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Review Article

Immunogenetics of the HLA System

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The HLA system is the major histocompatibility complex (MHC)in humans. It spans approximately 4,000 kb on the short arm(p21) of chromosome 6, and consists of a series of genes that are clustered in three distinct groups, designated class I, class II, and class III(Carroll *et al.* 1987). A genetic map of the HLA system is shown in Figure 1. Genes within the class I and class II regions encode HLA antigens, while class III region genes encode diverse proteins including complement components C2 and C4 of the classical pathway, factor B(Bf) of the alternate pathway, 21-hydroxylases(21-OH) A and B, and tumor necrosis factors (TNF) α and β. Although class III gene products are functionally related to the immune system, they do not function as major histocompatibility antigens. The HLA system is known to be the most polymorphic genetic loci in humans. This paper will discuss the structural basis of the HLA polymorphism and its functional implications.

HLA class I region

There are at least three class I loci, HLA-A, B, and C, that encode polymorphic cell surface molecules expressed on most nucleated cells. Class I molecules are composed of a glycosylated 44 kilodalton(kd) polypeptide chain (*heavy chain*) and noncovalently bound 12 kd beta$_2$-microglobulin (β_2 m, *light chain*). The latter chain is encoded by chromosome 15 and is known to be invariant in humans. Class I genes have the typical exon-intron organization that represents the domain structure of the molecule(Fig. 2). The heavy chain is an approximately 340 amino acid-long transmembrane protein with three extracellular domains(α1, α2, α3), a transmembrane region and a cytoplasmic portion. Recently, three additional "non-classical" class I genes, HLA-E, F, and G, have been identifed (Geraghty *et al.* 1987; Koller *et al.* 1988; Geraghty *et al.* 1990). HLA-G is expressed on the surface of trophoblasts in early gestational period. Otherwise, products of these genes are not detected on the cell surface, and their possible role in immune system remains to be studied.

HLA class II region

The class II region, also referred to as HLA-D, contains five subregions designated DP, DN, DO, DQ and DR, Each subregion has one to four loci called A or B followed by a number (Bodmer *et al.* 1990). Some A and B genes encode α and β peptide chains respectively, while others are pseudogenes or not expressed (Table 1). DP, DQ, and DR molecules are expressed on the cell surface as heterodimers made up of an α and β chain. Both chains are about 225 amino acid-long and consist of two extracellular domains(α1 and α2; β1 and β2), transmembrane and cytoplasmic portions. The α chain has a molecular weight of 33 kd, and the β chain 29 kd. Class II molecules are expressed on B lymphocytes, macrophages, and monocytes. Resting T lymphocytes do not express the class II molecules, but the expression is induced upon their activation.

STRUCTURE OF THE HLA MOLECULE

Tertiary structure of HLA molecule was revealed by X-ray crystallography of the HLA-A2 molecule (Bjorkman *et al.* 1987). The membrane-proximal α3 domain is paired with β_2 m forming an immunoglobulin constant region-like structure as was expected from the significant sequence homology between α 3 domain, β_2 m and constant regions. The membrane-distal α1 and α2 dnmains are paired to form a

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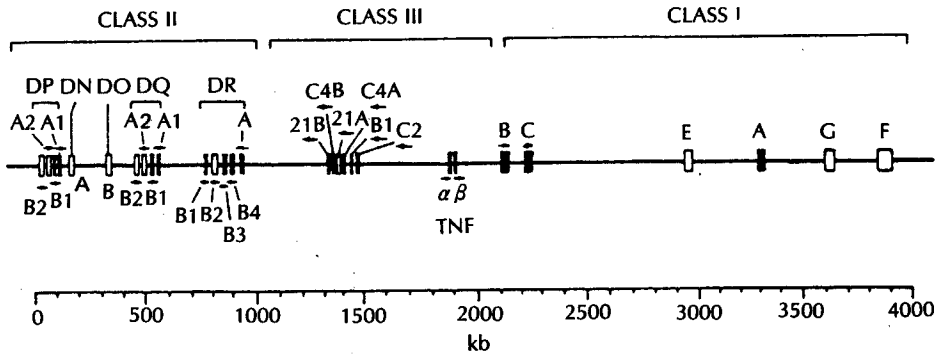

Fig. 1. *Genetic map of human major histocompatibility complex, HLA system. Solid boxes represent genes that are expressed, and open boxes represent genes that are not expressed.*

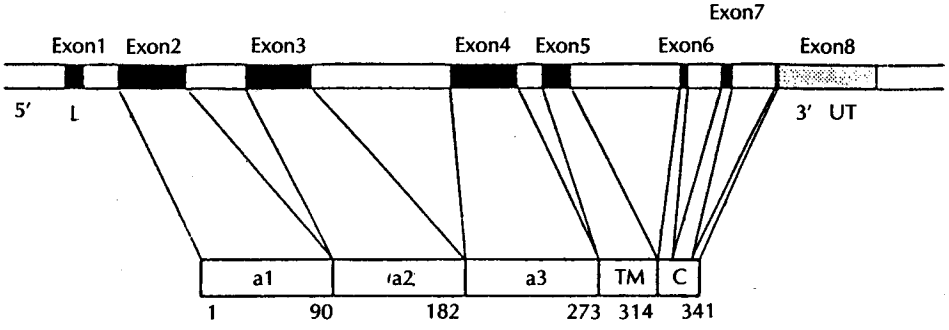

Fig. 2. *Exon-intron organization of HLA class I gene and its encoded protein. L, leader peptide; α1, α2, α3, three extracellular domains; TM, transmembrane region; and C, cytoplasmic portion. The number repesents the amino acid residues.*

Table 1. HLA-D region genes and their products[1]

Genes	Protein Products	Antigen Specificities
HLA-DRA	DR α-chain	
HLA-DRB1	DR β1-chain	DR1-DRw18
HLA-DRB2	pseudogene with DR-β-like sequences	
HLA-DRB3	DR β3-chain	DRw52(Dw24, Dw25, Dw26)
HLA-DRB4	DR β4-chain	DRw53
HLA-DRB5	DR β5-chain found in DR2 haplotypes	
HLA-DQA1	DQ α-chain	
HLA-DQB1	DQ β-chain	DQw1-DQw9
HLA-DQA2	DQ α-chain-related sequence, not expressed	
HLA-DQB2	DQ β-chain-related sequence, not expressed	
HLA-DOB	DO β-chain	
HLA-DNA	DN α-chain	
HLA-DPA1	DP α-chain	
HLA-DPB1	DP β-chain	
HLA-DPA2	DP α-chain-related pseudogene	
HLA-DPB2	DP β-chain-related pseudogene	

[1]Adapted from Bodmer *et al.* 1990

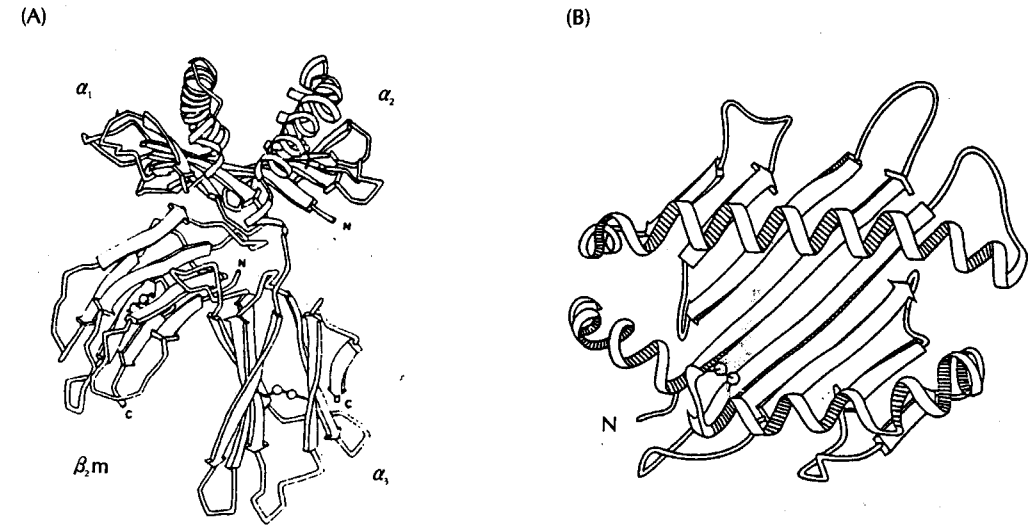

Fig. 3. *Tertiary structural model of the HLA class I molecule.*
A) The α1, α2 and α3 extracellular domains of the heavy chain are noncovalently bound to β_2 microglobulin(β_2m).
B) The α1 and α2 domains of the HLA class I heavy chain form eight anti-parallel β strands and two α-helices. (Adapted from Bjorkman et al. 1987)

β-sheet platform with eight antiparallel β-strands, covered by two α-helices(Fig. 3). A groove~25Å long, 10Å wide, and 10Å deep runs on top surface of the molecule, formed by the α1 and α2 domains. The sides of the groove are formed by side chains from the two α helices and the floor is formed by side chains from the central β-strands of β-sheet. This groove provides a binding site for processed peptide antigens. The size of the antigen binding cleft is sufficient to bind a peptide of 8-20 amino acids. The three-dimensional structure of a second HLA molecule, HLA-Aw68, showed a similarity to that of HLA-A2(Garrett *et al.* 1989). Eleven amino acid substitutions at polymorphic sites in the antigen binding cleft, however, altered the detailed shape and electrostatic charge of the antigen binding site. The structural differences between HLA-A2 and HLA-Aw68 molecules indicate that polymophism creates and alters subsites(pockets) positioned to bind peptide side chains, thereby suggesting the structural basis for allelic differences in foreign peptide binding.

Class *II* molecules are presumed to have a structure similar to that of class I (Brown *et al.* 1988). The membrane-proximal domains of two chains, α2 and β2, have an immunoglobulin-like structure; while the membrane-distal domains of two chains, α1 and β1, form the binding site for antigenic peptides.

STRUCTURAL BASIS FOR THE HLA POLYMORPHISM

Primary structural studies using amino acid and nucleotide sequencing provide the understanding on the polymorphism pattern in the HLA molecule. Comparison of class I sequences currently available reveals that amino acid substitutions are primarily located in two extracellular domains, α1 and α2 (Bjorkman and Parham 1990). Projection of hypervariable residues on tertiary structural model further reveals that the side chains of all these positions either point into or up from the antigen binding site indicating that these polymorphic amino acids are involved in interactions with peptide or T-cell receptor, respectively (Biorbman *et al.* 1987). Comparison of class II sequences shows that amino acid variations occur only in one domain in the N-terminal α1 and β1 domains of α and β chains (Gregersen *et al.* 1989; Bugawan *et al.* 1988). All genes at HLA-DRB, DQB and DPB loci are known to be polymorphic. DPA locus is polymorphic, but genes at DRA and DQA are less or non-polymorphic.

ROLE OF HLA MOLECULE

Alloantigens

HLA molecules play a role as major histocompatibility antigens to induce alloimmune response. HLA antigenic differences between donor and recipient are responsible for the rejection of allogenic tissue graft and graft-versus-host disease(GVHD) following transplantation of bone marrow cells containing immunocompetent cells. The relevance of HLA matching is well documented in bone marrow transplantation (Beatty *et al.* 1985; Anasetti *et al.* 1989; Anasetti *et al.*1990). The best transplantation results are obtained when an HLA-genotypically identical twin or sibling donates. Transplantation from phenotypically identical donors is less favorable, and mismatched transplantation has the lowest success rate. The impact of matching for class I and class II molecules stet equally important. Available data indicate that class I and class II variant molecules with only a minor amino acid substitution can function as alloantigens (Strachan, 1984; Taketani *et al.* 1984; Rojo *et al.* 1987; Rojo *et al.* 1989). Alloreactive cytotoxic T lymphocytes generated *in vitro* by allogeneic stimulation can distinguish a single amino acid substitution. These findings imply that precise HLA typing will be required in the clinical testing to achieve a higher sensitivity than with conventional serology. Biochemical and DNA typing techniques will achieve these goals(see below).

Antigen presentation

T-cell receptor molecules recognize peptide antigens in conjunction with class I or class II molecules, and this phenomenon is called MHC restriction. Cytotoxic T lymphocytes recognize processed peptide antigens with class I molecules, and helper T cells recognize antigens with class II molecules. Sequence comparison and tertiary structural model of the HLA molecule suggest that the restricting elements on the HLA molecules are determined by polymorphic amino acids located in the antigen binding cleft (Garrett *et al.* 1989; Brown *et al.* 1988; Bjorkman and Parham 1990; Bjorkman *et al.* 1987). This implies that each HLA molecule has the distinct capability of binding certain peptide fragments to present to T cells, thereby serving as an immune response determinant.

Disease association

A number of diseases are known to be associated with HLA antigens (Tiwari and Terasaki 1985). The best known is the association of HLA-B27 with ankylosing spondylitis (Brewerton *et al.* 1973; Schlosstein *et al.* 1973). The role of B27 molecule in the pathogenesis, however, is still unclear. Two hypotheses can be proposed (Yu *et al.* 1989). 1. HLA-B27 antigen may have a direct role in the pathogenesis as a molecule presenting peptides derived from microbial organisms or their products; or the B27 antigen may be crossreactive with pathogenic microbes thus serving as a target for an aberrant immune response. There is epidemiological and experimental evidence that some enteric bacteria are related to HLA-B27 associated diseases, but the pathogenic microbial antigen has not been identified. 2. HLA-B27 may simply be a marker for another as yet unidentified susceptibility gene. Chromosome walking studies of the area adjacent to the B locus identified several new genes, but no candidate susceptibility gene has been characterized in this region (Spies, Blanck *et al.* 1989; Spies, Bres nahan *et al.* 1989). According to recent reports, HLA-B27 transgenic animals may be useful in resolving whether the B27 molecule itself is capable of conferring susceptibility (Nickeson *et al.* 1990; Hammer *et al.* 1990). Animal model experiments, however, may not be informative if disease susceptibility depends on other genetic and environmental factors, or specific immune responsivenes by the T cell repertoire that are present in humans but absent in animals.

Class II antigens are also known to be associated with diseases. Most remarkable associations are found in rheumatoid arthritis associated with DR4 (DRB1*0401); insulin-dependent diabetes mellitus with DR3, DR4(DRB1*0401 and DRB1*0402), and DQw8(DQw3); and narcolepsy with DR2. Underlying mechanisms for the class II-disease associations are not known. Since genes in the HLA region are highly linked (*linkage disequilibrium),* it is sometimes dificult to determine whether observed associations between HLA antigens and diseases are due to the involvement of a particular HLA allele or the other closely linked genes.

DIVERSIFICATION OF HLA POLYMORPHISM

Sequence polymorphism among HLA alleles sho-

ws patchwork patterns (Bjorkman and Parham 1990). There are multiple variable regions with two or more polymorphic sequences at each region, and these variable sequences are shared by multiple alleles in different combinations. These patterns might have been derived from a combination of point mutations, homologous recombination, and gene conversion. It is worthwhile to note that the distribution of HLA alleles and their subtypes are distinct among different ethnic groups. It is not clear what driving force maintained the extremely high polymorphism in the HLA system. One may presume that the diversification in HLA polymorphism was driven by the selective advantage of being able to present a wide array of antigenic peptides. The ethnic difference in HLA allele distribution has been used as a tool in anthropological study to infer the evolution and migration of major ethnic groups.

HLA TYPING-CONVENTIONAL

HLA typing was initially introduced to select histocompatible tissue donors. Conventionally serological and cellular typings were used to define HLA antigens.

Serological typing

HLA-A, B, C, DR, and DQ antigens are defined by human alloantisera mainly obtained from multiparous women. Currently 20 A, 40 B, 10 C, 18 DR, and 9 DQ antigen specificities are officially recognized by the World Health Organization HLA Nomenclature Committee (Bodmer *et al.* 1989). Each individual inherits two sets of HLA genes, termed HLA haplotypes, from their parents. Thus each person has two antigens at each locus.

Cellular typing

HLA-D region compatibility can be determined by mixed lymphocyte culture(MLC) or mixed lymphocyte response(MLR). This testing measures the lymphocytic proliferation as a result of *in vitro* stimulation. MLC can be tested in "both ways" to determine the stimulation and responding capabilities of recipient and donor cells. Homozygous typing cells (HTC) are known to be homozygous for the HLA-D region, and these cells are used as stimulator in MLC reaction to determine the cellular-defined Dw specificities. DP antigens are not detected by serology or primary MLR, but can be defined in the secondary MLR, called primed lymphocyte test (PLT). Cell-mediated lympholysis (CML) is a test in which the allospecificity of cytotoxic T lymphocyte (CTL) against class I molecules is determined.

HLA TYPING-BIOCHEMICAL AND MOLECULAR

Recent studies on the HLA system using biochemical and molecular techniques have revealed that the degree of HLA polymorphism is far more extensive than previously defined by serological and cellular typing.

Biochemical study

One-dimensional isoelectric focusing (1-D IEF) of class I antigens and 2-D (combination of IEF and SDS-PAGE) gel electrophoresis of class II antigens show that structural variants exist among serologically well defined antigen specificities. A collaborative IEF analysis of HLA class I antigens from more than 900 individuals (Yang 1989). revealed that the number of IEF-defined HLA-A and B variants exceeded by more than 50% the number of serologically defined private alloantigens as summarized in Table 2. For example, HLA-B27 is a class I antigen specificity well defined by alloantisera, but consists of at least six IEF variants (Choo *et al.* 1986). Amino acid and nucleotide sequencing of the six IEF variants of HLA-B27 antigen showed that these differ by one to six amino acid substitutions (Choo *et al.* 1988). The extent of amino acid variation among the members of the HLA-B27 antigen family is limited compared to the number of amino acid differences found between serologically distinct alleles such as B27 and B7 (No=20). In general, variants among the well defined serological specificities such as A2, A3 B7, and B44 have a limited number of one to five amino acid substitutions. Available data, although limited, suggest that minor structural variants of class *I* antigens are functionally relevant, i.e., these behave as distinct alleles (Strachan *et al.* 1984; Taketani 1984; Rojo et al. 1987; Rojo *et al.* 1989).

Table 2. The number of HLA-A and B antigen specificities and their subtypes defined by IEF

Locus	Antigen specificities	IEF subtypes
HLA-A	20	36
HLA-B	40	56
Total Number	60	92

Molecular study

Molecular cloning and sequencing of genomic or cDNA clones directly demonstrated the nature of polymorphism at the primary structural level. Sequencing of HLA alleles will be the ultimately precise typing method, however, currently simple sequencing procedures are not available for clinical typing purposes. Restriction fragment length polymorphism (RFLP) is detected by Southern blot hybridization of genomic DNA digested with restriction endonucleases to radiolabeled genomic or cDNA probes. RFLP typing using DRB, DQA, and DQB probes can distinguish all the known serological specificities of DR and DQ by their unique patterns. The RFLP technique is very useful in typing class II alleles of leukemic cells since serological DR typing is frequently problematic with abnormal cells. Recent development of polymerase chain reaction (PCR) (Saiki *et al.* 1985). allows molecular typing of HLA alleles by amplifying specific genes and hybridizing with sequence-specific oligonucleotide probe (SSOP). Based upon the known sequences of HLA alleles, a panel of SSOP is designed to detect the polymorphic sequences. Allele assignment is achieved by interpreting the hybridization pattern of amplified genomic DNA with a panel of SSOP. PCR/SSOP has been used for DR and DP typing and shown that this technique can define sequence polymorphisms among antigen specificities that are otherwise not distinguished by serological and cellular technique (Bugawan *et al.* 1988; Petersdorf *et al.* 1990). PCR amplification also facilitates the sequencing studies. Since locus or allele specific amplification oligo-nucleotide primers are used, conventional molecular cloning becomes unnecessary (Petersdorf *et al.* 1990).

NEW DEFINITION OF HLA POLYMORPHISM

Conventionally HLA specificities have been defined by serological and cellular typing and their official designation given by the WHO Nomenclature Committee. Since molecular studies identify new genes in the HLA region, official designations are now extended to these new allelic sequences. For example, eight alleles are currently designated in the serologically defined DR4 antigen (Table 3). All these alleles are serologically typed as DR4. DR4 haplotypes can be divided into six subtypes according to Dw specificities (Dw4, Dw10, Dw13, Dw14, Dw15, and Dw "KT2") defined by HTC typing. There are at least two alleles belonging to Dw13 and Dw14 specificities respectively, indicating that only the sequence determination will reveal all the existing alleles. Similarly, there are six alleles in the HLA-B27 antigen family (Table 4). These six alleles are not distinguished by available alloantisera, but have originally been identified by distinct IEF patterns (Choo *et al.* 1986) and their unique sequences were determined (Choo *et al.* 1988).

Table 3. New definition of HLA-DR4 alleles according to the sequeness[1]

HLA-DR4 alleles	HLA-DR specificity	HLA-Dw specificity
DRB1*0401	DR4	Dw4
DRB1*0402	DR4	Dw10
DRB1*0403	DR4	Dw13
DRB1*0404	DR4	Dw14
DRB1*0405	DR4	Dw15
DRB1*0406	DR4	Dw"KT2"
DRB1*0407	DR4	Dw13
DRB1*0408	DR4	Dw14

[1]Adapted from Bodmer *et al.* 1990

Table 4. New definition of HLA-B27 alleles according to the sequences[1]

HLA-B27 allele	HLA-B specificity	IEF subtype
B*2701	B27	B27.1
B*2702	B27	B27.2
B*2703	B27	B27.3
B*2704	B27	B27.4
B*2705	B27	B27.5
B*2706	B27	B27.6

[1]Adapted from Bodmer *et al.* 1990

SUMMARY

The study of the HLA system was primarily initiated to understand the basis for the histocompatibility between recipients and tissue donors. HLA typing methods are being continuously improved and biochemical and molecular typing, in particular, are expected to provide precise typing of the HLA system. Conventional HLA typing methods can define antigen specificities, while biochemical and molecular methods will provide direct allele typing that is based on the actual sequence polymor-

phism. The precise tissue typing will definitely improve the outcome of transplantation. Structural studies have revealed the highly polymorphic nature of the HLA system and given insight to understanding the molecular basis of the HLA polymorohism. One big immunological puzzle remaining to be answered is how T-cell receptor molecules recognize peptide antigen in conjunction with the HLA molecule. The crystallization of the T-cell receptor molecule, an experiment currently underway, will eventually reveal the structural basis of the trimolecular interaction.

REFERENCES

Anasetti C, Amos D, Beatty PG, Appelbaum FR, Bensinger W, Buckner CD, Clift R, Doney K, Martin PJ, Mickelson E, Nisperos B, Ouigley J, Ramberg R, Sanders JE, Stewart P, Storb R, Sullivan KM, Witherspoon RP, Thomas ED, Hansen JA: Effect of HLA compatibility on engraftment of bone marrow transplants in patients with leukemia and lymphoma. *N Engl J Med 320: 197-204, 1989*

Anasetti C, Beatty PG, Storb R, Martin PJ, Mori M, Sanders JE, Thomas ED, Hansen JA: Effect of HLA incompatibility on graft-versus-host disease, relapse, and survival after marrow transplantation for patients with leukemia or lymphoma. *Hum Immunol 29: 79-91, 1990*

Beatty PG, Clift RA, Mickelson EM, Nisperos B, Flournoy N, Martin PJ, Sanders JE, Stewart P, Buckner CD, Storb R, Thomas ED, Hansen JA: Marrow transplantation from related donors other than HLA-identical siblings. *N Engl J Med 313: 765-771, 1985*

Bjorkman PJ, Parham P: Structure, function and diversity of class I major histocompatibility molecules. *Annu Rev Biochem 59: 253-288, 1990*

Bjorkman PJ, Saper MA, Samraoui B, Bennett WS, Strominger JL, Wiley DC: Structure of the human class I histocompatibility antigen, HLA-A2. *Nature 329: 506-512, 1987*

Bjorkman PJ, Saper MA, Samraoui B, Bennett WS, Strominger JL, Wiley DC: The foreign antigen binding site and T cell recognition regions of class I histocompatibility antigens. *Nature 329: 512-518, 1987*

Bodmer WF, Albert E, Bodmer JG, Dupont B, Mach B, Mayr W, Sasazuki: T, Schreuder GMTH, Svejgaard A, Terasaki PI: Nomenclature for factors for the HLA system, 1987. *Hum Immunol 26: 3-14, 1989*

Bodmer JG, Marsh SGE, Parham P, Erlich HA, Albert E, Bodmer WF, Dupont B, Mach B, Mayr WR, Sasazuki T, Shreuder GMTh, Strominger JL, Svejgaard A, Terasaki PI: Nomenclature for factors of the HLA system, 1989. *Tissue Antigens 35: 1-8, 1990*

Brewerton DA, Hart FD, Caffrey M, Nicholls A, James DCO, Sturrock RD: Ankylosing spondylitis and HL-A27. *Lancet 1: 904-907, 1973*

Brown JH, Jardetzky R, Saper MA, Samraoui B, Bjorkman PJ, Wiley DC: A hypothetical model of the foreign antigen binding site of class II histocompatibility molecules. *Nature 332: 845-850, 1988*

Bugawan TL, Horn GT, Long CM, Mickelson E, Hansen JA, Ferrara GB, Angelini G, Erlich HA: Analysis of HLA-DP allelic sequence polymorphism using the in vitro enzymatic DNA amplification of DP-α and DP-β loci. *J Immunol 141: 4024-4030, 1988*

Carroll MC, Katzman P, Alicot EM, Koller BH, Geraghty DE, Orr HT, Strominger JL, Spies T: Linkage map of the human major histocompatibility complex including the tumor necrosis factor genes. Proc Natl Acad Sci USA 84: 8535-8539, 1987

Choo SY, Antonelli P, Nisperos B, Nepom GT, Hansen JA: Six variants of HLA-B27 identified by isoelectric focusing. *Immunogenetics 23: 24-29, 1986*

Choo SY, St. John T, Orr HT, Hansen JA: Molecular analysis of the variant alloantigen HLA-B27d (HLA-B* 2703) identifies a unique single amino acid substitution. *Hum Immunol 21: 209-219, 1988*

Garrett TPJ, Saper MA, Bjorkman PJ, Strominger JL, Wiley DC: Specificity pockets for the side chains of peptide antigens in HLA-Aw68. *Nature 342: 692-696, 1989*

Geraghty D, Koller B, Orr H: A human major histocompatibility complex class I gene that encodes a protein with a shortened cytoplasmic segment. *Proc Nat Acad Sci USA 84: 9145-9149, 1987*

Geraghty D, Orr H, Koller B: HLA-F: An expressed HLA gene composed of a class I coding sequence linked to a novel transcribed repetitive element. *J Exp Med 171: 1-19, 1990*

Gregersen PK, Todd JA, Erlich HA, Long E, Serveniu S, Choi E, Kato HT, Lee JS: *First domain sequence diversity of DR and DQ subregion alleles.* In: Dupont B, (ed), *Immunobiology of HLA. Vol. 1,* New York, Springer-Verlag, *1989, 1027-1031*

Hammer RE, Maika SD, Richardson JA, Tang J-P, Taurog JD: Spontaneous inflammatory disease in transgenic rats expressing HLA-B27 and human β_2m: An animal model of HLA-B27-associated human disorders. *Cell 63: 1099-1112, 1990*

Koller B, Geraghty D, Shimizu Y, DeMars R, Orr H: A novel HLA class I gene expressed in resting T lymphocytes. *J Immunol 141: 897-904, 1988*

Nickeson CL, Luthra HL, Savarirayan S, David C: Susceptibility of HLA-B27 transgenic mice to Yersinia enterocolitica infection. *Hum Immunol 28: 382-396, 1990*

Petersdorf EW, Griffith RL, Erlich HA, Mickelson EM, Smith AG, Nisperos BB, Martin PJ, Hansen JA:

Unique sequences for two HLA-DRB1 genes expressed on distinct DRw6 haplotypes. *Immuno-genetics 32: 96-103, 1990*

Rojo S, Aparicio P, Choo SY, Hansen JA, Lopez de Castro J: Structural analysis of an HLA-B27 population variant, B27f. *J Immunol 139: 831-836, 1987*

Rojo S, Aparicio P, Hansen JA, Choo SY, Lopez de Castro J: Structural analysis of an HLA-B27 functional variant, B27d, detected in Amrican Blacks. *J Immunol 139: 3396-3401, 1989*

Saiki R, Scharf S, Faloona F, Mullis K, Horn G, Erlich HA, Arnheim N: Enzymatic amplification of β-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. *Sci 230: 1350-1354, 1985.*

Schlosstein L, Terasaki PI, Bluestone R, Pearson CM: High association of an HL-A antigen, W27, with ankylosing spondylitis. *N Engl J Med 288: 704-706, 1973*

Spies T, Blanck G, Bresnahan M, Sands J, Strominger JL: A new cluster of genes within the human major histocompatibility complex. *Sci 243: 214-217, 1989*

Spies T, Bresnahan M, Strominger JL: Human major histocompatibility complex contains a minimum of 19 genes between the complement cluster and HLA-B. *Proc Natl Acad Sci USA 86: 8955-8958, 1989*

Strachan T, Sodoyer R, Damotte M, Jordan BR: Complete nucleotide sequence of a functional class I HLA-gene, HLA-A3: Implications for the evolution of HLA genes. *EMBO J 3: 887-894, 1984*

Taketani S, Krangel MS, Spits H, deVries J, Strominger JL: Structural analysis of an HLA-B7 antigen variant detected by cyotoxic T lymphocytes. *J Immunol 133: 816-821, 1984*

Tiwari JL, Terasaki PI: *HLA and disease associations.* New York, Springer Verlag, *1985*

Yang SY: *Nomenclature for HLA-A and HLA-B alleles detected by ID-IEF gel electrophoresis. In:* Dupont B, (ed), *Immunobiology of HLA. Vol. 1,* New York, NY, Springer-Verlag. *1989, 54-57*

Yu DTY, Choo SY, Schaack T: Molecular mimicry in HLA-B27-related arthritis. *Ann Intern Med 111: 581-591, 1989*