II. LITERATURE REVIEW

A. Major Histocompatibility Complex

The major histocompatibility complex (MHC) or the human leukocyte antigen (HLA) in human is a region of highly polymorphism genes on the short arm of chromosome 6. These HLA genes consist of three regions: centromere-class II (~850 kb) -class III (~1100 kb) -class I (~2000 kb) -telomere (Fig. 1) (Kendall et al., 1991). The HLA class I and HLA class II regions contain genes that encode cell surface glycoproteins. One of the major role of their products in physiological immune response involves in antigen presentation to T cells. The segment of DNA in between these two regions, generally termed as the class III region, contains genes encoding proteins of diverse structure and function such as those encoding complement component 2 (C2), C4 and heat shock protein 70 (Hsp70) (Powis et al., 1995).

The HLA class I or class II region comprises multiple locus or loci. A locus is a single gene for coding a single product. Each individual has two variant forms of the product of a particular locus. Each variant form is called allele. Two allelic forms of each locus in everyone are expressed co-dominantly. The set of HLA locus that are inherited together on a single chromosome is termed the haplotype.

A. 1 Genomic organization of the HLA class I and class II genes

A. 1.1 The HLA class I genes

The α gene consists of 8 exons (Hansen et al., 1993). The first exon is short and contain the leader sequence. Exon 2, 3 and 4 are all similar in size. They code for the extracellular domains: α1, α2 and α3. The α1 and α2 domains of class I molecule are polymorphic. The fifth exon encodes the membrane binding region and also the highly basic C-terminal portion. The cytoplasmic section is encoded in several small exon 6, 7 and 8. The gene for β2 microglobulin is coded on chromosome 15. It is present as a single copy which consists of 4 exons but most of the coding region is found in the second exon (Austyn and Wood, 1993).

The class I loci are designated HLA-A, HLA-B and HLA-C by serological techniques. HLA-A and HLA-B were first extensively characterized. The serological

specificity of HLA-A and HLA-B are highly polymorphic, alternatively, those of HLA-C is less polymorphic. There are 24 forms of HLA-A, 52 of HLA-B and 11 of HLA-C antigens (McClosky et al., 1992). By the molecular techniques can be classified 50 allelic forms of HLA-A , 97 of HLA-B and 34 of HLA-C (Bodmer et al., 1994). The HLA class I like molecules, HLA-F, HLA-G, HLA-H, HLA-J and HLA-X, locate telomeric to the HLA-A and some are found between HLA-A and HLA-C. They are defined as pseudogenes and their functions still unclear. Some encode nonpolymorphic products associated with β_2 microglobulin (Abbas et al., 1994).

A. 1.2 The HLA class II genes

The HLA class II genes each contain five or six exons. The first exon of the class II α and β genes code for the hydrophobic signal sequence. The second and third exons code for the extracellular domain of the α and β chains; $\alpha 1\alpha 2$ and $\beta 1\beta 2$ domains. These region is very polymorphic especially the $\alpha 1$ and $\beta 1$ region. For the alpha genes the fourth exon encodes the transmembrane domain, the cytoplasmic domain and a portion of the 3' untranslated (3'UT) region of the mRNA, whereas the fifth exon encodes the remainder of 3'UT region. In contrast, the fourth exon of the β genes encodes the transmembrane region and the fifth encodes a portion of cytoplasmic region. The remainder exon 6 encodes the remaining cytoplasmic portion and 3' UT region (Hansen et al., 1993).

There are three main subregions in the order centromere -DP-DQ-DR-telomere. Each subregion contains at least one functional A and B gene. The other class II genes, HLA-DZ and HLA-DO lie between HLA-DP and HLA-DQ subregions. Their function has not been established (Fig. 1).

The HLA-DR subregion has only one α gene or A gene (termed HLA-DRA) while the β genes (termed HLA-DRB) have been assigned to 9 different genes comprising 4 expressed genes, DRB1, DRB3, DRB4 and DRB5, and 5 pseudogenes, DRB2 and DRB6-9 . The monomeric product of the DRA gene associates with one of the products of functional β genes (Anderson et al., 1994). Not all the HLA-DRB genes present on all haplotype and the combination of the HLA-DRB genes are haplotype specificity (Dyer et al., 1993, Anderson et al., 1994). There are divided into 5 main DR haplotypes. The genetic organization of each haplotype of HLA-DR region is shown in figure 2.

Each allelic form of HLA-DRB genes is typed due to serological role of HLA-DR antigen. At least 18 HLA-DR specificity can be detected serologically. Currently, more

than 135 alleles have been assigned to the HLA-DRB1 locus. The other three expressed HLA-DRB loci show less allelic polymorphism; four of HLA-DRB3, five of HLA-DRB4 and five of HLA-DRB5 (Bodmer et al., 1994).

In HLA-DQ and HLA-DP subrergions, each has two α chain genes and two β genes (DQA1, DQB1, DQA2, DQB2, DPA1, DPB1, DPA2 and DPB2). The first gene of each pair, DQA1, DQB1, DPA1 and DPB1, is functional (Abbas et al., 1994). Nine HLA-DQ serological specificities can be detected but the polymorphism of the HLA-DQ gene is greater than the molecule implied by serological specificities (Hansen et al., 1993). Fourteen HLA-DQA1 and 22 HLA-DQB1 alleles have been identified (Marsh and Bodmer, 1995). The serological specificity of HLA-DP can be detected in spite of lacking the alloantiserum by using monoclonal antibodies (Austyn et al., 1994). There are 6 antigenic specificities (Hansen et al., 1993). Eight HLA-DPA1 and 58 HLA-DPB1 allelic forms were identified (Bodmer et al., 1994).

A. 2 General feature of the HLA class I and class II antigens.

A. 2.1 The HLA class I molecule

The HLA class I antigen is cell surface glycoprotein consisting of two noncovalently association polypeptides. The heterodimer of two polypeptides compose of one α chain and one β_2 microglobulin chain. The α chain is about 40 kD polymorphic glycoprotein (Hansen et al., 1993). The β chain of class I molecules, is about 12 kD, encoded by a gene outside the HLA. This polypeptide is identical to a protein previously identified in human urine, called β_2 microglobulin for its electrophoretic mobility (β_2), size (micro) and solubility (globulin) (Abbas et al., 1994). The α chain can be divided into 4 regions: an amino terminal extracellular peptide binding region, an extracellular immunoglobulin(Ig)-like region, a transmembrane region and a cytoplasmic region (Abbas et al., 1994). The extracellular peptide binding region, is a prominent amino terminus, separated into 2 domains: α1 and α2. The interaction of both domain: two α helices form the sides of a cleft and eight β-pleated sheets form the floor of the groove (Brown et al., 1993). The amino acid side chains of either α helices or β strands that point into the cleft or toward the top of the helices are almost polymorphic (Austyn and Wood, 1993). The α 3 domain, locates in an extracellular immunoglobulin-like region, is highly conserved among all class I molecules and is homologous to Ig-like domain (Hansen et al., 1993).

The α3 and β_2-microglobulin domains interact with each other and sit on the membrane (Abbas et al., 1994). The transmembrane region is a carboxy terminus extends from the α3 segment. This region is believed to form an α-helix that passes through the hydrophobic region of the plasma membrane. These are followed by short, hydrophilic cytoplasmic tails approximately 30 amino acids. These segment locates in the cytoplasm (Abbas et al., 1994).

Over all structure of HLA class I is similar to that of HLA class II that reflect their specialized functions. The N-terminus of both molecules are formed as the binding baskets (Brown et al., 1993). Saper et al (1991) identified six pockets of the HLA class I molecule, denoted A through F that were localized within this peptide binding groove. A and F pockets are located between the two α-helices and pockets B, C, D and E are located at the junction of the β-pleated sheet and an α-helix (Saper et al., 1991). A comparisons of structures of four distinct HLA class I molecules indicated that individual amino acid substitutions result in local structure changes. Each allele examines thus far presents a distinct peptide-binding groove with characteristic deep and shallow pockets (Saper et al., 1991; Matsumura et al., 1992). A peptide, 8 to 10 amino acids long, bound within the groove could accomodate one or more of its side chain within these peptide-binding pockets. Peptides lie extended in the cleft by their main chain bonded to conserved residues of HLA class I and a few interaction involved the side chain of bound peptides (Hansen et al., 1993). Interactions within the pockets might be direct hydrogen bonds between the side chain of the anchor residue and the class I residue or might be mediated by water (Matsumura et al., 1992).

A. 2.2 The HLA class II molecule

The HLA class II molecules are quite similar to the HLA class I molecule (Brown et al., 1993). One α and one β polypeptide chians form the functional noncovalent α-β heterodimers. Each chain can be divided into 4 regions; 2 extracellular regions, transmembrane region and cytoplasmic region as the HLA class I molecule (Abbas et al., 1994). The extracellular portion of α and β chains have been subdivided into two domains: α1, α2, β1 and β2, respectively. The α1 and β1 domains form the peptide binding groove. Similar to the HLA class I structure, this binding groove composes of eight strands of β-pleated sheet and 2 strands of α-helices (Abbas et al., 1994). The polymorphism of class II molecules are concentrated in α1 and β1 domains. Especially the

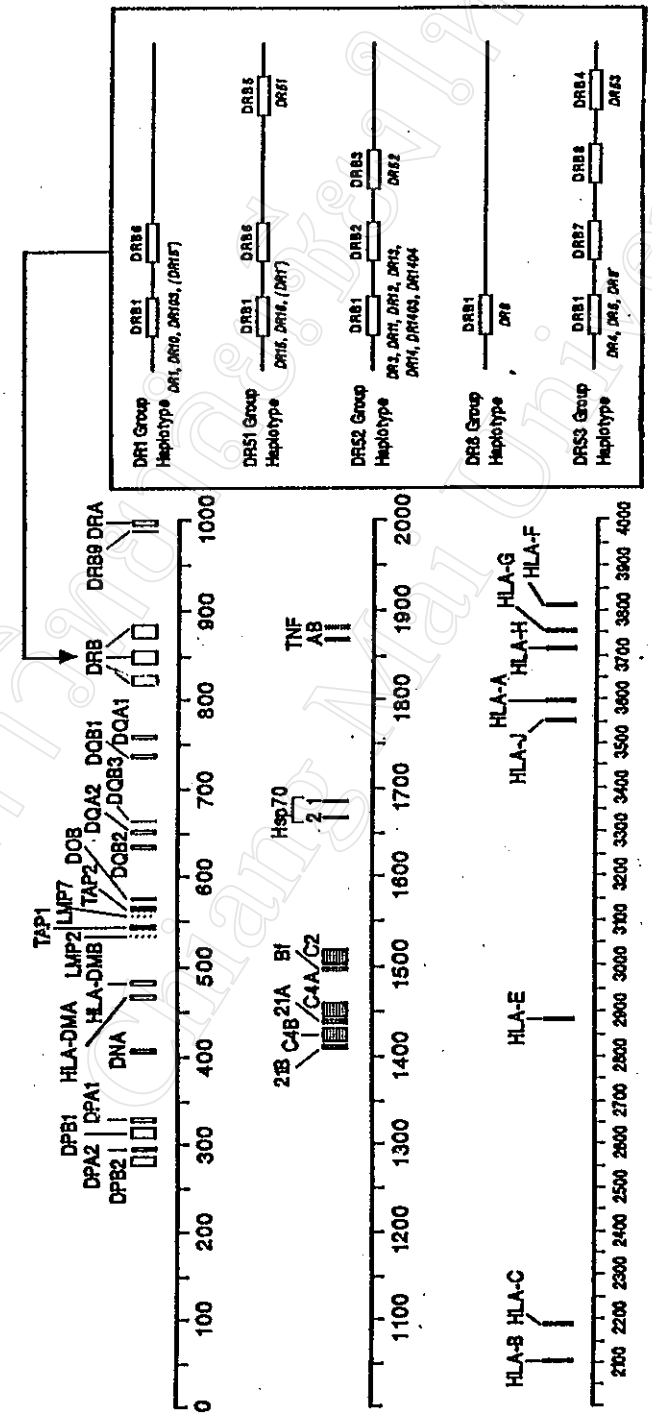

Figure 1 Map of the human major histocompatibility complex. (Trowsdale et al., 1991)

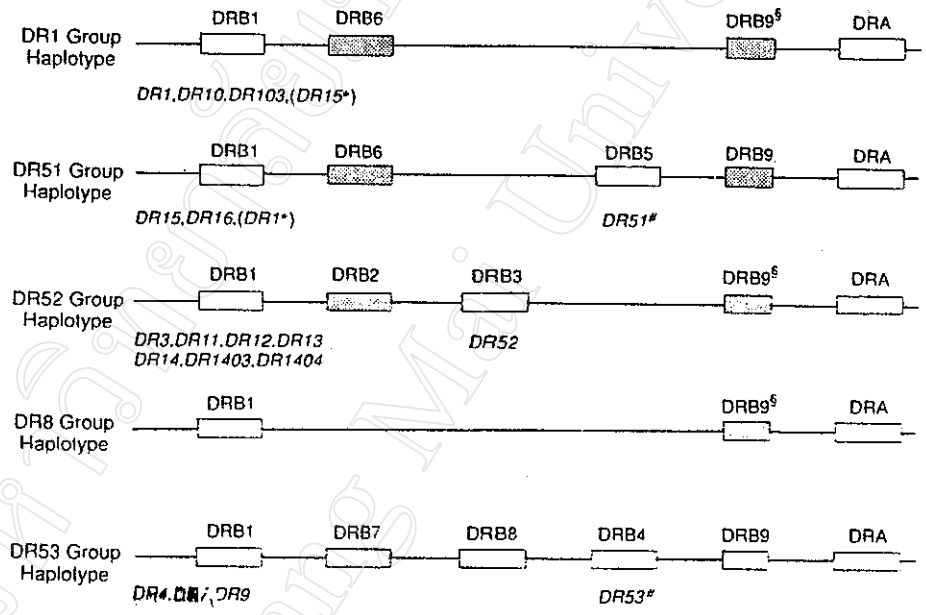

Figure 2 Genomic organization of the HLA-DR region and encoded products (specitficities). Psuedogenes are indicated by shaded boxes, expressed genes by open boxes. The serological specificity encoded by a gene is shown underneath in italics. (Dyer and Middleton, 1993)

*rarely observed haplotypes

#DR51 and DR53 may not be expressed on certain haplotypes

§the presence of DRB9 in these haplotypes needs confirmation

DR locus, the greater degree of polymorphism is found in β chain than α chain (Marsh and Bodmer, 1995). The greater polymorphism usually lie in the amino acid side chain of the α helices or β pleated sheet of the groove (Austyn and Wood, 1993). The α2 and β2 segment of class II molecules are the member of Ig superfamily, like the class I α3 and β2 microglobulin domains. They are nonpolymorphic among various alleles of class II molecules but show some differences among the different genetic loci (Abbas et al., 1994). The two α-chain domains, α1 and α2, of HLA-DR1 superimpose closely on the corresponding α1 domain and the β_2-microglobulin subunit. The two β-chain domains of HLA-DR1 superimpose on the α2 and less closely on the α3 domains, respectively (Brown et al., 1993). The transmembrane regions expand from the α2 and β2 domains in the carboxy terminus. They are approximately 25 amino acid stretches of hydrophobic residues. This region are followed by a short hydrophilic cytoplasmic segment. Their function is a little known (Abbas et al., 1994).

The three-dimension structural model of the HLA class II base on the structure molecule of the HLA-DR1 and -DR3 (Brown et al., 1993; Grosh et al., 1995). There are 9 possible pockets, termed 1-9, that accomodated the side chains of HA306-318 (Fig 3) and CLIP87-104 peptides (Stern et al., 1994; Ghosh et al., 1995) . The series of pockets; 1, 3, 4, 6, 7 and 9, accommodate the side chain of peptide in the binding site. Pocket 1 and 9 are formed as deep cavities at either ends of the site. Pocket 6 and 7 are smaller cavities in the center of the site (Ghosh et al., 1995). This HLA class II basket accommodated naturally processed peptides 10 to 34 amino acid in length (Chicz et al., 1993). The peptide was bound to HLA-DR1 as a straight extended strand with the peptide N and C termini projecting out of the open-ended antigen binding groove (Stern et al., 1994). There were 15-17 hydrogen bonds between HLA and main chain atoms of the bound peptides (Stern et al., 1994; Ghosh et al., 1995).

A. 3 HLA typing

In order to determine the HLA type, many techniques are performed base on their phenotype or genotype. The introduction of the PCR technology has improved the capacity for detecting genetic difference. The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method and polymerase chain reaction-specific sequence oligonucleotide (PCR-SSO) method are performed to determine the genotype. The allelic variability often be accurately investigated via molecular biology.

Only recently, the major technology has been applied to HLA class I. Conversely, HLA class I DNA typing has many troubles because of their abundance psuedogenes and the patchwork nature of polymorphisms in exon 2, intron 2 and exon 3 (Bidwell, 1994).

A. 3.1 Serological HLA typing method

A. 3.1.1 Leukoagglutination

The basis of leukoagglutination method is mediated by a specific antibody. The differentiation of HLA antigens can be analyzed by the patterns of a panel of antisera. The two important ingredients that are required for typing of the HLA antigens are a suitable cell suspension and quality reagents. For typing HLA class II antigen, B cell are needed. There are two forms of reagents; alloantibodies and monoclonal antibodies. The alloantibodies are produced by various sensitization procedures. The major source of alloantibodies are obtained from parous women. The HLA antibodies are found in sera depending on the number of pregnancies. The more pregnancies means the more antibody titer. The other sources derive from blood donors, transplanted recipients and immunization of volunteers with whole blood or lymphocyte suspensions (Middleton et al., 1993). Monoclonal antibodies can be used instead of supplement alloantisera but they are directed solely at a single epitope. In nature, the HLA antigens are complexity. It is impossible to obtain the necessary reagents which can be used to detect all class I and class II antigens. These techniques are limited by the availability of either monospecific antisera, monoclonal antibodies or homozygous typing cells.

A. 3.1.2 Microlymphocytotoxicity

The principle of the lymphocytotoxicity test is a cytolysis mediated by a specific antibody in the presence of complement. It needs the same important ingredients as the leukoagglutination method

A. 3.2 DNA-based HLA-typing methods

The application of molecular techniques to tissue typing has led to change in methodology for HLA class II typing. There are significant correlation between HLA class II phenotyping and PCR-based HLA typing (Bidwell et al., 1994).

A. 3.2.1 Restriction fragment length polymorphism (RFLP)

Restriction fragment length polymorphism (RFLP) technique involves the digestion of genomic DNA with a specific restriction endonuclease, electrophoretic resolution of endonucleolytic fragment in an agarose gel and in-situ denaturation of duplex DNA followed by transfer to a support membrane (Bidwell, 1994). The restriction enzyme Taq I is favored by many investigators because it permits distinction between the majority of DRB-DQB-DQA haplotypes (Bidwell et al., 1994). After single-stranded DNA is immobilized by crosslinking to the membrane, HLA class II sequences may be visualized by hybridization with homologous radiolabelled genomic or cDNA probes. Variation in nucleotide sequence between alleles of a given gene is reflected by allele-specific hybridization signal patterns.

A. 3.2.2 Polymerase chain reaction-sequence-specific oligonucleotide (PCR-SSO)

Polymerase chain reaction-sequence specific oligonucleotide (PCR-SSO) typing was the first PCR-based test to offer substantial improvements on RFLP. The test involves hybridization of a panel of SSO probes to PCR-amplified target HLA sequences. The target sequences are normally amplified from the polymorphic exons of HLA gene. The exons 2 and 3 with or without intron 2 of HLA class I genes and exon 2 of HLA class II genes are amplified (Bidwell, 1994). The SSO probes were designed and standardized during the 11^{th} international histocompatibility workshop. Each probe is complementary to a different motif within hypervariable region of HLA sequences. The amplified DNA are perfectly match with a panel of SSO probe. The specific patterns of SSO probe hybridization will be used to identify individual alleles or the combination of alleles.

There are 2 formats of PCR-SSO hybridization. The solid phase dot-blot, slot blot and reverse dot-blot were the first format. They are based on the membrane solid phase. In

recently, this technique was modified in order to use for the large scale studies. The second method was based on the microtiter tray formats such as the oligocapture sandwich assay and the dual-phase oligocapture assay. The third strategy has been developed to uses a single fluid-phase and employs acridinium-ester-labelled SSO probes and automated luminometry (Bidwell, 1994).

A. 4 HLA and diseases

The occurrence of disease associated with certain HLA alleles has been recognized for a long time. There are over 40 diseases which linked to different HLA haplotypes (Stastny et al., 1983). Some of the infectious diseases, inflammatory diseases and autoimmune diseases are strongest associated with particular HLA allele. It would be expected that the immune response genes of the HLA region should influence resistance or susceptibility to disease. The mechanism is still unclear. One of the causes of the allelic diversity is driven by the pathogen-selection. The infection have provided mechanism for natural selection and may be the driving force for the remarkable polymorphism of the HLA system (Hill et al., 1994). In the study of the association between severe *Plasmodium falciparum* malaria and HLA class II, the different allelic HLA-DR conferred resistance to severe malaria. The HLA-DR*0101 allele was remarkable resistance allele in East Africa whereas HLA-DRB1*1302 conferred resistance in West Africa (Powis et al, 1995).

HLA genes may affect predisposition to autoimmune diseases by several mechanisms. The association is not unexpected, since autoimmune diseases are T-cell dependent and all T-cell-mediated response are HLA restricted (Gergely, 1992). Several mechanisms are not mutually exclusive including the T-cell receptor repertoire, peptide selection and presentation and peptide transport (Theofilopoulos, 1995). Histocompatibility genes are involved in selective binding of autoantigenic peptide and expansion of autoreactive T cells (Carson, 1992).

The HLA-linked autoimmune diseases are including rheumatoid arthritis (RA), insulin dependent diabetes mellitus (IDDM), thyroiditis, Goodpasture's syndrome, myasthenia gravis and multiple sclerosis (Stastny et al., 1983). HLA class II molecules are logical candidates for gene products that associated with susceptibility to autoimmune disease. HLA-DR3 and HLA-DR4 are important susceptibility marker in RA and IDDM among Caucasian (Segall, 1988; Winchester et al., 1994). During this period,

improvement in HLA typing led to an increase in the relative risk for the disease. The association with IDDM was the strongest with HLA-DQ. Todd noticed that all class II haplotypes IDDM possessed an aspartic acid at position 57 in the β chain of HLA-DQ was neutral or negatively associated (Todd et al.,1987). The substitution of amino acid 57 of DQβ with an alanine, a valine or a serine was positive associated with IDDM. Various HLA antigens were found associated with disease in many different ethnic. HLA-DR7 haplotypes appeared to confer disease susceptibility in blacks although neutral in Caucasian (Charron, 1991). Goodpasture's syndrome was associated with HLA-DR2 in Caucasian. Rheumatoid arthritis was associated with HLA-DR4 in most populations except Jews(Stastny et al., 1983).

One possible mechanism is that the selective nature of the peptide-binding groove of the HLA molecule ensures that only certain alleles are capable of presenting pathogenic peptide. The good event was provided by coeliac disease which strongly associated with HLA-DQA1*0501 and HLA-DQB1*0201. The antigen of this diease is the gliadin. Gliadin reactive T cells have been isolated from biopsy material. They were restricted to HLA-DQA1*0501 and HLA-DQB1*0201 (George et al., 1995).

B. Graves' disease

Graves' disease is a disease of thyroid gland. It is named after Robert James Graves for his description in 1835 (Gossage et al, 1991). It is the one of the autoimmune endocrine disease. Graves' patients present diffusely enlarged thyroid gland and hyperthyroidism. They have marked lymphocytic infiltration in their thyroid gland. Female are mostly found with this disease than male in ratio 7:1 to 10:1 (Feliciano, 1992). The most common clinical presentation in Graves' disease is hyperthyroidism. The thyroid gland is diffusely enlarged with remarkably lymphocytic infilltration. The function of the gland is overstimulated by the autoimmune process and cause the hypersecretion of the thyroid hormone. The other manifestation, which are less common, are ophthalmopathy, either infiltrative or non-infiltrative, and dermopathy. Clubbing finger and acropachy can also be found (Feliciano, 1992, McDougall, et al, 1991).

The eye changes occur in 50% to 100% of patients. The noninfiltrative component of eye changes is a reflection of hyperthyroidism-induced sympathetic stimulation. It stimulates the levator palpebrae superioris muscle of the eye lid that cause the upper eye lid retract. The infiltrative component of eye changes is caused by

mucopolysaccharide deposition and infiltration of the orbit and extraocular muscle. Skin changes occur in 5% to 10% of patients and usually coexists with ophthalmopathy. The deposition of mucopolysaccharide is the cause of dermopathy especially in the pretibial area (Feliciano, 1992). Acropachy is present in less than 1% of patients.

The diagnosis of Graves' disease may be difficult in patients with atypical presentation. The physical examination is confirmed by laboratory tests of the elevated level of triiodothyronine (T_3) and thyroxine (T_4) and suppressed thyrotropin (TSH) (Feliciano, 1992; McDougall et al., 1991).

B. 1 Etiology and pathogenesis

Graves' disease is a common disorder which still has uncertain etiology. The entire immune system including T and B lymphocytes, monocytes, granulocytes and humoral factors participate in the autoimmune process (Gergely, 1992). The development of immunological mechanism of all endocrine disease represent a failure to develop or maintain tolerance to self antigen (Herold et al, 1995). Susceptibility to Graves' disease is supposed to have multifactorial model of disease (Weetman et al., 1994). The ingestion of iodine, infectious agents and stress are nongenetic factor that involved in pathogenic of disease. The increased ingestion of iodine is associated with an increase in lymphocytic infiltration of the thyroid gland and increase in detectable thyroid autoantibodies in circulation. The antigen of infectious agents, *Yersinia enterocolitica* envelop proteins, *gag* proteins of the human foamy virus and heat shock protein 70 are associated with Graves' disease. The cross reactive antigens caused the increasing thyroid autoantibodies in Graves ' patients that could change the thyroid function (Weetman et al., 1994).

There are many attempts to point the stressful event as a causative of the illness. Many paper have been unconvincing because of the small number of patients, lack of the suitable control subjects and the imprecision of the technical term of stress. Gray and colleague studied life events of 50 thyrotoxic patients and 50 control subjects which occurred before and after six months of the onset of symptom. They have not found the positive evidence of a causative role of stress in the etiology of thyrotoxicosis (Gray et al., 1985).

In 1956, Adams and Purves found a factor in serum of hyperthyroidism patients. This factor caused stimulation of animal thyroid with a longer time than thyroid-stimulating hormone (TSH) reactivity (Adams et al., 1956). It was called long-acting

thyroid stimulator (LATS). For the meanwhile, this factor was proven to be the thyroid-stimulating immunoglobulins (TSIg or TSAb). These autoantibody, belonged to IgG class, mimicked most of the effect of TSH by stimulate the thyroid stimulating hormone receptor (TSHR) (Smith et al., 1974). They bound to the amino acid positions 25-30 at the amino terminal of the extracellular region of the receptor (Nagayama et al., 1992). There are other reports of TSH antagonist activity in the sera of patients with Graves' disease. They inhibited the binding of ^{125}I-labelled TSH to the preparation of the thyroid membrane (Creagh et al., 1985). The blocking antibodies was usually found in the patients after the radioiodine treatment (Hashim et al., 1986). The correlation of both activities is still unknown (Macchia et al., 1988).

Volpé proposed that the environmental factor may caused a reduction in generalized suppressor T cells. The result was to reduce suppression of a thyroid-directed helper T cell (Th) population. In the presence of monocytes and the specific antigen, the specific Th cells would produce interferon-γ (IFN-γ) and also stimulated specific B cells to produce TSIg (Volpé, 1991). The suppressor T cells from Graves' patient showed a significant lower activation by the thyroid specific antigens (Yoshikawa et al., 1992). There were also T helper defective. A particular subset of CD4+ T cells, namely T helper 1 cells, contributed to the pathogenesis of IDDM and experimental autoimmune encephalomyelitis (EAE) (Liblau et al., 1995). Th1 cells secrete IL-2, IFN-γ and tumor necrosis factor (TNF). IL-2 production by PHA stimuation was reported to be reduced in Graves' PBMC cultures (Eisenstein et al., 1994). Conversely, Marezuela examined the IL-2 production, the proliferative response to mitogenic stimulators and the expression of HLA-DR antigen in untreated and treated T-cells derived from Graves' disease. Those of untreated T-cells were significantly enhanced as compared to those of treated patients and healthy controls (Marazuela et al., 1994). Alternatively, monoclonal antibodies specific for human thyrotropin receptor, a subset of pathologic autoantibodies, could induce the up-regulation of MHC mRNA and proteins in human and murine thyroid epithelial cells as well as IFN-γ (Ropars et al., 1994). This can lead to an increased efficiency of the presentation of autoantigens and activation of autoreactive T cells.

Genetic factors in the etiology of disease is well established. It was assessed by the high concordance rate between monozygotic twins and between sibling. The identical sibling had 50% chance to develop the disease when the other one had disease. The risk was 30% for nonidentical twin (McDougall et al, 1991).

B. 2 HLA antigen associated with Graves' disease

McGregor's report indicated that over 90% of Graves' patients who were HLA-DRw3 positive relapsed within 12 months after withdrawal of antithyroid treatment (McGregor et al., 1980). The HLA-DRw3 positive individual also had less efficient Fc-mediated function. This was measured by slower clearance of circulating immune complex which have been found in Graves' patients (Lawley et al., 1981). The thyroid gland section and the thyroid cell cultures from Graves' thyroidectomy specimens displayed aberrant HLA-DR expression (Hanafusa et al., 1983). This may be due to the stimulation by local immune response because it was known that MHC class II mRNA and antigens of thyroid cells could be upregulated by IFN-γ (Bottazzo et al., 1983). The expression of HLA-DR antigens was detectable in most cases, followed by HLA-DP, and HLA-DQ on thyrocytes (Bottazzo et al., 1986).

In 1974 Grumet and colleagues reported that the HLA antigen B8 associated with Graves' disease in American population. The incidence of the HLA-B8 in patient group was twice of the control group (Grumet et al., 1974). Later, other Caucasian populations, the HLA-DR3 antigen mostly related to Graves' disease (Dahlderg et al., 1981; Farid et al., 1979; Schlensener et al., 1983). This DR3 antigen is thought to be the most specific indicator of liability to Graves' disease. Two studies, Yanagawa et al. (1994) and Badenhoop et al. (1995) reported that the HLA-DQA*0501 was significantly increased in white Graves' patients. They found that this haplotype was distincted in male group (Yanagawa et al., 1994). Controversy, the study in Oriental populations, the association of Graves' disease in Beijing showed the strong association with HLA-DR4 (Luo et al., 1994). The HLA-B46 and the HLA-DR9 were strong associated with male Singaporean patients (Chan et al., 1993) and male Hong Kong Chinese (Caven et al., 1994). In Japanese patients, the HLA-A2 and the HLA-DPB1*0501 were reported with the most relative risk (Dong et al., 1992; Onuma et al., 1994). The HLA antigens or alleles of a high risk group for Graves' disease were shown in Table 1.

The HLA-DR3 was also claimed that it was a specific marker for those who relapse after a course of antithyroid drug (McGregor et al., 1980). The PCR-RFLP allophenotyping subdivide the HLA-DR3 (-DRβ17) into 2 forms, HLA-DRβ17^1 and -DR β17^2 (Bidwell and Jarrold, 1986). The high remission rate was found in the homozygous of HLA-DQA2 U positive allele which co-existed with HLA-DRβ17^1 (Ratanachaiyavong et al., 1990). Festenstein described HLA-DQA2 (originally known as DXα) polymorphism

by RFLP analysis. It consisted of 2.1 kilobases (upper = u) and 1.9 kilobases (lower = u) fragments (Festenstein et al., 1986). The degree of linkage disequilibrium between HLA-DQA2 and HLA-DR/DQ complex dose not strong as those of HLA-DRB1-DQA1-DQB1 haplotype (Ratanachaiyavong et al., 1991). Ratanachaiyavong proposed that the HLA-DQA2 U was unlikely a susceptible allele but it might lead to perpetuation of disease by increasing the opportunity for providing a variety of appropriate antigen binding sites (Ratanachaiyavong et al., 1990). Our project did not study the association between HLA-DQA2 locus and disease because of the failure to detect its product and function. Thus, it is useless to identify the non-functional gene while the functionally susceptible gene has not been clarified.

Table 1 HLA antigens and alleles significantly associated with a high risk for Graves' disease in representative studies

Race	Antigens and allleles with positive or negative association with Graves' disease (RR)*	No. tested patient	control	Method	References
Caucasian (Indo-Aryans♦) group					
USA	A8 (47% vs 21%)	62	113	Standard fluorochro-masia microcytotoxicity	Grumet 1974
USA	DR3a (7.37), DQw2	65	65	RFLP	Mangklabruks 1991
USA	DQA1*0501 (3.71), DR3 (2.46)	94	75	PCR-SSO	Yanagawa 1993
USA	DQA1*0501 (8.35)♣	131	104	PCR-SSO	Yanagawa 1994
USA	DQA1*0501 (2.5)	271	271	PCR-SSO	Badenhood 1995
Germany	DQA1*0501 (3.0)†, DR3 (7.3)††	218	-	Sequence specific PCR	Cuddihy 1996
Canada	B8 (50% vs 26%), DRw3 (68% vs 28%)	41	50	Serotyping, two-step complement-dependent microcytotoxicity	Farid 1979
Sweden	B8 (4.43), DR3 (3.89)	78	100	Microlympho-cytotoxicity	Dahlberg 1981
Hungary	A1 (2.39), B8 (3.48), DR3 (4.80)	256 107	380 380	Standard microcytotoxicity	Stenszky 1985
Wales	DRw17, DRβ17-DQα2-DQβ2a± DQA2 U±	51	-	Standard lyphocyte microcytotoxicity, RFLP	Ratanachaiyavong 1990
Italy	A1-B8-Cw7-DR3 (11.29)±	105	6682	Serotyping, magnatic micospheres	Baldini 1995
Turkey	B8 (2.54), B49 (2.98), DR3 (1.97)	70	306	Serological specificity	Orhan 1993
India♦	A10 (2.9)¶, B8 (4.1)¶, DQw2 (5.5)¶	57	134	Standard two-stage microlymphocytotoxicity	Tandon 1990
Oriental group					
China	DR4-Dw4 (DRB1*0401) (13.0% vs 1.6%) DR8-Dw8.3 (DRB1*0803) (11.7% vs 4.9%)	30	225	PCR-SSO	Luo 1994
China	Bw46 (4.2), DR9 (3.2)♣	159	330	NIH lymphocyte microcytotoxicity	Yeo 1989
Hong Kong	B46 (6.04)♣, DR9 (3.85)♣ DQB1*0303 (6.53)♣	97	105	Standard lymphocyte microcytotoxicity, PCR-SSO	Cavan 1994
Singapore	B46 (8.2)	35	80	PCR-SSO, Standard microlymphocytotoxicity	Chan 1993
Japan	DQw4 (2.79), DRw8 (3.09)	88	186	Serotyping, RFLP	Inoue 1992
Japan	A2 (2.86), B46 (2.22), Cw11 (2.94) DPB1*0501 (5.32)	76	317	Standard complement dependent microcyto-toxicity, PCR-SSOP	Dong 1992
Japan	B46 (13.78)♥, DPB1*0501 (6.96)¶	106	100	Serotyping, RFLP	Onuma 1994
Japan	DRw8 (62.3% vs 20.0%)♠	35	263	NIH standard micro-cytotoxicity	Tamai 1986
Korea	B13 (3.8), DR5 (4.4)	137	220	Standard micro-lymphocytotoxicity	Cho 1986

± patients with persistent activity of disease, ♠ patients with thyroidtoxic periodic paralysis, ♣ only male,
¶ Significant increase was observed in the early-onset patients, † only female patients, ♦ Indo-Aryan,
♥ Significant increase was observed in the late-onset patients, †† total patients and female group, * relative risk

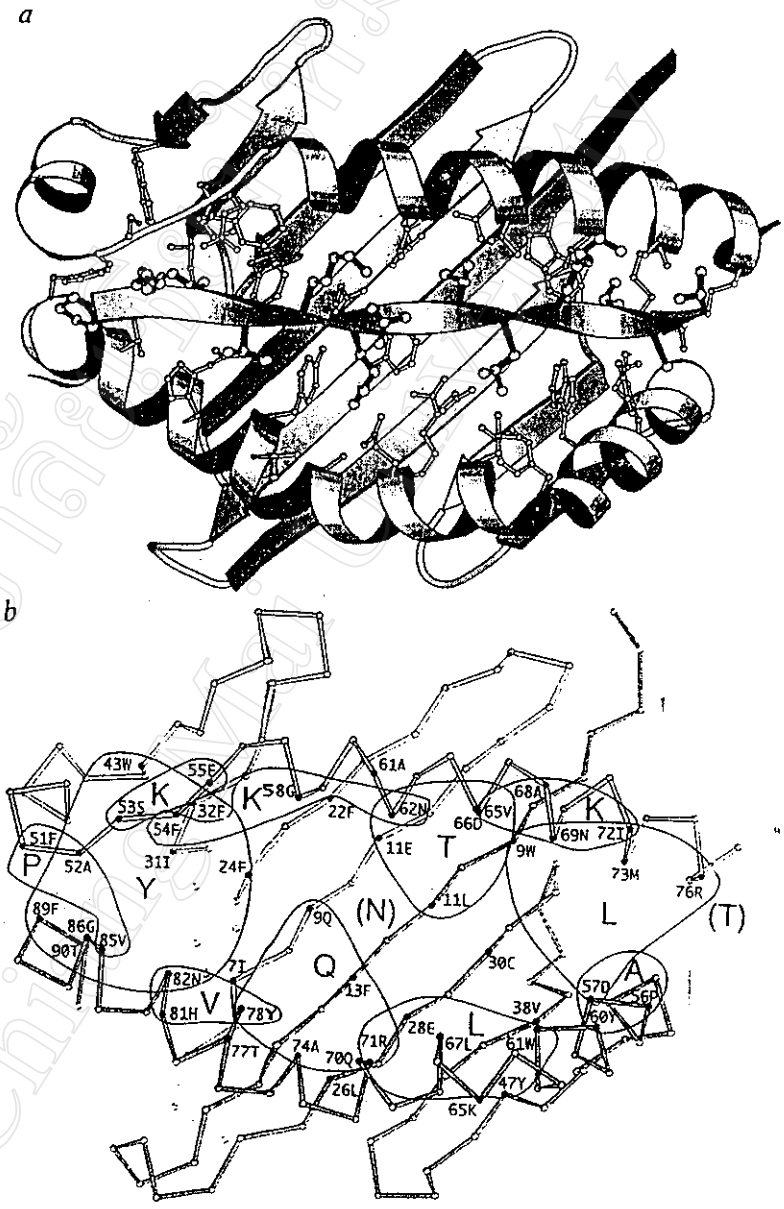

Figure 3 Map of HLA-DR1/HA peptide contracts. (a) Ribbon diagram, top view of the peptide-binding side. HLA side chains that contact the peptide or are buried by interaction with the peptide are shown by light bonds, HA peptide side chains by dark bond. (b) Each HA side chain and HLA-DR1 side chian inner the binding groove, labelled in the one-letter amino acid code (Stern et al., 1994).