EDITORIAL

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Role of Human Leukocyte Antigens and T Lymphocytes in the Immune Response

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Human leukocyte antigens (HLA) may play some role in the pathogenesis of every disease that involves an immune response. In the oncological field, HLA is implicated to be strongly associated with some cancers such as cervical carcinoma, nasopharyngeal carcinoma, thyroid carcinoma, Hodgkin's disease etc. and now HLA is essential to analyze the immunogenetic mechanisms underlying many diseases. Important technical advances of molecular biology in recent years have made it possible to characterize the HLA gene region at the DNA level.

This paper provides a comprehensive overview of the etiology of various immunologic disorders with emphasis on the role of HLA, a self/not-self discrimination mechanism, and of T lymphocytes.

Self/not-self discrimination

On earth, there are reportedly one million or more animal species and at least 500 thousand plant species¹⁾. In addition, each species shows considerable individual variation (polymorphism) at the protein and gene level despite their similarity in appearance. If a species did not have such individual genetic variations, it could not adapt flexibly to changing environmental conditions and would be eliminated by natural selection. The most important mechanism for production of genetic polymorphism is the continual exchange of genes between individual members of the species during reproduction.

The exchange of genes must only occur between individuals of the same species, and this is achieved by various mechanisms which allow same-species genetic material to be recognized. The development of such

mechanisms also means the development of mechanisms by which other species (or other individuals of the same species) can be recognized. Thus, a self/not-self discrimination mechanism for biological defense has developed, by which invading pathogenic microorganisms such as bacteria and viruses can be recognized as foreign (not-self) and can be eliminated.

Plants are also provided with a genetically controlled mechanism termed self-incompatibility, by which self-fertilization is blocked through self/not-self discrimination (i.e., the pistil of the flower rejects pollen from the same plant)²⁾. Going upstream along the genealogical tree, protozoa conjugation also only occurs between cells of different mating types, suggesting the presence of a self/not-self discrimination mechanism. Furthermore, considerable evidence has been obtained for the presence of a distinct self/not-self discrimination mechanism in each of the following phyla : porifera, coelenterata, platyhelminthes, annelida, echinodermata, prochordata and vertebrate. Among the various mechanisms involved, the major histocompatibility complex system, which has been demonstrated to function in mammalia and aves, seems to be the most sophisticated and elaborate.

Major histocompatibility complex (MHC)

In experimental transplantation between mouse siblings, Gorer found that matching a series of antigens (H-2 complex) between the donor and the recipient resulted in marked prolongation of graft survival in the recipient³⁾. Among these so-called transplantation antigens, the most important one was termed the major histocompatibility system³⁾. Later, Snell and Benacerraf et al. determined the loci of multiple genes which encode these antigens and termed them the major histocompatibility complex (MHC)⁴⁻⁶⁾. The human

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Fig.1 Gene organization of the HLA region

The gene organization of the HLA region was constructed according to our results of pulsed-field electrophoresis analysis. See ref. 69 for further details. Arrows indicate the orientation of transcription (5'→3').

HSP 70 : heat shock protein 70. 21 : 21-hydroxylase. BAT : HLA-B associated transcript. TNF : tumor necrosis factor

■ : expressed gene. □ : pseudogene. ▨ : gene of which protein product has not been identified despite the presence of its transcript

MHC is the HLA complex located on the short arm of chromosome 6, while the mouse MHC is the H-2 complex located on chromosome 17 and the rat MHC is the RT-1 complex located on chromosome 20. HLA are a group of cell membrane proteins encoded by the HLA complex with a high level of genetic polymorphism, and regulate the immune response by self/not-self discrimination as described above. HLA act as a marker of self for the human body : foreign antigens such as those on bacteria and viruses are checked against the self HLA and are recognized as not-self or foreign. As a result, T cells are stimulated to eliminate the foreign organisms or antigens. Thus, HLA play an important role in biological defenses against foreign organisms.

A widely accepted concept in population genetics is that mutant genes which are disadvantageous to the survival of the population are rapidly eliminated (negative natural selection), so that most of the existing alleles (allelomorphs present in one gene) are the result of so-called neutral mutations which have no specific survival advantages or disadvantages (neutral mutation random drift hypothesis)^{7,8}. On the other hand, it is thought to be rare for positive natural selection to occur, a process which actively makes a certain mutant gene predominant in a species. However, the human

MHC shows a high incidence of non-synonymous substitutions (mutations by which substitution of a DNA base causes amino acid exchange)⁹, indicating that positive natural selection has operated in the MHC system.

Ongoing investigation on the MHC of various vertebrates other than aves and mammalia have suggested that there might have been a common ancestor MHC before that divergent evolution of the vertebrate into various species. Thus, the diversity of the MHC appears to have been sustained much longer than that of other genes and must have been established by continual positive natural selection during the long history of evolution.

HLA and HLA genes

Classification, structure, and expression of HLA

HLA can be classified into class I and class II with respect to their structure, function, and specificity of expression. Between the HLA class I and class II gene regions, there is a group of genes that encodes complement components (C2, C4, and Bf) and other products. This region is generally termed the HLA class III

Table 1 HLA alloantigen specificity

A	B	C	D	DR	DQ	DP
A1	B5	Cw1	Dw1	DR1	DQ1	DP1
A2	B7	Cw2	Dw2	DR103	DQ2	DP2
A210	B703	Cw3	Dw3	DR2	DQ3	DP3
A3	B8	Cw4	Dw4	DR3	DQ4	DP4
A9	B12	Cw5	Dw5	DR4	DQ5 (1)	DP5
A10	B13	Cw6	Dw6	DR5	DQ6 (1)	DP6
A11	B14	Cw7	Dw7	DR6	DQ7 (3)	
A19	B15	Cw8	Dw8	DR7	DQ8 (3)	
A23 (9)	B16	Cw9 (w3)	Dw9	DR8	DQ9 (3)	
A24 (9)	B17	Cw10 (w3)	Dw10	DR9		
A2403	B18		Dw11 (w7)	DR10		
A25 (10)	B21		Dw12	DR11 (5)		
A26 (10)	B22		Dw13	DR12 (5)		
A28	B27		Dw14	DR13 (6)		
A29 (19)	B35		Dw15	DR14 (6)		
A30 (19)	B37		Dw16	DR1403		
A31 (19)	B38 (16)		Dw17 (w7)	DR1404		
A32 (19)	B39 (16)		Dw18 (w6)	DR15 (2)		
A33 (19)	B40		Dw19 (w6)	DR16 (2)		
A34 (10)	B41		Dw20	DR17 (3)		
A36	B42		Dw21	DR18 (3)		
A43	B44 (12)		Dw22			
A66 (10)	B45 (12)		Dw23	DR51		
A68 (28)	B46		Dw24	DR52		
A69 (28)	B47		Dw25	DR53		
A74 (19)	B48		Dw26			
	B49 (21)					
	B50 (21)					
	B51 (5)					
	B5102					
	B5103					
	B52 (5)					
	B53					
	B54 (22)					
	B55 (22)					
	B56 (22)					
	B57 (17)					
	B58 (17)					
	B59					
	B60 (40)					
	B61 (40)					
	B62 (15)					
	B63 (15)					
	B64 (14)					
	B65 (14)					
	B67					
	B70					
	B71 (70)					
	B72 (70)					
	B73					
	B75 (15)					
	B76 (15)					
	B77 (15)					
	B7801					
	Bw4					
	Bw6

Fig.2 Schematic structure of HLA
 $\alpha 1, \alpha 2, \alpha 3$: $\alpha 1, \alpha 2, \alpha 3$ domains. $\beta 2m$: $\beta 2$ -microglobulin
 $\beta 1, \beta 2$: $\beta 1, \beta 2$ domains. TM: transmembrane region
 CY: cytoplasmic region. CP: connecting region
 S-S: disulfide bond

region, but it actually has no functional relationship to HLA (Fig.1). HLA have a high level of genetic polymorphism that allows functioning as a self/not-self discrimination system, and are known to have many alloantigens (alleles) (Table 1).

Class I antigens

A typical class I molecule is bound to the cell membrane and is formed by a glycopeptide with a molecular weight (M.W.) of 45,000 (H-chain, α chain) that is non-covalently associated with a polypeptide having an M.W. of 12,000 (L-chain, $\beta 2$ -microglobulin) (Fig.2). The H-chain is composed of the $\alpha 1$ domain, $\alpha 2$ domain, $\alpha 3$ domain, TM region (the region bound to the cell membrane), and CY region (the intracellular region). The peptide-binding region corresponds to the $\alpha 1$ and $\alpha 2$ domains, which form the upper portion of the class I molecule. The N-terminal side of each of these domains forms 4 antiparallel β strands on a single plane, and 8 such strands from the two domains constitute a platform. Above this structure, the C-terminal sides of the two domains form two long winding parallel α helixes with a deep intervening cleft (Fig.3).

Genetic polymorphism is maintained by alloantigen determinants concentrated in these two domains. The $\alpha 3$ domain and $\beta 2$ -microglobulin, which form the extracellular portion of the class I molecule, have structures very similar to the domain of immunoglobulin. In fact, these components are members of the immunoglobulin super gene family, having evolved from the same ancestor gene as that for class II HLA, the T cell receptor, CD2, CD3, CD4,

CD8, and neural-cellular adhesion molecule (N-CAM). The domains which constitute the peptide-binding region ($\alpha 1$ and $\alpha 2$ domains) do not belong to this family. Their origin was previously unknown, because they had little homology with any other protein.

However, recent analysis of their secondary structure has revealed similarity to HSC70, a member of the heat shock protein (HSP) family, and it has been hypothesized that the $\alpha 1$ and $\alpha 2$ domains were generated by divergent evolution from this family, which seems to have appeared very early in the evolutionary process¹⁰. Taken together, the findings mentioned above suggest that HLA formed when the $\alpha 3$ domain and $\beta 2$ -microglobulin (members of the immunoglobulin super gene family) were combined with the $\alpha 1$ and $\alpha 2$ domains generated by divergent evolution from the HSP family. $\beta 2$ -microglobulin also plays an important role in transporting the H-chain synthesized in the Golgi apparatus to the cell membrane¹¹. The gene which encodes this molecule is located on chromosome 15, and not on chromosome 6 which contains the major members of the HLA genes.

HLA-A, -B, and -C have a high level of polymorphism and have long been known as class I antigens. They are widely distributed on the cell membrane of almost every tissue except for erythrocytes and spermatozoa, and their expression is particularly marked in cells of the lymphocytic series (T cells and B cells). Recent analysis of gene clones has led to the successive discovery of HLA-E¹², HLA-F¹³, and HLA-G¹⁴ as new or non-classified class I antigens.

The HLA-F and HLA-G genes have been mapped to the locus corresponding to that of the mouse Qa/TL antigen gene, but whether there is functional homology between the human and mouse antigens remains unknown. HLA-G has been found to be expressed on cell membrane, while the expression of HLA-E and HLA-F appears to be entirely intracellular. HLA-E, -F, and -G differ from the previously identified class I antigens in that they have a low level of polymorphism and are expressed in specific tissues. It has recently been found that HLA-G is expressed by trophoblasts in the embryonal period^{15,16}.

Since the expression of HLA-A, -B, and -C by trophoblasts is suppressed at this time, HLA-G may act as an inducer of the immune response in place of these other antigens. The fetal HLA-A, -B, and -C have an alloantigen specificity which differs from that of maternal HLA (semi-allograft), but their expression by trophoblasts, which face the maternal tissue of the placenta, is suppressed and this enables the fetus to

Fig.3 Three dimensional structure of HLA-A2¹⁸
 This conformation of HLA-A2 was clarified by Bjorkman et al. using X-ray analysis.
 (Bjorkman PJ, Saper MA, Samraoui B, et al. with permission of the authors and publishers of Nature)

avoid maternal immunological attack. In place of these antigens, HLA-G, which has a low level of genetic polymorphism and is thus a poor alloantigen, is expressed in the embryonal period and may protect the fetus from invading bacteria and viruses by inducing an immune response. It is hoped that future studies will clarify the detailed function of these non-classified antigens.

Class II antigens

A typical class II molecule is bound to the cell membrane and is formed by two glycopeptides, the α chain (M.W. 33,000~35,000) and the β chain (M.W. 27,000~29,000), that are non-covalently associated with each other (Fig.2). The α chain is composed of the α -1 domain, α -2 domain, TM region, and CY region while the β chain is composed of β -1 and β -2 domains as well as the TM and CY regions. The peptide-binding region corresponds to the α -1 and β -1 domains, which form the upper portion of the class II molecule.

The genetic polymorphism of class II antigens is arose by variations in alloantigen determinants concentrated in the β -1 domain of the β chain (as well as in the α -1 domain of the α chain in the case of HLA-DQ). The α -2 and β -2 domains have structures similar to that of immunoglobulin and belong to the

immunoglobulin super gene family. Thus, class II antigens, like class I antigens, were formed by combining two domains from the immunoglobulin super gene family with the α -1 and β -1 domains generated by divergent evolution from the HSP family. Based on this information on the high dimensional organization of HLA, Kasahara et al. have suggested that class I antigens, which have a more mobile and flexible 3-domain structure (H-chain), evolved earlier than the less mobile class II antigens¹⁰.

However, many other immunologists have argued against this suggestion on the basis of the higher polymorphism of HLA class II genes, and no consensus has been so far reached. An invariant chain (Ii chain) of M.W. 33,000 has been identified as the third component of class II antigens^{17,18}, and the gene encoding this chain is located on chromosome 5.

There are three types of class II antigens, which are designated as HLA-DR, -DQ, and -DP. There are known to be 4 other new class II genes, DNA (formerly DO α or DZ α)¹⁹⁻²¹, DMA²², DMB²², DOB (formerly DO β)²³ that have been recently discovered by gene cloning and nucleotide sequence analyses, although their products have not been identified biochemically or serologically. In addition, HLA-D is identified by cellular typing in mixed lymphocyte culture (MLC). However, this does not mean that a

Table 2 Correlation of HLA-DR, HLA-D, -DR51/52/53 and -DQ

HLA-DR		HLA-D	HLA-DR51/52/53,	-D	HLA-DQ
HLA-DR1		Dw1			DQ5 (DQ1)
		Dw20			DQ5 (DQ1)
HLA-DR103		Dw 'Bon'			DQ1
HLA-DR2	DR15	Dw2	DR51		DQ6 (DQ1)
	DR15	Dw12	DR51		DQ6 (DQ1)
	DR16	Dw21	DR51		DQ5 (DQ1)
	DR16	Dw22	DR51		DQ7 (DQ3)
HLA-DR3	DR17	Dw3	DR52a,	Dw24	DQ2
	DR17	Dw3 variant	DR52b,	Dw25	DQ2
	DR18	Dw 'RSH'	DR52a,	Dw24	DQ4
HLA-DR4		Dw4	DR53		DQ7 (DQ3)
					DQ8 (DQ3)
		Dw10	DR53		DQ8 (DQ3)
		Dw13	DR53		DQ7 (DQ3)
					DQ8 (DQ3)
		Dw14	DR53		DQ8 (DQ3)
		Dw15	DR53		DQ4
HLA-DR5	DR11	Dw5	DR52b,	Dw25	DQ7 (DQ3)
	DR11	Dw 'DB2'	DR52b,	Dw25	DQ1
	DR12	Dw 'DB6'	DR52b,	Dw25	DQ7 (DQ3)
	DR13	Dw18	DR52a,	Dw24	DQ6 (DQ1)
HLA-DR6	DR13	Dw18	DR52b,	Dw25	DQ1
	DR13	Dw19	DR52c,	Dw26	DQ6 (DQ1)
	DR14	Dw9	DR52b,	Dw25	DQ5 (DQ1)
	DR14	Dw16	DR52a,	Dw24	DQ7 (DQ3)
HLA-DR7		Dw7	DR53		DQ2
		Dw17	DR53		DQ2
		Dw11	blank		DQ9 (DQ3)
		Dw11	DR53		DQ9 (DQ3)
		Dw 'DB1'	DR53		DQ2
HLA-DR8		Dw8.1			DQ4
		Dw8.2			DQ4
		Dw8.3			DQ1
		Dw8.3			DQ7 (DQ3)
HLA-DR9		Dw23	DR53		DQ9 (DQ3)
HLA-DR10		Dw new			DQ5 (DQ1)

() : indicates an original alloantigen from which HLA-DQ split antigens are divided. Dw 'DB1', Dw 'DB2', Dw 'DB6' and Dw new are newly identified specificities which have not been officially recognized.

particular antigen designated as HLA-D is present, but instead indicates that a D-antigen epitope is formed by both HLA-DR and -DQ antigens. The HLA-DR and -DQ genes are located very close to each other, resulting in linkage disequilibrium, so the expression of HLA -DR, -DQ, and -D alloantigens is well correlated (Table 2). The HLA-DP gene is located on the most centromeric side of the HLA region and there is a hot spot for recombination between the HLA-DP and -DQ genes. Therefore, there is only a weak correlation between expression of the DP alloantigens on one hand and the DR, DQ, and D alloantigens on the other hand.

Unlike class I antigens, class II antigens are only expressed by certain kinds of cell : in human they are specifically expressed by macrophages (antigen-presenting cells ; APC), B cells, Langerhans cells, dendritic cells, activated T cells, and other cells of the immune system. They are also expressed by thymic epithelial cells, as well as by some fibroblasts,

endothelial cells, ordinary epithelial cells, and tumor cells (e.g., malignant melanoma).

Another feature of class II antigens is that they are not expressed by mature blood cells, such as neutrophils, erythrocytes and plasma cells, although they are expressed by undifferentiated myeloid cells such as granulocyte/macrophage colony forming units (CFU-GM) and erythroid colony forming units (CFU-E). This finding suggests that class II antigens may also play a role in cellular development and differentiation.

Processing of foreign antigens

Foreign antigens undergo intracellular digestion into short peptide fragments before binding to either an HLA class I or class II molecule. Whether a peptide fragment binds to a class I molecule, a class II molecule, or both mainly depends on the origin of

Fig.4 Processing of foreign antigens by HLA class I and class II molecules

foreign antigens, endogenous (class I) or exogenous (class II) proteins (see later for details) and also on the intrinsic structure of the peptide, or more specifically on the antigenic determinant (aggretope) of the peptide. The antigenic determinant is the part of a foreign antigen (a peptide region consisting of 9-17 amino acids) which determines the class of the HLA molecule to which it binds. For example, a protein produced by the influenza virus has both an aggretope for HLA class I molecules and another aggretope for HLA class II molecules^{24, 25}.

Other foreign antigens which are known to have different aggretopes recognizable by different classes of HLA antigen include ovalbumin²⁶, myelin basic protein²⁷, lysozyme^{26, 28}, and cytochrome²⁹. The mechanisms involved in the processing of exogenous antigens are discussed next.

Antigen processing by the HLA class I system

Class I antigens are expressed on the cell membranes of almost all nucleated cells. When a cell is infected by a virus or bacterium, mRNA is synthesized from viral or bacterial genes by the virus or bacteria-derived RNA polymerase or the nuclear RNA polymerase of the infected cell. This is followed by translation of the mRNA to produce viral or bacterial proteins (endogenously synthesized antigens). Subsequently, these proteins are transferred to the

proteasome, a low molecular-mass polypeptide (LMP) complex with multiple proteolytic activities, where they are partially digested³⁰⁻³⁶. The peptides are then selectively incorporated into the endoplasmic reticulum (ER) by the TAP transporter on the ER membrane³⁶⁻⁴⁴. In the ER, these peptides derived from endogenously synthesized antigens are bound to class I molecules. For example, only peptides consisting of 9 amino acids with the second amino acid being leucine can bind to HLA-A2 to form an HLA class I-foreign peptide complex⁴⁵, which then appears on the cell membrane via the Golgi apparatus. If no foreign peptide is present in the cleft of an HLA class I molecule, the molecule has an unfixed α -helical region and an unstable lower strand region.

Therefore, free class I molecules unbound to an antigenic peptide rarely appear on the cell membrane. HLA class II molecules are already bound to the invariant (Ii) chain in the endoplasmic reticulum, and this prevents these molecules from binding to any peptides derived from endogenously synthesized viral or bacterial antigens and incorporated into the ER (Fig.4). The HLA class I-foreign peptide complexes which appear on the cell membrane are generally recognized by CD8⁺ cytotoxic T lymphocytes (CTL).

Recent studies using RMA-S or 174/T2 cell lines deleted in the MHC class II gene region have revealed that the genes encoding proteases constituting a subunit

of the proteasome, human LMP2 and LMP7 genes (also termed RING12³³) and RING10³¹), and those encoding the TAP transporter, human TAP 1 and TAP 2 genes (also termed RING4³⁸ or PSF1³⁹, and RING11⁴³ or PSF2⁴⁴), are located between the DOB gene and the DNA gene in the MHC class II region³⁰⁻⁴¹. It is of great interest that the genes encoding these proteins, which are involved in antigen presentation by class I molecules, are located in the MHC class II region, although Spies et al. suggest that proteasome subunits encoded by the MHC are not essential for the processing of peptides bound by MHC class I molecules^{34,39}. It is possible that various diseases which appear to show correlations with class II antigens may actually be related to these genes, which produce products involved in the processing of endogenous antigens bound to class I molecules. The polymorphism of the genes encoding the TAP transporter^{43, 46-50} or LMP2⁵¹ is also currently being investigated.

To better understand the mechanism of various diseases, especially those having a known correlation with class II antigens, it seems necessary to investigate the polymorphism of these genes. It has also been suggested that the genes encoding proteins involved in antigen presentation by class II antigens may be located in this region⁵².

Antigen processing by the HLA class II system

Class II molecules are associated with the β_2 chain in the endoplasmic reticulum and Golgi apparatus and are thus prevented from binding to peptides derived from endogenous antigens. It is thought that the β_2 chain also plays a role in the transportation (localization) of class II molecules to the endosome. Class II antigen- β_2 chain complexes are transferred to the endosome, where the antigen molecule is dissociated from the β_2 chain under acidic conditions. Soluble exogenous antigens are actively incorporated into antigen-presenting cells (endocytosis), and are subsequently digested into peptide fragments by lysosome-derived enzymes in the endosome. These peptide fragments then bind to free class II antigen molecules, forming HLA class II antigen-foreign peptide complexes which are transferred to the cell membrane (Fig.4)^{17, 18, 53}. This type of complex is generally recognized by CD4⁺ helper T lymphocytes.

One exception is the DQ antigen-foreign peptide complex, which is thought to be involved in the induction of suppressor T lymphocytes. Class II molecules are stable even when they are not bound to any foreign antigen. Therefore, unlike class II

molecules, many free class II molecules unbound to antigenic peptides appear on the cell membrane, but they cannot be recognized by T cells due to MHC restriction.

Gene structure of the HLA region

The human MHC (HLA gene region) is located on the short arm of chromosome 6⁵⁴, and is a large region of 4,000 kb that has grown to this size through repeated gene duplication and conversion during the evolutionary process⁵⁵. The size of this region is comparable to that of the E. coli genome. The MHC class I region is about 1,500 kb in size and the MHC class II region is about 1,000 kb. The remainder comprises the MHC class III region^{56,57}, which contains genes encoding complement components (C2, C4, and Bf factor)⁵⁸ and 21-hydroxylase⁵⁹ as well as various other genes (Fig.1).

However, not all genes located in this HLA region have been cloned and some may still remain unidentified. For example, we have discovered several new HTF (CpG) islands in the class I region and obtained data suggesting the presence of new housekeeping genes in this region (Mizuki et al., in preparation).

In addition, there is a 400 kb region lying between the class III and class I regions. This region has been completely cloned and has been found to contain at least 20 non-HLA genes (genes with no functional relation to HLA), including tumor necrosis factor- α and - β (TNF- α , - β)⁶⁰⁻⁶³, heat shock protein-70 (Hsp-70)^{64, 65}, B144⁶⁶, and HLA-B associated transcript (BAT)^{67,68} genes.

Table 3 shows the genes which have been identified in the HLA region. Further information on the non-HLA genes can be found elsewhere^{69,70}. It is of great interest that various non-HLA genes involved in the immune response are also located in the HLA region on the short arm of human chromosome 6. This finding suggests that this important chromosomal region may play a pivotal role in biological defenses and immune response.

HTF (CpG) islands and housekeeping genes will only be briefly mentioned in this section⁷¹. HTF (*Hpa* II tiny fragment) islands are human gene regions which are very abundant in C-G sequences and therefore are also referred to as CpG islands. The term "*Hpa* II tiny fragment" is derived from the fact that the restriction enzyme used at the time of discovery of this island was *Hpa* II. CpG islands can also be recognized by other restriction enzymes recognizing C, G rich sequences,

Fig.5 Gene structure of HLA class II region

In the DRB gene region, the DR3, DR5 or DR6 (DR52 group) haplotype is illustrated. In the other haplotypes, the structures of the DRB gene region are slightly different. See Fig. 6 and Table 4.

Table 3 The number of HLA and non-HLA genes so far identified in the HLA region (total 96)

expressed gene	:	75
class I gene	:	6
class II gene		
{ α chain	:	5
{ β chain	:	6**
class III gene	:	4*
non-HLA gene	:	54
pseudogene	:	21
class I gene	:	12
class II gene		
{ α chain	:	2
{ β chain	:	5**
non-HLA gene	:	2

* Class III genes are included in non-HLA genes.

** The number of the class II β genes depends on the HLA-DR haplotype.

Not I, *Bss*HII, *Sac* II, *Eag* I, and *Mlu* I, but these are referred to as rare cutter enzymes since they only recognize a few sites in the human genome.

Therefore, CpG islands can also be defined as small DNA regions (2-3 kb or less) that contain sites recognized by two or more of the rare cutter enzymes. These islands are often located on the 5'-terminal side of a housekeeping gene, and 80% or more of the *Not* I sites are associated with expressed housekeeping genes⁷². Housekeeping genes are the genes expressed in all cells and tissues, as opposed to tissue-specific genes which are expressed only by specific cells or tissues. The class I to III regions of the human MHC are abundant in C-G sequences and contain many housekeeping genes, while the HLA class II region is abundant in A-T sequences and tends to contain tissue-specific genes.

Class I MHC

The individual class I antigens (HLA-A, -B and -C) are encoded by single genes of the HLA-A, -B, and -C loci. Therefore, each HLA class I antigen gene has a corresponding serologically determined specificity. As described previously, there are also 3 other class I antigen genes (HLA-E, -F, and -G) that are known to be expressed. Including these genes and 11 pseudogenes, a total of about 17 genes have been identified in the class I region.

Class II MHC

In the class II region of the human MHC (Fig. 5), the DP gene subregion, DNA gene, DMA gene, DMB gene, DOB gene, DQ gene subregion, and DR gene subregion are located from the centromere in this order. The products (antigenic proteins) of the DNA and DOB genes (which are classified as class II antigen genes) have not been identified, although synthesis of mRNA from these genes has been observed^{19-21, 23}. New class II antigen genes named DMA (DM α : RING6 gene) and DMB (DM β : RING7 gene) were recently identified between the DOB and DNA gene regions. The products of these genes have not been identified, either, but antigen production from them seems very likely to occur because mRNA synthesis has been detected by Northern hybridization²³. Unlike class I antigens, each class II antigen is encoded by two genes, with one encoding the α chain (A) and the other encoding the β chain (B).

Therefore, each class II molecule in the endoplasmic reticulum is a heterodimer of the two chains encoded by the respective genes. As HLA-DR has polymorphism only for the β chain (encoded by the DRB1 gene), the

Fig.6 Gene structure of HLA-DR region with the various haplotype^{75,76}
(Bodmer JG, Marsh SGE, Albert ED, et al. with permission of the authors and publishers of Tissue Antigens)

antigen exists in the form of two types of heterodimer originating from two chromosomes. In contrast, HLA-DQ has polymorphism for both the α (DQA1 gene) and β chains (DQB1 gene) and therefore exists in the form of 4 types of heterodimer arising from cis- and trans-combinations of the DQA1 and DQB1 genes on two chromosomes (it has only a single type if it exists as a homozygote)^{73,74}. In addition, different haplotypes have different numbers and compositions of HLA-DR genes (Fig.6 and Table 4)^{75,76}. In serological studies, DR3, DR5, and DR6 show a 100% correlation with DR52, while DR4, DR7, and DR9 show a 100% correlation with DR53. These findings are explained by the differences in gene composition between different haplotypes.

The nomenclature of HLA genes will be briefly explained next. Before the international nomenclature was established, many laboratories employed their own naming systems, but this naturally caused confusion in this field of research. Since the 10th International Histocompatibility Workshop and Conference in 1987, a new principle for HLA nomenclature has been introduced and an official allelic name has been given only to genes for which the nucleotide sequences or the amino acid sequences of the products have been determined.

According to the new nomenclature, a gene encoding an α chain is termed A and a gene encoding a β chain is termed B. Further details of this system can be found

elsewhere. The new system provides standardized and simple notations for HLA genes and the former terms should be abandoned (Fig.7).

This paper does not deal with the correlations between HLA genes and serological findings due to space limitations^{75,76}. Many new alleles have been discovered in association with the results reported at the 11th International Histocompatibility Workshop and Conference. For further details, see "HLA in 1991" published by Oxford University Press, etc⁷⁵⁻⁷⁷.

Functions of HLA

HLA have a high level of genetic polymorphism, and act as a marker for self/not-self discrimination. The genetic polymorphism of HLA is essential for self/not-self discrimination, because recognition of a foreign antigen as well as a product of the host MHC (HLA) by T cells is required before they become activated to attack the foreign antigen (MHC restriction or altered self theory). As described previously, a deep cleft is present between the α -helical region and the underlying β -strand region of each HLA molecule (Fig.3). When this deep cleft is occupied by a peptide derived from a foreign antigen by partial digestion, the peptide is presented as an antigen to T cells (hot dog model)⁷⁸.

Recent studies have identified at the molecular level the amino acid sites at which foreign antigenic peptides associate with HLA molecules and the amino acid sites

Table 4 Gene of class II antigens with various HLA-DR haplotypes

haplotype	gene structure	function
DR antigen	DR1 Group (DR1, DR10, DR103)	DRA polymorphism(−), encodes DR1 α chain DRB1 polymorphism(+), encodes DR1 β chain pseudogene DRB6 pseudogene DRB9
	DR51 Group (DR15, DR16)	DRA polymorphism(−), encodes DR2 α and DR51 α chains DRB1 polymorphism(+), encodes DR2b β chain pseudogene DRB6 pseudogene DRB5 polymorphism(+), encodes DR2a α β and DR51 β chains DRB9 pseudogene
	DR52 Group (DR3, DR11, DR12, DR13, DR14, DR1403, DR1404)	DRA Polymorphism(−), encodes one of DR3, DR5, DR6 α chains and DR52 α chain DRB1 polymorphism(+), encodes one of DR3, DR5, DR6 β chains pseudogene DRB2 pseudogene DRB3 polymorphism(−), encodes DR52 β chain DRB9 pseudogene
	DR53 Group (DR4, DR7, DR9)	DRA polymorphism(−), encodes one of DR4, DR7, DR9 α chains and DR53 α chain DRB1 polymorphism(+), encodes one of DR4, DR7, DR9 β chains pseudogene DRB7 pseudogene DRB8 pseudogene DRB4 polymorphism(+), encodes DR53 β chain DRB9 pseudogene
	DR8	DRA polymorphism(−), encodes DR8 α chain DRB1 polymorphism(+), encodes DR8 β chain DRB9 pseudogene
	DQ antigen	DQA1 polymorphism(+), encodes each DQ antigen α chain DQA2 pseudogene DQB1 polymorphism(+), encodes each DQ antigen β chain DQB2 pseudogene DQB3 pseudogene
	DP antigen	DPA1 polymorphism(+), encodes each DP antigen α chain DPA2 pseudogene DPB1 polymorphism(+), encodes each DP antigen β chain DPB2 pseudogene

(1) Class I antigen (2) Class II antigen

HLA - A * 0201
① ② ③ ④HLA - DRB1 * 0405
① ② ③ ④ ⑤ ⑥ ⑦

Fig.7 Nomenclature of HLA alleles

- (1) ① represents a class I antigen gene locus (A,B,C,E,F, or G).
 ② may be omitted if the gene locus is contained in the MHC class I region, but if it is omitted, italic notation is required.
 ③ should be a figure (basic number) representing the serologically defined specificity with the most strong relationship.
 ④ should be a figure representing a further characterized allele among 3.
- (2) ① means that this antigen is classified as a class II antigen.
 ② represents the name of the subregion (R, Q, P, O, N or M).
 ③ should be A if this gene encodes an α chain and should be B if this gene encodes a β chain.
 ④ represents a number distinguishing multiple α or β chains
 ⑤ is an asterisk essential for naming of class II alleles
 ⑥ has basically the same meaning as 3 for class I antigens
 ⑦ has the same meaning as 4 for class I antigens

at which T cells recognize the HLA-foreign antigen complex⁷⁹. In this regard, some evidence has been obtained to suggest that formation of a 3-molecule complex between HLA, the peptide derived from a

foreign antigen by digestion, and the T cell receptor is essential for T cell recognition of foreign antigens^{80, 81}. A self HLA molecule that is not bound to foreign antigen is not recognized by T cells. Thus, T cell responses should always be thought of as occurring to HLA-peptide complexes.

As described above, the peptides derived from endogenously synthesized antigens are generally presented by HLA class I molecules, while antigens incorporated into a cell (exogenous soluble antigens) are generally presented by HLA class II molecules. HLA class I-peptide complexes are recognized by CD8⁺ cytotoxic T lymphocytes and HLA class II-peptide complexes are recognized by CD4⁺ helper T lymphocytes. In addition, HLA-DQ-peptide complexes are also thought to induce suppressor T lymphocytes⁸².

Role of T lymphocytes in the immune response

How do T lymphocytes eliminate not-self antigens in response to the signals delivered by HLA? It is thought that the T lymphocyte immune response occurs by the following mechanism, but the actual *in vivo* response may be complicated by various other factors.

Antigen recognition by the T cell receptor (TCR)

The TCR is composed of α and β chains, which directly recognize HLA-peptide complexes and 5 supporting protein chains (γ , δ , ϵ , ζ , η or γ , δ , ϵ , ζ , η : CD3 complex)^{83, 84}. Like immunoglobulins, the α and β chains are composed of a variable (V) region and a constant (C) region. The V region of the α chain is encoded by the V and J genes, while the V region of the β chain is encoded by the V, J, and D genes⁸⁵. The very large repertoire of the TCR is attained by the recombination and rearrangement of each of these genes with polymorphism^{86, 87}. Thus, the V region contains 3~4 hypervariable regions, and these regions presumably form a complementarity determining region (CDR) that binds to HLA-peptide complexes. The 5 protein chains forming a supporting structure are also involved in signal transduction (transmission of signals for the activation of T lymphocytes). After the α and β chains of the TCR are bound to an HLA-peptide complex, the CD4 or CD8 molecule also binds to the constant region of the HLA molecule to reinforce the bond between the TCR and the HLA-peptide complex and to facilitate signal transduction^{88, 89}. Binding of the TCR to an HLA-peptide complex is also reinforced by intercellular adhesion molecule-1 (ICAM-1), as well as lymphocyte function associated antigen-1 (LFA-1), LFA-3, and LFA-2 (CD2), which facilitate intercellular adhesion between antigen-presenting cells and T lymphocytes. Several kinds of tyrosine kinase, including *lck* and *fyn*, are associated with the TCR (Fig.8).

These enzymes phosphorylate many intracellular substrates and membrane proteins (especially ζ) which are important in the signal transduction process for T cell activation. After the TCR recognizes an MHC antigen complex, the tyrosine kinases (*lck* and *fyn*) are dephosphorylated and activated by interaction with CD45 tyrosine phosphatase, and then cause the phosphorylation of many intracellular substrates mediating signal transduction, resulting in phosphatidylinositol degradation and protein kinase C (PKC) activation among other changes.

Induction of cytotoxic T lymphocytes (CTL)

The induction of cytotoxic T lymphocytes requires involvement of helper T cells and HLA class II-foreign peptide complexes presented by antigen-presenting cells (APC). Presentation of the HLA class I-foreign peptide complex to precursor CTL induces IL-2 receptor expression on the precursor CTL. However,

Fig.8 T cell recognition and its adhesion molecules
TCR : T cell receptor, ICAM-1 : intracellular adhesion molecule, LFA-1, -2, -3: lymphocyte function associated antigen type 1, 2, 3, *lck*, *fyn* : lymphocyte specific tyrosine kinase (membrane associated oncogene products, which activate, proliferate and differentiate T cells)

their growth and differentiation to mature CTL requires the assistance of helper T cells that recognize HLA class II-foreign peptide complexes on APC⁹⁰. That is, precursor CTL are stimulated by IL-2 released from APC-activated helper T cells to differentiate into CTL with killer activity.

It is unknown whether the interaction between precursor CTL and helper T cells occurs via a receptor or due to simple contact at sites of inflammation. Although there is some uncertainty regarding the details of this mechanism, CTL that mature in this way selectively kill cells which express the HLA class I-foreign peptide complex by injecting perforin, that is cytotoxic in the presence of Ca ions⁹¹ (Fig.9).

There are two helper T cells subsets designated as Th1 and Th2, and the subset involved in induction of CTL is thought to be Th1. This subset is activated to produce IL-2 and IFN- γ through signal transduction mediated by protein kinase A^{92, 93} (Table 5).

Induction of antibody-forming cells (B cells)

Helper T cells are also involved in the induction of antibody-forming cells. When a helper T cells recognize HLA class II-foreign peptide complexes on APC, they also produce IL-4, IL-5, and IL-6 and stimulate B cells to divide and differentiate into antibody-forming cells. The antibody formed by these cells are specific to a particular soluble exogenous antigen and eliminate this antigen through activation of the complement system and induction of antibody-dependent cellular cytotoxicity (ADCC) (Fig.9).

The helper T subset involved in this process is Th2, and its functioning requires IL-1 expression as a costimulatory signal^{94~96}. It is thought that Th2 express

Fig.9 Immune response of T cells after antigen presentation

APC : antigen presenting cell. TCR : T cell receptor
 Th1 : helper T cell type 1. Th2 : helper T cell type 2
 pCTL : precursor of cytotoxic T lymphocyte. B : B cell
 CTL : cytotoxic T cell. NK : natural killer cell
 IL-1, -2, -4, -5, -6 : interleukin-1, -2, -4, -5, -6. IFN- γ : interferon- γ
 ADCC : antibody-dependent cellular cytotoxicity
 ● : foreign antigen peptide bound to HLA class I antigen
 ▲ : foreign antigen peptide bound to HLA class II antigen

Table 5 Comparison of two helper T cells subsets, Th1 and Th 2

helper T cell (Th)	signal transduction	lymphokine production	costimulatory signal	main immune response
Th1	protein kinase A, Ca^{2+}	IL-2, IFN- γ IL-3, GM-CSF	no involvement of IL-1 (no expression of IL-1 receptor)	induction of CTL
Th2	protein kinase C, Ca^{2+}	IL-4, IL-5, IL-6, IL-10 IL-3, GM-CSF	involvement of IL-1 (expression of IL-1 receptor)	antibody production

These can be tentatively but not definitely classified at present.

the IL-1 receptor and are activated through signal transduction by protein kinase C to produce IL-4, IL-5, and IL-6^{92, 97} (Table 5).

Recognition of allogeneic MHC (alloantigen)

The not-self antigens that induce the most intense immune response are alloantigens which form the allogeneic major histocompatibility system. The elimination of these powerful antigens (e.g., in allograft rejection) involves the mechanism for eliminating ordinary soluble exogenous antigens as well as another

response mechanism. In the former classical mechanism, allogeneic HLA class I molecules are bound to HLA class II molecules on APC to form HLA class II -alloantigen complexes which present the alloantigens to CD4⁺ helper T cells. In the latter mechanism, foreign allogeneic class I antigens are directly recognized by CD8⁺ helper T cells without the mediation of APC or HLA class II molecules^{98, 99}. CD8⁺ helper T cells are only involved in the immune response to allogeneic HLA class I molecules and certain viral antigens, and appear to have a small repertoire. However, this subset is present in similar numbers to

CD4⁺ helper T cells¹⁰⁰.

In contrast, recognition of allogeneic HLA class II molecules is performed by CD4⁺ helper T cells, regardless of whether or not the recognition follows processing and antigen presentation by HLA class II. It is also known that two subsets of cytotoxic T cells, CD8⁺CTL and CD4⁺CTL, undergo differentiation in response to helper T cells and act as the final effectors in the immune response to alloantigens.

In summary, allogeneic MHC antigens are eliminated by CD8⁺CTL with the aid of CD4⁺ or CD8⁺ helper T cells as well as through direct rejection by these two helper T cell subsets (CD4⁺Th/eff and CD8⁺Th/eff mechanism)¹⁰¹. Thus, several elimination mechanisms are responsible for the intense rejection of allografts and represent important determinants of the prognosis of clinical transplantation.

Induction of self tolerance

The human body has highly sophisticated immunological mechanisms by which almost all foreign antigens can be eliminated, so how is immunological unresponsiveness to autoantigens obtained? The thymus plays a major role in the establishment of self tolerance. Although some authors have suggested that all autoantigens in the body are present in the thymus¹⁰² or reach the thymus from other tissues through the circulation^{103, 104}, this hypothesis is relatively unlikely. Currently, the most widely accepted idea is that immunological tolerance to autoantigens which are not presented in the thymus is induced in other tissues. Thus, induction of self tolerance is achieved by the previously known thymic system as well as by a non-thymic (peripheral) system.

Autoimmune diseases can be defined as being caused by the recognition of autoantigens as not-self as a result of the failure of established self tolerance, and HLA probably have a significant role in the pathogenesis of such diseases. The following hypotheses have been proposed regarding the actual mechanism of induction of self tolerance.

Clonal selection

Precursor T cells produced in the bone marrow travel via the circulation to the thymus. These precursor cells (CD3⁻) are initially negative for both CD4 and CD8 expression, and are then differentiated via a double-positive stage (CD4⁺CD8⁺) to a single-positive mature T cells (CD4⁺CD8⁻ or CD4⁻CD8⁺) by negative and positive selection in association with expression of the

α and β chains of the TCR¹⁰⁵. Thus, after the T cell repertoire which is completely complementary to autoantigens and the repertoire which has no complementation with autoantigens have been deleted (negative selection or clonal deletion)¹⁰⁶⁻¹¹⁰, only the T cell clone repertoire which is partially complementary to autoantigens is selected and expanded (positive selection)¹⁰⁹⁻¹¹¹. This model suggests that T cells recognize HLA molecules bound to foreign antigens (HLA-foreign peptide complexes), i.e., markers of self which have undergone slight morphological alterations due to binding the foreign antigen peptide (altered self). This has been proposed as a basic hypothesis which accounts for the interactions between HLA and T cells. The cells which present autoantigens together with HLA in the thymus and induce self tolerance are probably derived from myeloid stem cells, although it has also been suggested that thymocytes themselves may induce self tolerance¹¹².

Though the identity of the actual antigen-presenting cells (APC) remains a matter of controversy, the T cell clone repertoire formed by selection in the presence of HLA-autoantigen complexes should vary considerably between individuals. In other words, the T cell clone repertoire which has been selected by the presentation of various peptide fragments, which were derived by digestion from intracellular autoproducts (autoantigens) and bound to particular HLA alleles, should have a spectrum completely different from that of the T cell clone repertoire selected by the presentation of various peptides bound to other HLA alleles.

Clonal anergy

Clonal anergy is the term for a condition in which a T cell clone for a given autoantigen exists in the body but fails to show an immune response due to dysfunction. In the narrow sense, clonal anergy means that a given T cell clone does not proliferate in response to signals delivered via the TCR despite the lack of TCR down regulation or any related modifications, but is able to respond to exogenously administered IL-2. Many recent reports have discussed clonal anergy. Transgenic mice prepared by the introduction of allogeneic MHC antigens remain normal without any autoimmune diseases. V β -positive T cells corresponding to the introduced allogeneic MHC antigens can be detected in their circulation but show no immune response to the alloantigens.

In an *in vitro* study, reduction of signaling via the TCR by anti-Ia or anti-CD4 antibodies induced anergy of T cell clones which should have been activated or

eliminated, suggesting that quantitative or qualitative changes in signaling to T cells alter the consequences of T cell stimulation from clonal elimination/activation to clonal anergy¹¹³.

Various subsequent experiments using anti-V β monoclonal antibodies have indicated that a T cell clone becomes unresponsive if an antigen is not presented to the clone by professional APC but by non-professional APC such as thymic epithelial cells and pancreatic cells¹¹⁴⁻¹²⁰. Thus, inadequate antigen presentation and stimulation of T cells by cells non-professional APC appears to induce clonal anergy. Inadequate stimulation may be defined as stimulation unassociated with costimulatory signals (second signals)⁹⁴⁻⁹⁶.

It is rare for the activation of T cells to be induced solely by a 3-molecule complex consisting of the TCR, HLA, and the foreign antigen, and activation generally requires the simultaneous presence of a costimulatory signal via other receptors. Antigen presentation without such a signal (by cells with an inadequate antigen-presenting ability) induces unresponsiveness of the T cell clone rather than its activation.

Interaction between the BB-1/B7 molecule expressed by APC and the CD28 molecule expressed by T cells has been suggested to be an important costimulatory signal that can be delivered only by professional APC. The BB-1/B7 molecule is a membrane protein expressed by activated B cells and memory B cells, and presumably causes marked stimulation of IL-2 production when bound to the CD28 molecules on T cells. Thus, modulation of the expression of the IL-2 gene seems to make a primary contribution to the activation/inactivation of T cell clones^{121, 122}. IL-1 is a well known costimulatory signal for Th2 cells^{92, 96, 97}, although it has no direct relationship with clonal anergy. This clonal anergy mechanism acts both in the thymus and in nonthymic peripheral tissues.

Suppression by T cells

This hypothesis has been proposed on the basis of the analysis of an experimental animal model of autoimmune thyroiditis (Hashimoto's thyroiditis). In this model, animals remain normal without the development of thyroiditis despite the existence of both T and B cells responsive to thyroglobulin, an autoantigen^{123, 124}. Autoimmune thyroiditis only develops after a certain T cell subset is deleted, and its development is suppressed if the T cell subset is re-injected¹²⁵.

The T cell clone which responds to the autoantigen is neither deleted nor inactivated by this process. In

normal individuals, such clones may be antagonized by other T cell clones and the response to autoantigens may be suppressed in this manner. The molecular mechanisms involved in the functioning of these inhibitory T cell clones (possibly suppressor T cells) remain to be clarified.

Molecular mechanisms of associations between HLA and diseases

HLA may play a role in the pathogenesis of every disease that involves an immune response. Recent studies have proved that particular HLA genes are related to the risk of certain diseases. That is, HLA alloantigen types (alleles) are host-related determinants of the liability to develop certain illnesses. The molecular mechanisms involved have been accounted for by the following 3 hypotheses.

① Different HLA allotypes have differing abilities to present foreign antigens and make T cells recognize them. Such differences sometimes result in the induction of an excessive or insufficient immune response, leading to the development of disease. Typical diseases and the related HLA allotypes which are thought to be explained by this hypothesis include juvenile diabetes mellitus-HLA-DQ β 57-non-aspartic acid^{126, 127}, rheumatoid arthritis-HLA-DR β 70-glycine and 71-arginine¹²⁸, narcolepsy-HLA-DR β 30-aspartic acid and 37-aspartic acid, and pemphigus vulgaris s-HLA-DQ β 57-aspartic acid¹²⁹.

② HLA may be a mere genetic marker, and the true genes determining susceptibility or resistance to a disease may be nearby non-HLA genes which show linkage disequilibrium with HLA. Abnormalities or particular alleles of such genes may then lead to the development of disease, e.g., if a non-HLA gene encodes a biologically important protein, its deletion may lead to the development of pathology. As a concrete example, it has been demonstrated that congenital adrenal hyperplasia (which is linked with HLA-B47) develops due to deletion of the gene encoding 21-hydroxylase that is located in the MHC class III region¹³⁰, while C2 deficiency (linked with HLA-B8) has been proven to develop due to deletion of the gene encoding C2 that is located also in the MHC class III region¹²⁷.

③ Homology of amino acid sequences between HLA and foreign antigens may cause antibodies or killer T cells directed against the antigens to also attack self HLA due to cross reactivity, leading to the development of autoimmune disorders (molecular mimicry theory). This hypothesis is based on the premise that the foreign

antigens differ sufficiently from HLA that immunological tolerance cannot occur and also have enough similarity to HLA to produce cross-reactions. For example, ankylosing spondylitis shows a high correlation with HLA-B27, but molecular homology between a *Klebsiella pneumoniae* cell component (nitrogenase reductase) and a B27 (HLA-B*2705) alloantigen suggests that the bacterial cell component may actually be responsible for the development of this disease¹³¹.

As mentioned previously, these disease models can be applied to cases where HLA or adjacent genes make a primary contribution to the development of a disease or where the host HLA allele determines the liability to a disease. There are many diseases that have not yet been shown to have any definite association with HLA although some connection seems very likely. In fact, only a few diseases have been definitely related to HLA.

Thus, it has been suggested that although HLA play a major role in antigen presentation and antigen recognition by T cells, the differences among HLA allotypes may not be large enough to alter the intensity of these immune responses and may not be directly involved in the pathogenesis of many diseases.

Conclusions

This article reviewed the established theories and recent findings concerning the role of HLA and T lymphocytes in the immune response. Marked progress is being made and new findings are constantly being reported in this field. In the future, the molecular mechanisms of immune response involving HLA and T lymphocytes will be clarified in more detail to provide a more thorough understanding of the pathogenesis of various immunologic disorders including malignant tumour at the molecular level.

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