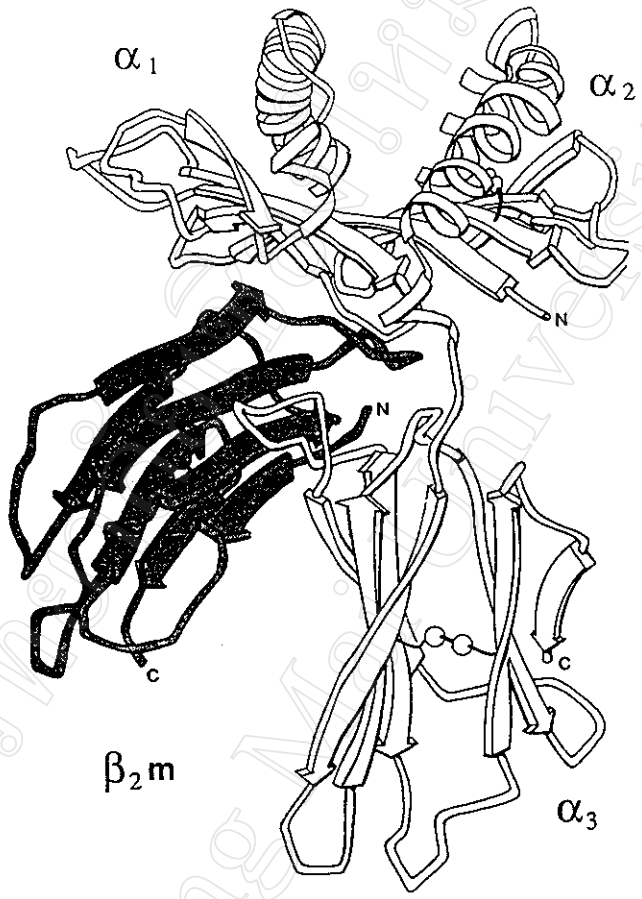

Figure 2.3 MHC class I molecular structure (Virella, 1998).

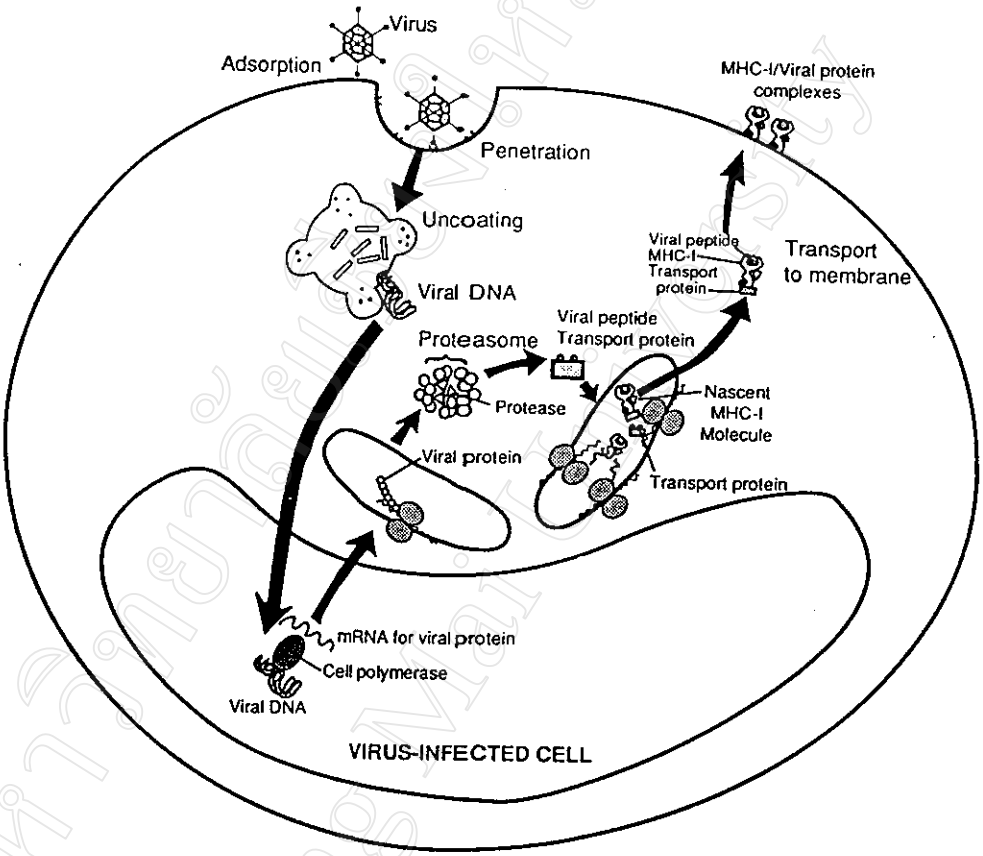

Figure 2.4 Diagram of MHC class I molecule presented peptides to CD8+ T lymphocytes (Virella, 1998).

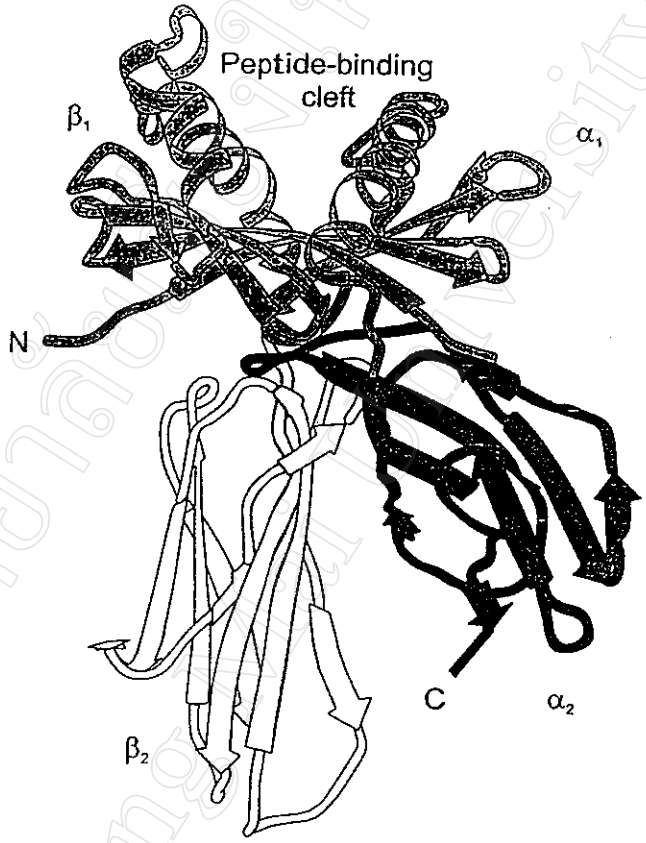

Figure 2.5 MHC class II molecular structure (Cruse and Lewis, 1999).

highly conserved. Both α1 and β1 domains contain a highly hydrophilic connecting peptide that links the membrane proximal domain to the hydrophobic transmembrane domain. HLA class II molecules are expressed on B lymphocytes, antigen-presenting cells (monocytes, macrophage and dendritic cells, thymic epithelium) and activated T lymphocytes (Abbas, 1991). These cells are presented peptides antigen to the receptors of T cells. The class II HLA molecules present antigens, such as those derived from bacteria, which pass through the exogenous pathway to the CD4+ T lymphocyte (Figure 2.6), that functions as T-helper cells (Long, 1989).

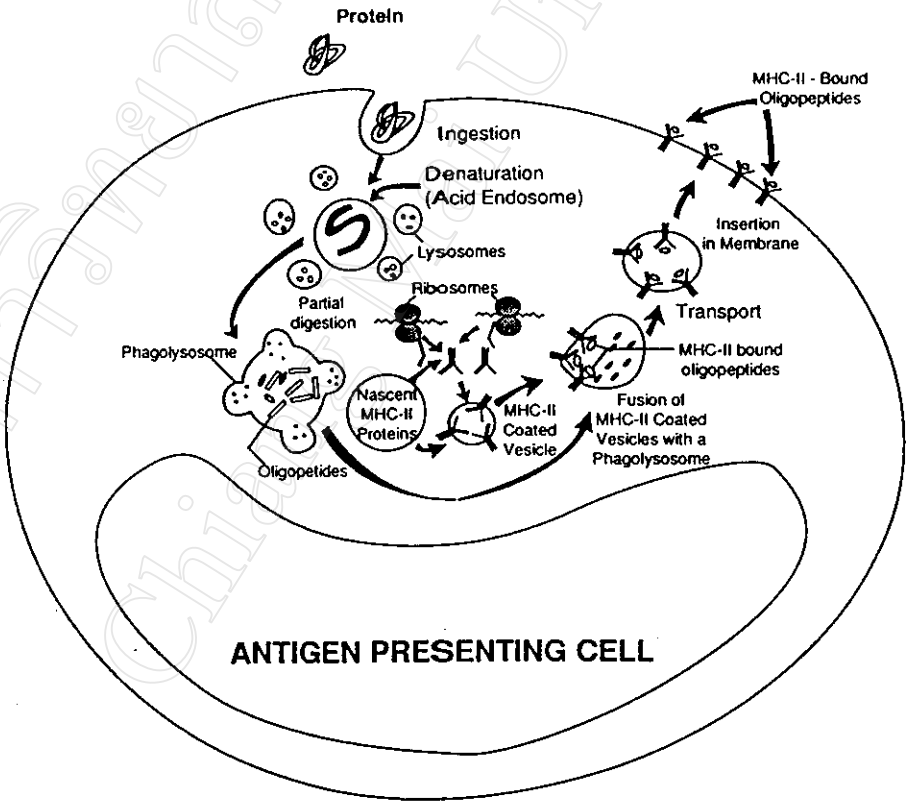

Figure 2.6 Diagram of MHC class II molecule presented peptide to CD4+ T lymphocytes (Virella, 1998).

2.2 HLA Typing

2.2.1 Serological Typing of HLA Antigens

In the late 1950s, leukoagglutination was initially tested for HLA antigens by clinicians who were interested in blood typing (van Rood, 2000). The transfused patients produced antibodies that were agglutinated leukocytes, and different donors gave various leukoagglutinin patterns. Since that time, there has been an explosion of interest and understanding of the human leukocyte antigen (HLA) system.

In 1960, the cytotoxicity test replaced the leukoagglutination one because it was more reproducible and generally applicable (van Rood, 2000). The complement-dependent microlymphocytotoxicity test developed by Terasaki and McClelland (1964) has been used as standard for serologic typing of HLA class I and HLA class II antigens. The lymphocytes of an individual are isolated and mixed with a serum of known HLA specificities. The antibody will bind to the corresponding HLA antigen on the surface of the lymphocytes. Complement obtained from rabbit serum is added. During the incubation period, the cells are injured by the process of cytolysis. Damage to the cell membrane can be detected by the addition of vital dye. The dead cells' permeable membranes allow the dye to enter. Cells that have no attached antibody, no activated complement, and no damaged permeable membrane keep the vital dye from penetrating. The HLA-ABC or DR typing can then be determined by the pattern of antisera reactions.

2.2.2 Cellular Typing

The mixed lymphocyte culture (MLC) is perhaps the most widely used of cellular assays by mixing peripheral blood mononuclear cells from two individuals in

vitro and observing cellular proliferation (Mickelson et al., 1993). The one-way MLC test is performed by mixing responder T cells with irradiated stimulator cells. After 5 or 6 days, the level of DNA synthesis resulting from responder cells is determined by pulsing the culture with tritiated thymidine. Reactive MLC results indicate that the HLA-D region is incompatible.

Although MLC is extremely useful in assessing the overall proliferative response to stimulation, it cannot be used to define cellular specificities or distinguish the reactivity to individual gene products.

MLC has been used clinically for donor selection, predominantly bone marrow transplantation. With the advent of DNA-based HLA-typing methods, MLC is used less often for donor selection, but more frequently for following the recipient's post transplant donor antigen-specific immune status (Kaminski et al., 1988).

2.2.3 Molecular Typing of HLA Alleles

Serological and cellular typing have been utilized to define HLA types. However, there are limitations to these techniques. It is known that serological typing has been considered the gold standard in HLA typing, but limitations of the test are as follows: (i) Serological testing depends on the strength and specificity of the serologic reagents used in the assay. It is difficult to maintain consistent sensitivity and specificity from lot to lot of the reagent that came from the same source and may be in short supply because it derived from humans. Although, monoclonal antibodies are used instead of human sera, their consistency is a big problem. (ii) Viable cells are required for the lymphocytotoxicity test, and the samples must be submitted to the laboratory as quickly as possible after collection. (iii) The quality of the complement is required for good results. (iv) It is difficult to obtain the appropriate cells for typing from some patients ; such as those with Leukemia, Aplastic anemia, chronic renal

failure etc. (v) Cross-reactivity is observed by using serological typing. (vi) Variants or subtypes of HLA alleles are indistinguishable by serological typing.

Although the MLC was used to identify the difference between potential donors and recipients, there are many limitations to the test, as follows: (i) Sufficient numbers of the appropriate live cells must be available for testing. (ii) The test is difficult to interpret when there is a high background level of cellular proliferation. (iii) There is inconsistency and poor reproducibility from test to test to generate and maintain cellular typing reagents. (iv) The MLC could be influenced by the health of the patient, type of disease, medications the patient has received and a history of prior transfusions.

For these reasons the assays of HLA are being replaced by DNA-based typing methods. The advantages of DNA-based testing include the following: (i) The specificity of each DNA typing reagent is clearly defined based on a specific nucleotide sequence. (ii) DNA typing reagents are synthetic oligonucleotides, there is no lot to lot variation in specificity and they are not limited. (iii) The test can be carried out at several levels of resolution, depending on the typing requirements and the time available. (iv) DNA typing does not require live cells and only a small amount of samples are required. (v) The results of the test are not influenced by the health of the patient.

Molecular HLA typing of class II began in 1980 (Bidwell et al., 1988). The first technique used was a restriction fragment length polymorphism (RFLP) southern blotting analysis. RFLP analysis is based on nucleotide sequence locus specific probes, which can detect a specific pattern of restriction endonucleases that recognize and cut certain DNA sequences. The human genomic DNA is digested with an appropriate restriction enzyme, and the digested DNA fragments are resolved by electrophoresis. The DNA is then transferred onto nitrocellulose and is hybridized with

labeled locus specific oligonucleotide probes. The HLA alleles can be identified by autoradiography. Although RFLP can identify HLA alleles more finely than the serologic methods, the number of restriction sites recognized by a given enzyme is limited in each gene and, thus, only a few selected sequence polymorphisms are detected. The precision of the defined allele depends on the quality of the enzymes. RFLP analysis is no longer used for clinical typing.

In recent advances in molecular genetics, the polymerase chain reaction (PCR) using Taq DNA polymerase became available to amplify and study HLA genes (Uryu et al.,1990). Synthetic oligonucleotide primers were designed to initiate DNA amplification of specific HLA genes. PCR-based HLA typing was developed using the sequence-specific oligonucleotide probe (SSOP or SSO) technique (Figure 2.7) (Tiercy et al., 1991). The polymorphic sequences of HLA genes are amplified from genomic DNA by PCR reaction. The amplified PCR products are reacted under stringent hybridization conditions with a panel of synthetic oligonucleotide probes corresponding to variable regions of the genes. The SSO hybridization requires the knowledge of the DNA sequence of recognized alleles. The SSO probes can discriminate down to a single base pair difference of the HLA alleles. This method is rapid, more efficient and appropriate for distinguishing multiple DNA sequence variations and comfortable for large batches of DNA samples, but it is unsuitable for a low number of tests. Disadvantages of the test include potential difficulty in the interpretation of results and the need to use multiple filters to perform collective hybridizations.

A variation of this technique, the reverse dot blot hybridization (Figure 2.8), eliminates the need for multiple filters and hybridizations by the incorporation of a label into the PCR product during its amplification from genomic DNA (Buyse et al., 1993). The PCR product is then hybridized to a membrane containing SSOs, and its

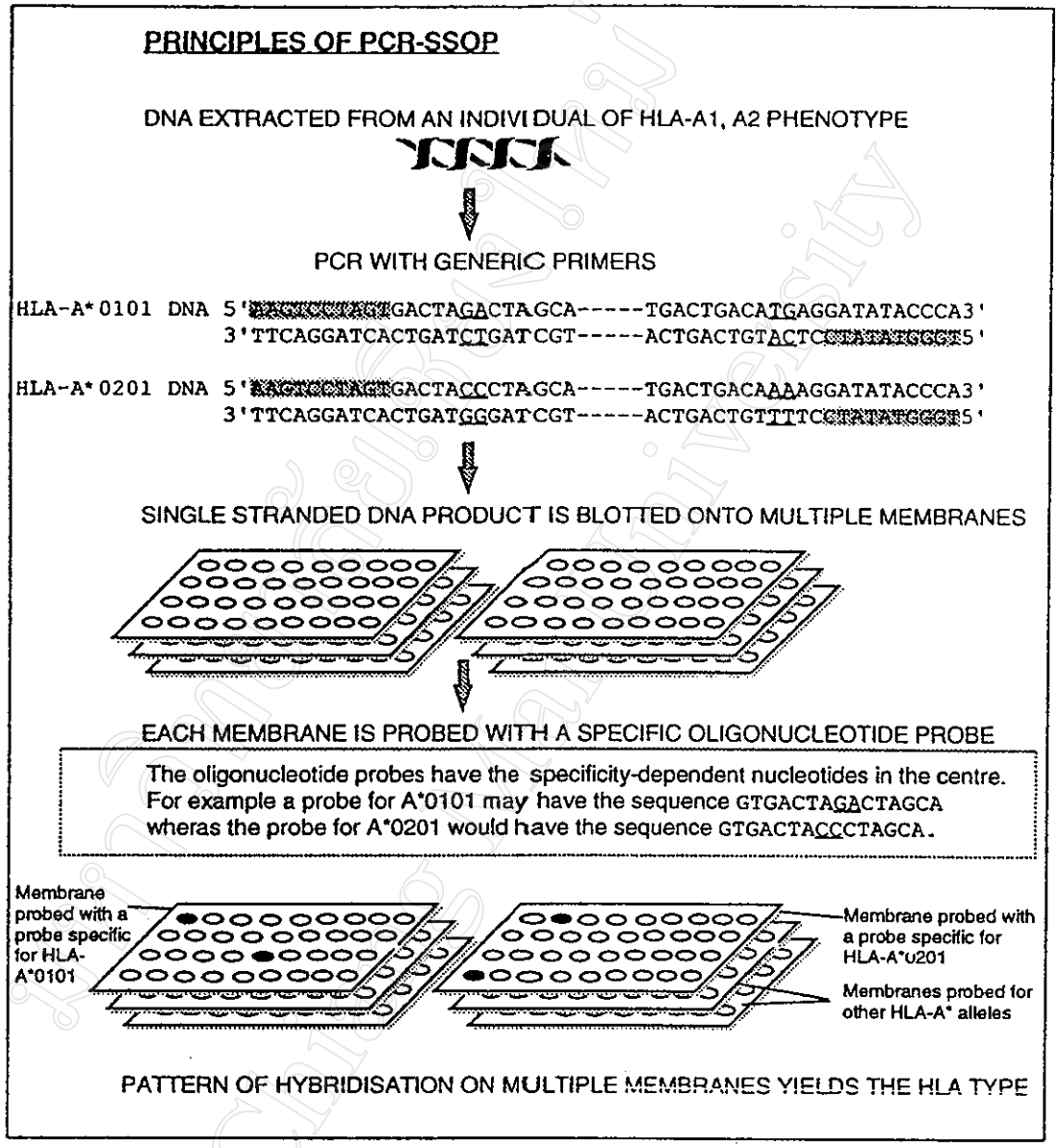

Figure 2.7 Principles of PCR-SSOP (Bunce et al., 1997).

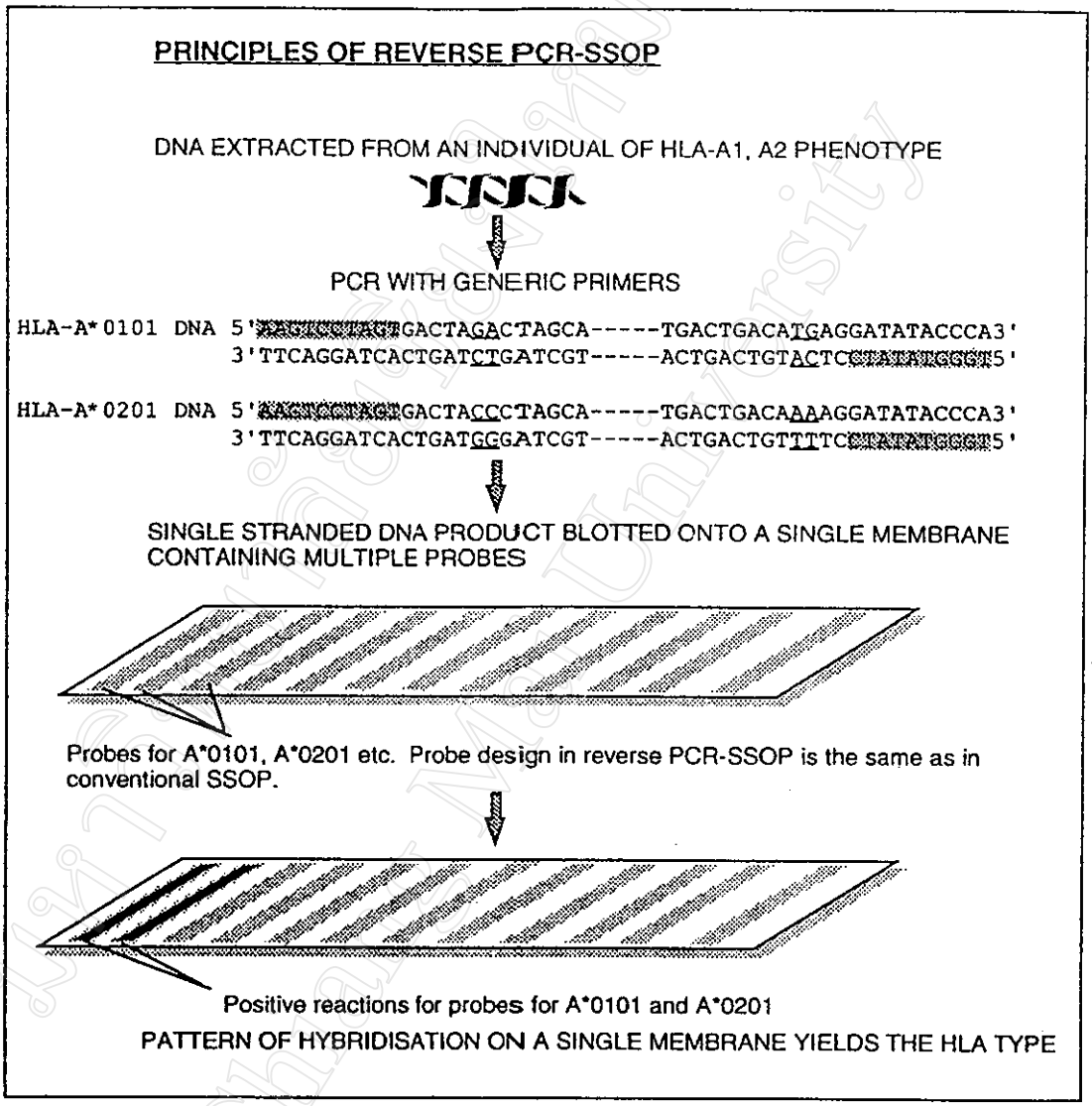

Figure 2.8 Principles of reverse PCR-SSOP (Bunce et al., 1997).

pattern of hybridization with the SSOs is revealed by the detection of the incorporated label. This method is more comfortable, but expensive for routine HLA typing.

Alternatively, polymorphic DNA sequences can be used as amplification primers by using sequence-specific amplification. This strategy of DNA typing is called the sequence-specific primers (SSP) method (Figure 2.9), and it detects sequence polymorphism at given areas by the presence of a particularly amplified DNA fragment (Bunce et al., 1997).

PCR-SSP specificity derived from matching the terminal 3'-nucleotide of the primers with the target DNA sequence. Taq DNA polymerase can extend 3'-matched primers, but not 3'-mismatched ones. Consequently, only target DNA that are complementary to both primers are efficiently amplified. PCR-SSP works because Taq DNA polymerase lack 3' to 5' exonucleolytic proof-reading activity (Tindal and Kunkel, 1988).

The first comprehensive PCR-SSP HLA typing system was described by Olerupand Zetterquist (1992) for low resolution HLA-DRB1 typing, including group-specific detection of DRB3 and DRB4 using 19 PCR-SSP reactions. This technique was applied to detect the polymorphism in other class II loci such as HLA-DQA1, DQB1 (Olerup et al., 1993) and DPB1 (Knipper et al., 1994), and in class I loci for HLA-A, HLA-B and HLA-C. In 1995, Bunce and co-workers from Oxford Transplant Center reported the PCR-SSP system (Phototyping) allowing simultaneous detection of HLA-A, B, C, DRB1, DRB3, DRB4, DRB5 and DQB1 alleles in a single assay (Bunce et al., 1995).

The PCR-SSP has an overall resolution equivalent to high-quality serological typing for HLA and takes only 2-3 hours to complete, making it suitable as the sole method for genotyping cadaver donors. The development of automatic equipment has facilitated the use of PCR-SSP in many laboratories by using a single PCR-SSP

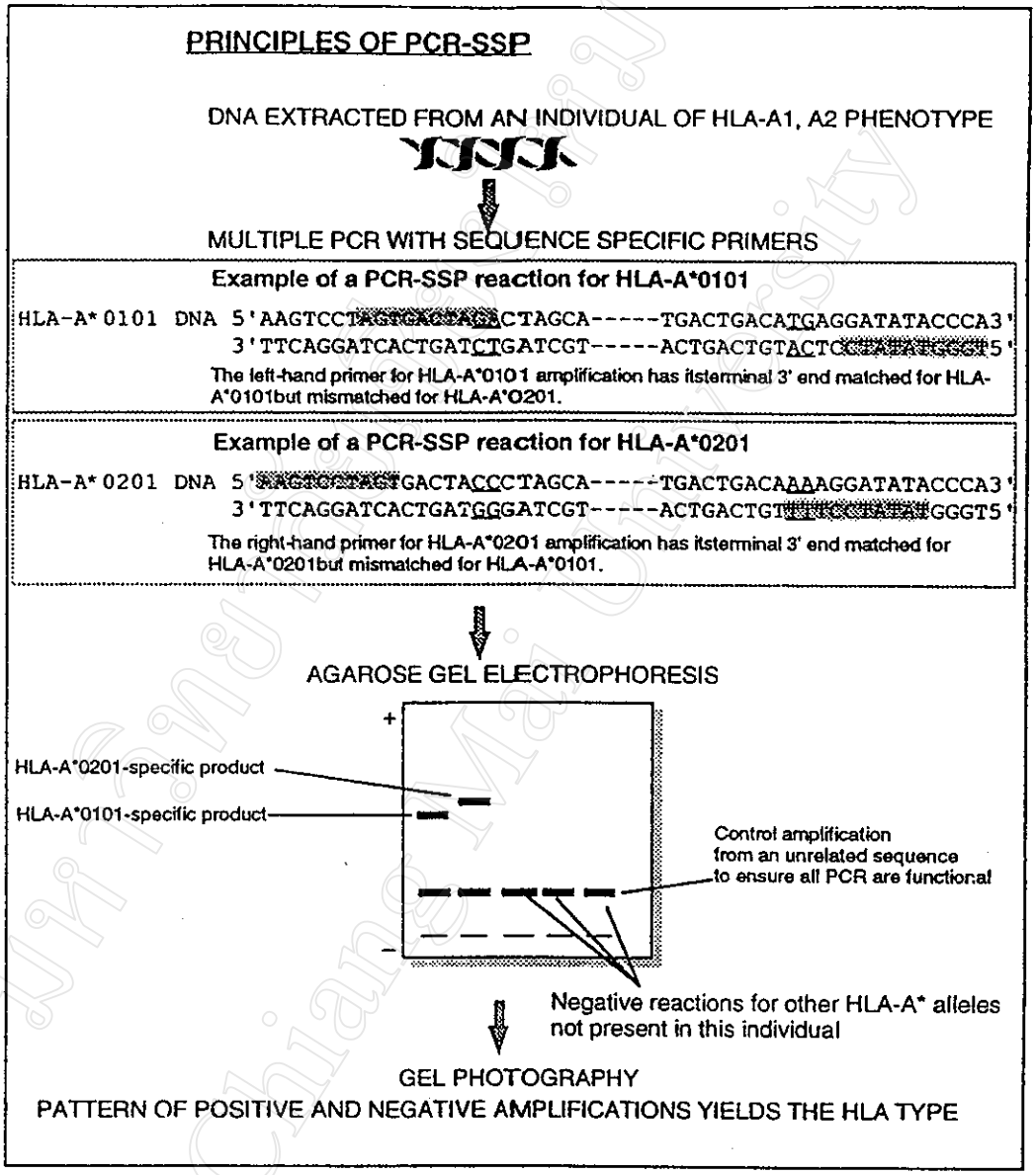

Figure 2.9 Principles of PCR-SSP (Bunce et al., 1997).

protocol and one PCR thermocycling program without changing any parameters. This greatly facilitates a routine for HLA and non-HLA genes.

One advantage that PCR-SSP has over PCR-SSO methods is that PCR-SSP detects polymorphism linked on an individual chromosome (cis), whereas PCR-SSO detects polymorphism on both DNA chromosomes (cis or trans) (Welsh and Bunce, 1999). In this PCR-SSP technique, a potentially new allele is mapped by using a sense primer that recognizes the allelic variant in combination with multiple anti-sense primers in multiple reactions. It has been used successfully to identify many new HLA class I (Barnardo et al., 1997) and class II alleles (Aldener and Olerup, 1993), which were proved correct by sequencing.

PCR-SSP and PCR-SSO methods are limited within the context of existing sequence information: new alleles may be missed by both PCR methods. On the other hand, new alleles are often identified by PCR-SSO and PCR-SSP by unique patterns; but these always require confirmation by sequencing before a new allele is accepted by the nomenclature committee.

The principles of sequence-based typing (SBT) are that the polymorphic regions of any given allele are amplified by flanking PCR primers. Group specific amplification is performed in order to limit the number of alleles in any sequencing template, otherwise DNA from both haplotypes would be present. The resulting PCR product is sequenced by any one of a variety of subtype methods and analyzed by computer to ascertain the type. Computer analysis is required because the sequenced product from a heterozygous individual will contain two superimposed sequences that need to be aligned with all previously known sequences in order to be identified and separated. Suitable computer software is one of the greatest problems with this technique and the most critical step is the amplification reaction (Bunce et al., 1997).

SBT was initially described for HLA-DRB1, DQB1 and DQA1 by Santamaria et al (1992) and for HLA-DPB1 by Rozemuller et al (1993). SBT is admirably suited to class II typing because the vast majority of the functional polymorphism is located in exon 2. SBT of class I exon 2, 3 and 4 was first described by Santamaria et al (1993). Elegant alternative SBT approaches for class I have been developed that concentrate on exon 2 and 3, where the majority of class I polymorphisms are located (Petersdorf and Hansen, 1995). SBT is the only technique which detects the nucleotide sequence of an allele directly, thus, allowing an exact assignment. The main drawbacks of SBT are the equipment costs and the time required to fully sequence one individual. However, at present, allele-specific typing whether by SBT, SSO, or SSP present the researcher with a powerful analysis tool.

HLA-DNA typing are more reliable and a large number of additional alleles have been assigned compared to the serology typing. Table 2.2 showed the No. of HLA serologic equivalent and HLA alleles of the WHO Nomenclature.

Table 2.2 Comparison of the number of presently known HLA alleles as presented in the WHO Nomenclature reports 1997 (Bodmer et al., 1997) and 1998 (Bodmer et al., 1999) and their known serologic equivalents as presented in the previous (Hurley et al., 1997) and the present "dictionary" (Schreuder et al., 1999)

Locus	1997		1998	
	WHO alleles n	Serologic equivalents n (%)	WHO alleles n	Serologic equivalents n (%)
HLA-A	78	40 (51%)	119	90 (76%)
HLA-B	173	88 (51%)	245	190 (77%)
HLA-DRB1	162	80 (49%)	201	145 (72%)

2.3 The HLA System and Transfusion

HLA antigens are highly immunogenic and can cause adverse immunologic effects in transfusion therapy. HLA antibodies can be induced from previous alloimmunization episodes and can cause platelet refractoriness, febrile transfusion reaction, transfusion-related acute lung injury and post transfusion graft versus host disease.

2.3.1 HLA Alloimmunization

HLA alloimmunization by blood transfusion is caused by the leukocytes in cellular blood products. The incidence of HLA alloimmunization following transfusions can vary with the patient's diagnosis and therapy (Abou-Elella et al., 1995). However, leukocyte reduction to less than 5×10^6 can reduce the development of primary HLA alloimmunization (Van Marwijk-Kooy et al., 1991).

2.3.2 Platelet Refractoriness

HLA class I antigens are expressed on platelets and the development of antibodies to HLA or platelet specific antigens can cause the immune destruction of platelets, resulting in a refractoriness to random-donor platelet transfusions. Platelet-specific and HLA antibodies can be distinguished in platelet assays (Pulkrabek, 1996). Most patients are immunized for HLA, and immunization for platelet-specific antigens is mush less frequent. Prevention of alloimmunization would be highly desirable for some selected patients who have not been previously immunized by transfusion and are expected to need long-term platelet transfusions (Friedberg et al., 1994).

2.3.3 Febrile Non-hemolytic Transfusion Reaction

Fever and chills are the most common reactions, observed in up to 5% of transfused patients. Febrile non-hemolytic transfusion reaction (FNHTR) is frequently associated with the transfusion of platelets stored for more than 3 days. It is believed that the patient's sera may contain HLA antibodies or antibodies to granulocytes or platelets. These leukocyte antibodies may play a role in FNHTR reactions and the release of cytokines capable of causing fever (Thulstrup, 1971). The increasing use of leukocyte-depleted blood components, probably reduces the incidence of FNHTR (Menitove et al., 1982).

2.3.4 Post-transfusion Graft versus Host Disease

Transfusion-associated graft versus host disease (GVHD) is caused by viable lymphocytes contained in blood components. The immunocompetent patient may develop GVHD after receiving blood from a donor who is homozygous for one of the recipient's HLA haplotypes, and the donor's leukocytes are not recognized as foreign and, therefore, not destroyed (Petz et al., 1993).

2.4 HLA Testing and Transplantation

Both T and B lymphocytes are important in graft rejection. T lymphocytes recognize donor derived peptides in association with the HLA molecules on the graft. HLA-A, HLA-B and HLA-DR are known as major transplantation antigens. The role of HLA-C and other class II antigens remains to be studied (Cecka, 1997).

Transplantation of bone marrow, kidney, and more recently heart, liver, lung and pancreas has become the standard treatment for many otherwise fatal diseases.

For marrow and kidney transplant patients, the ideal donor is a sibling who has a 25% chance of inheriting the same two HLA haplotypes from the parents (Tyler, 1999). As a result, transplant physicians have turned to the utilization of unrelated donors. For patients receiving transplants from either related or unrelated donors, the closer the HLA match the greater the likelihood of success (Terasaki et al., 1996). Patients who belong to minority racial groups have an even lower probability of receiving matched transplants. This problem of inequity could be solved by increasing recruitment efforts among minority racial groups, thus providing equal access for patients in each racial group to optimal, HLA-matched organ or marrow donors.

2.5 Parentage Testing

The HLA system is highly polymorphic and the antigens are well developed at birth, which has proven useful in parentage testing. HLA typing alone can exclude about 90% of falsely accused males. With the combination of red cell antigen typing, red cell enzymes and serum proteins, the exclusion rate rises to 99% (Tyler, 1999). Gene and haplotype frequencies are used in these calculations with racial differences. A DNA-based assay technique, commonly used for paternity testing, permits excellent determination of inheritance patterns.

2.6 HLA and Disease Associations

Many factors determine the susceptibility to diseases. Genetic and environmental factors may play a role, but the cause of these associations is not yet known. Certain diseases, especially of autoimmune etiology are frequently associated with particular HLA types. The HLA molecules associated with these disorders

appear to bind self peptides by the peptide-binding grooves of the HLA molecules that stimulate autoreactive T cells (Wucherpfenning and Strominger, 1995).

The degree of association between HLA type and disease is described in terms of relative risk (RR), which is a measure of how much more frequently a disease occurs in individuals with a specific HLA type when compared to individuals not having that HLA type. However, because the HLA system is very polymorphic, an increased possibility of finding an association between HLA antigens and disease may be by coincidental.

The most prominent associations are Ankylosing spondylitis with HLA-B27 (Sonozaki et al., 1975), narcolepsy with HLA-DQ6 (Thorsby, 1997), and celiac disease with HLA-DQ2 (Lundin et al., 1993). Type I diabetes mellitus creates an increased risk with HLA-DQ8, while HLA-DQ6 confers a decreased risk (She, 1996). For these diseases there is an absolute association in individual families between transmission of diseases and inheritance of certain HLA genes or haplotypes.

2.7 Population Study and Anthropology

The HLA gene is an extremely polymorphic system with large allele frequency differences between various groups of people. The polymorphism is not only at one locus, but also at many linked loci and patterns of haplotypes, which are held together by a linkage disequilibrium; a phenomenon in which certain alleles occur together in the same haplotype happens more often than would be expected by chance. For example, in Whites, the actual frequency of the HLA-A1 and HLA-B8 combination is 7-8%, while the expected frequency is only 1.5% (Tyler, 1999). The pattern of haplotype frequencies in Caucasian populations suggests that this disequilibrium has persisted for at least 5 to 10 thousand years (Bodmer, 1997).

The 12th International Histocompatibility Workshop, 1996 was conclusive to anthropology study in which carefully defined information was obtained about the populations studied. The information allowed the populations to be classified linguistically and made it possible to identify those which were a homogeneous group, and in a stable position, where some genes had been handed down by parents and grandparents. Those groups might contain mixtures of genes from different populations, due to recent migrations. A very clear analysis of these results has been carried out by using this information combined with the frequencies of alleles and haplotypes (Bodmer, 1997).

2.8 HLA-A11

2.8.1 Historical Background of Discovery

The HLA-A11 antigen was originally defined by serology using complement-fixation, and was considered as part of the HLA system. Dausset et al (1967), included HLA-A11 in the HU-1 system. In the 8th International Histocompatibility Work- shop, Raffoux and Streiff (1980) reported that HLA-A11 was well defined and segregated in families.

The distribution worldwide of HLA-A11 is shown in Fig.2.10. The highest frequencies (40-50%) are found in southern China, The Philipines, Thailand, Malasia and Nepal. The frequencies decrease westward to European Caucasians (12%), and eastward to Japan (16%). Australian aborigines have a frequency of 18%, but A11 was low frequency from African blacks (Hammond, 1986). At present, several subtypes of HLA-A11 have been detected by various methods.

Figure 2.10 The worldwide distribution of HLA-A11 (Hammond, 1986).

2.8.2 HLA-A11 Subtypes

The existence of two subtypes of HLA-A11 in Hong Kong Chinese was first proposed in 1985 according to the reaction patterns of anti-HLA-A11 antisera observed against expressing cells (Hawkins, 1985). Neefies et al. (1986), also reported two distinct forms of HLA-A11 using the one dimensional - isoelectric focusing (1D-IEF) method. These subgroups were initially called A11Long and A11Short. At the 11th International Histocompatibility Workshop, 1991 the HLA-A11 antigen was clearly defined as being divided into two splits, HLA-A11.1 (A11Short) and HLA-A11.2 (A11Long), by using both serological typing and 1D-IEF methods (Chandanayingyong et al., 1992, Juji et al., 1992, Kaidoh et al., 1992). It was previously shown that the serologically defined A11.1 split was encoded by the A*1101 sequence and that the A11.2 was encoded by the A*1102 (Lin et al., 1994).

HLA-A11.1 is a common antigen worldwide, whereas A11.2 is observed mainly in Asian populations (Lin et al., 1994). Although HLA-A11 is a very common antigen in Asian populations, the gene frequency is different among various races, as shown in Table 2.3, or even among the same race, as seen in Table 2.4.

In Thailand, HLA-A11 was the most frequent serologic type reported by Greiner et al, (1978). In a recent study, the gene frequency of HLA-A11.1 was 25.36% and A11.2 was rare and found only 3.64% in present day Thai (Chandanayingyong et al., 1997). The gene frequency of HLA-A11 in Northeastern Thai populations was 25.84% (Romphruk et al., 1997). Fongsatikul et al. (1997), reported that the HLA-A11.1 and A11.2 in northern Thai populations were 21.92% and 4.55%, respectively.

Six alleles of HLA-A11 (A*1101, A*1102, A*1103, A*1104, A*1105 and A*1106) have been reported to the GenBank data base (www.ncbi.nlm.nih.gov) shown in Figure 2.11. So far, three full-length HLA-A11 associated DNA sequences

have been described, A*1101, A*1102 and A*1104. For the A*1105 allele, only exon 2 and 3 sequences have been published.

Table 2.3 HLA-A2, A9, and A11.1 gene frequencies (%) in non-Thai SE Asian populations studied in 11IHWS

HLA	Mainland SE Asian populations (11IHWS)*				
	S. Han (n=138)	Buyi (n=69)	Miao (n=70)	Viet (n=149)	Sing. C (n=73)
A2	33.7	42.8	30.7	25.9	37.0
A9	19.9	16.7	16.4	13.5	16.4
A11.1	31.9	29.7	42.1	26.3	26.0

*(Imanishi et al., 1992)

Table 2.4 HLA-A2, A9 and A11 gene frequencies for Mainland Chinese from southern, middle and northern China

HLA	Mainland Chinese*		
	South (n=172)	Middle (n =211)	North (n=152)
A2	32.26	32.81	28.82
A9	19.76	14.88	16.38
A11	31.39	26.15	18.00

*(Shaw et al., 1999)

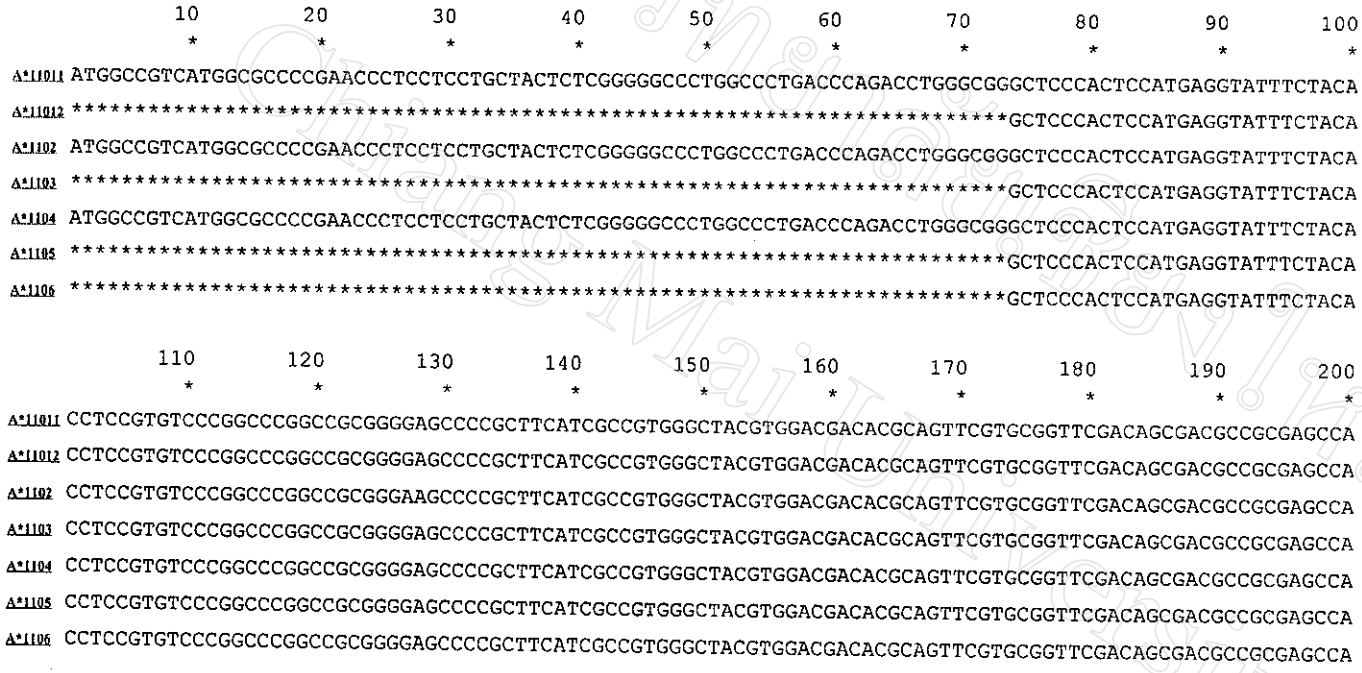

Figure 2.11 HLA-A*11 nucleotide sequence alignments (written by James Robinson for the IMGT/HLA database).

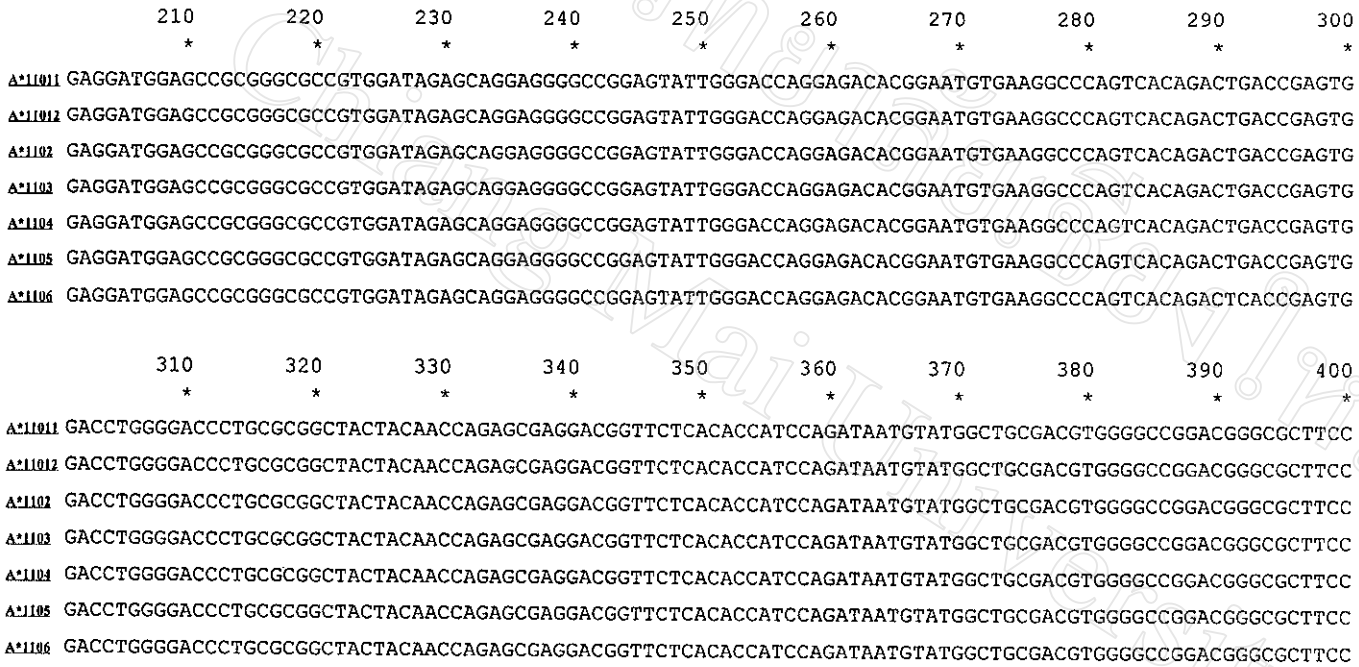

Figure 2.11 HLA-A*11 nucleotide sequence alignments (written by James Robinson for the IMGT/HLA database). (continued.)

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                410        420        430        440        450        460        470        480        490        500
                 *          *          *          *          *          *          *          *          *          *
A*11011 TCCGCGGGTACCGGCAGGACGCCTACGACGGCAAGGATTACATCGCCCTGAACGAGGACCTGCGCTCTTGGACCGCGGCGGACATGGCAGCTCAGATCAC
A*11012 TCCGCGGGTACCGGCAGGACGCCTACGACGGCAAGGATTACATCGCCCTGAACGAGGACCTGCGCTCTTGGACCGCGGCAGACATGGCAGCTCAGATCAC
A*1102  TCCGCGGGTACCGGCAGGACGCCTACGACGGCAAGGATTACATCGCCCTGAACGAGGACCTGCGCTCTTGGACCGCGGCGGACATGGCAGCTCAGATCAC
A*1103  TCCGCGGGTACCGGCAGGACGCCTACGACGGCAAGGATTACATCGCCCTGAACGAGGACCTGCGCTCTTGGACCGCGGCGGACATGGCAGCTCAGATCAC
A*1104  TCCGCGGGTACCGGCAGGACGCCTACGACGGCAAGGATTACATCGCCCTGAACGAGGACCTGCGCTCTTGGACCGCGGCGGACATGGCAGCTCAGATCAC
A*1105  TCCGCGGGTACCGGCAGGACGCCTACGACGGCAAGGATTACATCGCCCTGAACGAGGACCTGCGCTCTTGGACCGCGGCGGACATGGCAGCTCAGATCAC
A*1106  TCCGCGGGTACCGGCAGGACGCCTACGACGGCAAGGATTACATCGCCCTGAACGAGGACCTGCGCTCTTGGACCGCGGCGGACATGGCAGCTCAGATCAC

                510        520        530        540        550        560        570        580        590        600
                 *          *          *          *          *          *          *          *          *          *
A*11011 CAAGCGCAAGTGGGAGGCGGCCCATGCGGCGGAGCAGCAGAGAGCCTACCTGGAGGGCCGGTGCGTGGAGTGGCTCCGCAGATACCTGGAGAACGGGAAG
A*11012 CAAGCGCAAGTGGGAGGCGGCCCATGCGGCGGAGCAGCAGAGAGCCTACCTGGAGGGCCGGTGCGTGGAGTGGCTCCGCAGATACCTGGAGAACGGGAAG
A*1102  CAAGCGCAAGTGGGAGGCGGCCCATGCGGCGGAGCAGCAGAGAGCCTACCTGGAGGGCCGGTGCGTGGAGTGGCTCCGCAGATACCTGGAGAACGGGAAG
A*1103  CAAGCGCAAGTGGGAGGCGGCCCGTGAGGCGGAGCAGCAGAGAGCCTACCTGGAGGGCCGGTGCGTGGAGTGGCTCCGCAGATACCTGGAGAACGGGAAG
A*1104  CAAGCGCAAGTGGGAGGCGGCCCATGCGGCGGAGCAGCAGAGAGCCTACCTGGAGGGCACGTGCGTGGAGTGGCTCCGCAGATACCTGGAGAACGGGAAG
A*1105  CGAGCGCAAGTGGGAGGCGGCCCATGCGGCGGAGCAGCAGAGAGCCTACCTGGAGGGCCGGTGCGTGGAGTGGCTCCGCAGATACCTGGAGAACGGGAAG
A*1106  CAAGCGCAAGTGGGAGGCGGCCCATGCGGCGGAGCAGCAGAGAGCCTACCTGGAGGGCCGGTGCGTGGAGTGGCTCCGCAGATACCTGGAGAACGGGAAG
```

Figure 2.11 HLA-A*11 nucleotide sequence alignments (written by James Robinson for the IMGT/HLA database). (continued.)

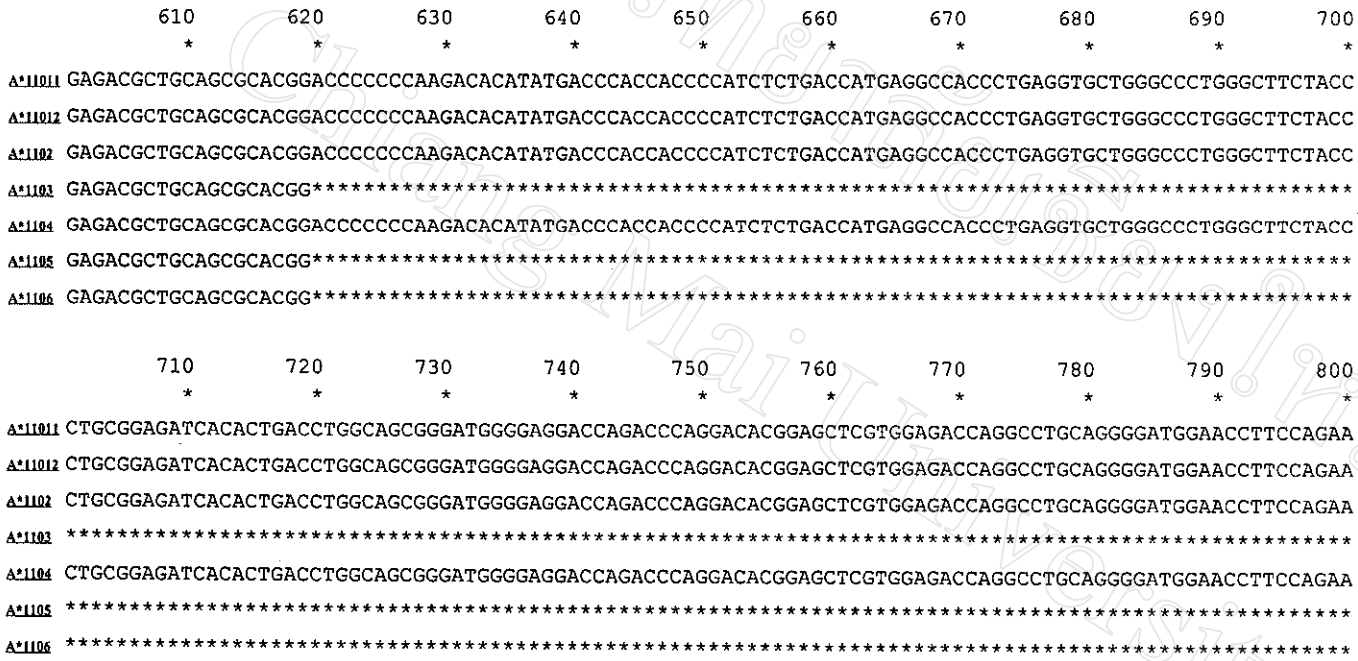

Figure 2.11 HLA-A*11 nucleotide sequence alignments (written by James Robinson for the IMGT/HLA database). (continued.)

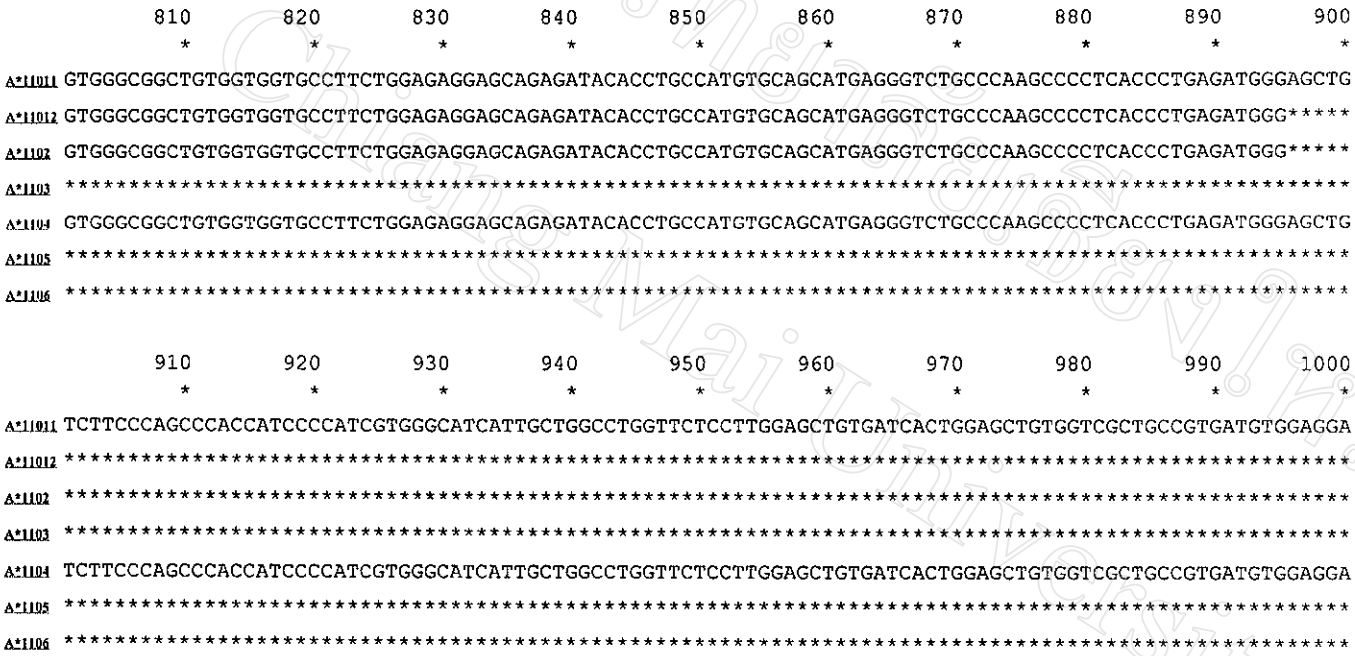

Figure 2.11 HLA-A*11 nucleotide sequence alignments (written by James Robinson for the IMGT/HLA database). (continued.)

HLA-A*1101 and A*1102 differ only for a single point mutation in exon 2 (codon 19), whereas A*1104 differs from both other A*11s at codon 163 (ACG, shared with the class I consensus sequence). In the regions sequenced, A*1105 follows A*1101 except for codon 144 (exon 3). Alignment of A*1103 sequence with other known A*11 alleles reveals two additional polymorphic codons, 151 and 152 (exon 3) positions within the A*11s as previously reported (Figure 2.12) (Tijissen et al., 2000).

	Exon 2	**Exon 3**	**Exon 3**	**Exon 3**	**Exon 3**
			Codon		
Alleles	**19**	**144**	**151**	**152**	**163**
A*1101	GAG	AAG	CAT	GCG	CGG
A*1102	A - -	- - -	- - -	- - -	- - -
A*1103	- - -	- - -	-G -	- A -	- - -
A*1104	- - -	- - -	- - -	- - -	AC-
A*1105	- - -	G - -	- - -	- - -	- - -

Figure 2.12 The difference codon positions of HLA-A*11 alleles (Tijissen et al., 2000).

2.8.3 The Important of HLA-A11

HLA-A11 is one of the most common alleles worldwide and is mostly encoded by A*1101 (Chujoh et al., 1998). The pool sequencing analysis of HLA-A*1101 self binding peptides has previously shown that Lys at the C-terminus is the primary anchor residue, while positions 2 (P2), 3 (P3) and 7 (P7) are secondary anchors for HLA-A*1101 (Chujoh., 1998). This suggests a strong interaction between positively

charged residue at the C-terminus and the F-pocket of HLA-A*1101 molecules. Indeed, since the F-pocket of HLA-A*1101 possesses a strong negatively charged environment formed by three Asp residues at positions 74, 77 and 116, it is assumed that HLA-A*1101 favors peptides carrying positively charged residues at the C-terminus. HLA-A*1101 can bind 10 and 11 mer peptides as effectively as 9 mer peptides, as suggested by the pool sequencing analysis. These HLA-A11 restricted epitope analyses showed a strong interaction between HLA-restricted CTL response and virus evolution.

Several HLA-A11 restricted CTL epitopes have been identified in EBV (Gavioli et al., 1993), hepatitis C virus (Koziel et al., 1995) and HIV (Culmann-Penciolelli et al., 1994). A possible explanation is that they are high affinity peptides for HLA-A*1101 molecules.

Disease associated HLA molecules preferentially bind and present immunogenic peptides to T cells that are involved in the pathogenesis of the disease, leading to susceptibility, or preferentially present peptides to T cells involved in a protective immune response. Depending on whether the selected T-cells are involved in the pathogenesis of the disease or protection, this may lead to HLA associated disease susceptibility or protection.

The distribution of and differences in HLA typing in each ethnic group would be valuable not only for the application of transfusion medicine and disease association, but also selected types and alleles would enhance a basic knowledge for the understanding and mechanism of the immune response.