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The Human Leukocyte Antigen (HLA) System

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ABSTRACT The discovery of Major Histocompatibility Complex (MHC) and its involvement in graft rejection, immune response and the genetic basis of disease associations lead to the birth of this new field of science called Immunogenetics. This field is important not only in basic biomedical research but also in clinical medicine. The growth of this field was further substantiated by the various International Histocompatibility Workshops. Solutions to idiopathic syndromes and autoimmune diseases come from the field of Immunology, Immunogenetics and Molecular biology. In studying the genetic basis of disease susceptibility in human beings, one has to have a different approach because predictive experimental mating cannot be achieved in humans as in animal models; moreover the generation time is longer. One approach is through a study of randomly selected patients and comparing their results with those of controls and the other is through a study of affected families (nuclear or extended) and looking for the mode of inheritance of the disease with relation to genetic markers. Nonetheless the sampling stratification, sample heterogeneity, disease heterogeneity, and age at onset, epidemiological conditions and other socio-biological factors limit these studies. Essentially attempts are to be made to control these parameters and achieve a meaningful conclusion. The current concepts of antigen presentation to immunocompetent cells indicate that antigen processing takes place in the acid environment of the endosomes of antigen presenting cells. Proteolytic degradation of the antigenic proteins results in peptides of different fragments, which are subsequently presented by MHC molecules after they are bound to peptide binding site of the MHC molecules which can bind a variety of peptides having in common, a peptide backbone. These immunogenic peptides (held snugly in the groove by hydrogen bonds between the MHC protein and the peptide backbone) on antigen presenting cells, with the MHC molecule and T-cell receptor on T-cells form a stable trimolecular complex. Thus MHC molecules and alpha beta T-cell receptors or gamma delta T-cell receptor play a major role in the subsequent immune response. The polymorphism of the MHC molecules and T-cell receptor may play an important role in the antigen recognition process. It is possible that a few of the many alleles of a given HLA locus may provide a more stable trimolecular complex than others, thus resulting in a 'high responder status' of an individual than the rest of the alleles. This field of HLA system has been evolving very fast and therefore an updated HLA system review has been provided.

INTRODUCTION

The term HLA refers to the Human Leucocyte Antigen System, which is controlled by genes on the short arm of chromosome six. The HLA loci are part of the genetic region known as the Major Histocompatibility Complex (MHC) (Hugh et al. 1984). The MHC has genes (including HLA) which are integral to normal function of the immune response. The essential role of the HLA antigens lies in the control of self-recognition and thus defense against microorganisms. The HLA loci, by virtue of their extreme polymorphism ensure that few individuals are identical and thus the population at large is well equipped to deal with attack (McDevitt 1985). Because some HLA antigens are recognized on all of the tissues of the body (rather than just blood cells), the identification of HLA antigens is described as "Tissue Typing" or "HLA Typing".

HISTORY

The early development of HLA typing sprang from attempts by red cell serologists to define antigens on leucocytes using their established agglutination methods. These methods, however, were plagued with technical problems and a lack of appreciation for the extreme polymorphism of the system. Although Jean Dausset reported the first HLA antigen, MAC (HLA-A2,A28) in 1958, the poor reproducibility of leuco-agglutination was hindering progress. It was five years later that the first glimpse of the polymorphic nature of the HLA system appeared (Terasaki 1990). The definition of the 4a/4b series by Jan van Rood in 1963 and the definition of LA1, LA2 and LA3 (HLA-A1, HLA-A2, HLA-A3) by Rose Payne and Walter Bodmer in 1964 indicated a need for International Standardization and thus was born a series of International Workshops, starting in 1964 (Glen 1991).

A summary of the events occurring at these workshops provides a chronicle of the milestones of achievement in HLA research (Roitt et al. 1998):

1964 * Acceptance of Cytotoxicity over agglutination

1965 * Allelism of HLA antigens proposed 1967 * Segregation of Alleles demonstrated in families

1970 *"single" locus now two - HLA-A, HLA-B

1972 * 60 world populations typed by 75 laboratories

1975 * Third locus, HLA-C, demonstrated

1977 * HLA-D defined by Homozygous Typing Cells

1977 * The serum-detected, D-related, HLA-DR defined

1984 * HLA and Disease associations explored

1984 * Studies of gene structure

1984 * Worldwide Renal Transplantation databases

1984 * Definition of MB (later to be HLA-DQ)

1987 * DNA techniques with serological, biochemical and cellular methods

1987 * Definition of HLA-DP and HLA-DQ

1992 * Use of Polymerase Chain Reaction - e.g. for SSOP

1996 * Molecular definition of HLA-Class I

1996 * Roles of HLA-G, E, DM, Tap & LMP's better understood

2002 * Molecular characterization of HLA alleles and Non HLA genes

2003 * Nomenclature of KIR genes better defined

THE HLA ANTIGENS

Based on the structure of the antigens produced and their function, there are two classes of HLA antigens, termed accordingly, HLA Class I and Class II.. The overall size of the MHC is approximately 3.5 million base pairs (Fig.1). Within this the HLA Class I genes and the HLA Class II genes each spread over approximately one third of this length. The remaining section, sometimes known as Class III, contains loci responsible for complement, hormones, intracellular peptide processing and other developmental characteris-

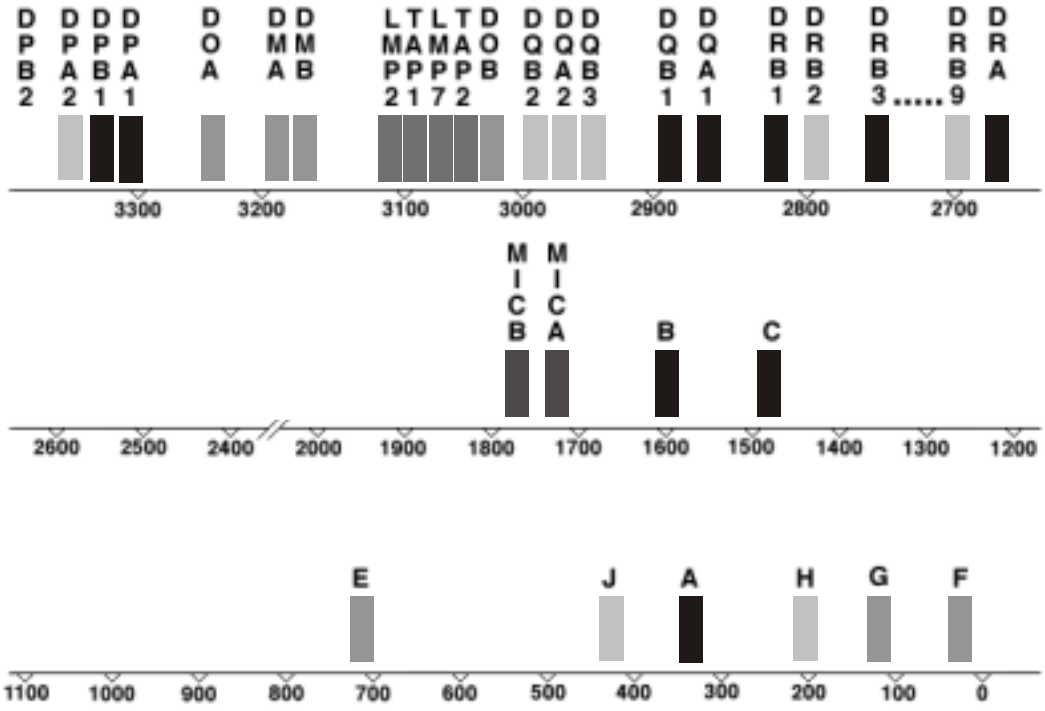

Fig. 1. The HLA gene complex

tics (Sanfilippo and Amos1986). Thus the Class III region is not actually a part of the HLA complex, but is located within the HLA region, because its components are either related to the functions of HLA antigens or are under similar control mechanisms to the HLA genes.

HLA Class I Antigens

The cell surface glycopeptide antigens of the HLA-A, -B and -C series are called HLA Class I antigens (Roitt et al. 1998). A listing of the currently recognized HLA Class I antigens are expressed on the surface of most nucleated cells of the body. Additionally, they are found in soluble form in plasma and are adsorbed onto the surface of platelets. Erythrocytes also adsorb HLA Class I antigens to varying degrees depending on the specificity (e.g. HLA-B7, A28 and B57 are recognizable on erythrocytes as so called "Bg" antigens).Immunological studies indicate that HLA-B (which is also the most polymorphic) is the most significant HLA Class I locus, followed by HLA-A and then HLA-C. There are other HLA Class I loci (e.g. HLA-E,F,G,H,J,K and L), but most of these may not be important as loci for "peptide presenters".

The HLA Class I antigens comprise a 45 Kilodalton (Kd) glycopeptide heavy chain with three domains, which is non-covalently associated with b - 2 microglobulin, which plays an important role in the structural support of the heavy chain. The HLA Class I molecule is assembled inside the cell and ultimately sits on the cell surface with a section inserted into the lipid bilayer of the cell membrane and has a short cytoplasmic tail (Fig. 2).

Fig. 2. Biochemical structure of the HLA Class I and Class II molecule.

The general structure of HLA Class I, HLA Class II and IgM molecules show such similarity of subunits, that a common link between HLA and immunoglobulins, back to some primordial cell surface receptor is likely. The full 3-dimensional structure of HLA-A Class I molecules has been determined from X-ray crystallography (Browning and Mc Michael 1996). This has demonstrated that the molecule has a cleft on its outermost surface, which holds a peptide (Fig. 3). In fact, if a cell becomes

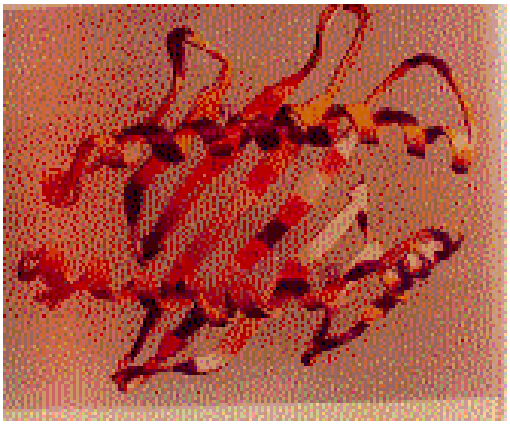

Fig. 3. Top view of the HLA molecule depicted by X- ray crystallography.

infected with a virus, the virally induced proteins within the cell are broken down into small peptides and these are the peptides which are then inserted into this cleft during the synthesis of HLA Class I molecules. The role of HLA Class I molecules is to take these virally induced peptides to the surface of the cell and by linking to the T-Cell receptor of a Cytotoxic (CD8) T Cell, demonstrate the presence of this virus. The CD8 T Cell will now be "educated" and it will be able to initiate the process of killing cells which subsequently has that same viral protein/HLA Class I molecule on its surface. This role of HLA Class I, in identifying cells, which are changed (e.g. virally infected), is the reason why they need to be present on all cells (Browning and Mc Michael 1996).

HLA Class II Antigens

The cell surface glycopeptide antigens of the HLA-DP, -DQ and -DR loci are termed HLA Class II (Sanfilippo and Amos 1986). The tissue distribution of HLA Class II antigens is confined

to the "immune competent" cells, including B-lymphocytes, macrophages, and endothelial cells and activated T-lymphocytes. The expression of HLA Class II, on cells, which would not normally express them, is stimulated by cytokines like interferon g and in a transplant, this is associated with acute graft destruction. HLA Class II molecules consist of two chains each encoded by genes in the "HLA Complex" on Chromosome 6 (Fig. 2). The T Cells, which link up to the HLA Class II molecules, are Helper (CD4) T cells. Thus the "education" process which occurs from HLA Class II presentation, involves the helper-function of setting up a general immune reaction which will involve cytokines, cellular and humoral defense against the bacterial (or other) invasion. This role of HLA Class II, in initiating a general immune response, is the reason why they need only be present on "immunologically active" cells (B lymphocytes, macrophages, etc.) and not on all tissues (Browning and Mc Michael 1996).

The HLA Nomenclature

There are a number of ways that you may see an HLA antigen is written. For example, you may see HLA-DR3, HLA-DR17, HLA-DRB1*03 or HLA-DRB1*0301. These could all refer to the same antigen! What do they mean? Firstly, as you know, "HLA" is the name for the gene cluster which tends to be inherited en-bloc on human chromosome number 6. These HLA antigens are responsible for the presentation of "foreign" peptides (antigens) to the immune competent cells of the immune system. H.L.A. stands for "Human Leucocyte Antigen" - a name that has been kept more as a tribute to history than actual function.

The second part – e.g. DR - is the name of the specific locus. There are 6 loci (Fig. 1) to which people normally refer. These are A, B, C, DR, DQ and DP. The HLA-A, B and C loci produce molecules (antigens) that normally present peptides of viral origin and are expressed on all nucleated cells. The HLA-A, B, C antigens are termed Class I. The HLA-DR, DQ and DP loci produce antigens that normally present peptides which have been broken down from bacterial or other proteins that have been engulfed by the cell in a process of immune surveillance. They are only expressed on cells actively involved in the immune response, e.g. B lymphocytes monocytes and activated T lymphocytes.

The HLA-DR, DQ and DP antigens are termed Class II. There are other Class I loci besides A, B and C and there are other Class II loci besides DR, DQ and DP. However, these loci are not normally tested for and their significance is not entirely clear. The third part - the number, e.g. 3, 17, 03, 0301, refers to the actual antigen at the locus. For example, the DNA in the gene region that we call the HLA-DR locus tends to be different from person to person. This difference will result in a different type of HLA-DR molecule. These different types of HLA-DR molecules are given names, such as DR17. Actually, HLA-DR17 is the old way of writing this antigen - based on using antibodies that react to the antigens on the cells. Now we can look directly at the DNA and therefore the accuracy is of much greater clarification. The problem is that now we can see a lot more variation between the different antigens and so we need a different way of writing them! So, when we look at the antigens above: HLA-DR3 is the broadest description of the antigen. It is the name for a specific group of antigens. The DR3 group can be divided into HLA DR17 and HLA-DR18 by using antibodies (serology). When we look at this antigen at the DNA level we call the DR locus DRB1 (because there are others termed A and B2,B3,etc) and the antigen 03 (for the general antigen) and 01 for the specific variant of the 03. So, HLA-DR17 is now called HLA-DRB1*0301.This is similar for other antigens in the system, at either HLA Class II or Class I. e.g. HLA-B60 (HLA-B*4001 molecularly).

How Many HLA Loci are There?

The currently recognized loci are given below (Marsh et al. 2002). Notice that HLA-DRB1 is the normal DR locus and the old DRw52 and DRw53 are DRB3 and DRB4, respectively:

The Common HLA Antigens and Their Molecular Diversity

The number of alleles that can now be recognized by molecular techniques is huge and is being increased rapidly. The tables 1 and 2 will help to demonstrate the antigens with the greatest diversity and to show the most frequent molecular variants.

Table1: The currently recognized HLA loci genes and those that are being routinely typed are presented.

HLA Class	*The HLA-loci (genes)*	*Routinely typed?*
Class I	HLA-A	YES
Class I	HLA-B	YES
Class I	HLA-C	YES
Class I	HLA-E	-
Class I	HLA-F	-
Class I	HLA-G	-
Class I	HLA-H	-
Class I	HLA-J	-
Class I	HLA-K	-
Class I	HLA-L	-
Class II	HLA-DRA	-
Class II	HLA-DRB1	YES
Class II	HLA-DRB2	-
Class II	HLA-DRB3	YES
Class II	HLA-DRB4	YES
Class II	HLA-DRB5	YES
Class II	HLA-DRB6	-
Class II	HLA-DRB7	-
Class II	HLA-DRB8	-
Class II	HLA-DRB9	-
Class II	HLA-DQA1	-
Class II	HLA-DQB1	YES
Class II	HLA-DQA2	-
Class II	HLA-DQB2	-
Class II	HLA-DQB3	-
Class II	HLA-DOB	-
Class II	HLA-DMA	-
Class II	HLA-DMB	-
Class II	HLA-DNA	-
Class II	HLA-DPA1	-
Class II	HLA-DPB1	not routine
Class II	HLA-DPA2	-
Class II	HLA-DPB2	-

GENETICS OF HLA

Routine Tissue Typing identifies the alleles at the three HLA Class I loci (HLA-A, -B, and -C) and the three class II loci (HLA-DR, -DP and -DQ). Thus, as each chromosome is found twice (diploid) in each individual, a normal tissue type of an individual will involve 12 HLA antigens (Sullivan and Amos 1986). These 12 antigens are inherited co-dominantly - that is to say, all 12 antigens are recognized by current typing methods and the presence of one does not affect our ability to type for the others. There are a number of genetic characteristics of HLA antigens, they are:

Polymorphism

The polymorphism at the recognized HLA loci is extreme. As the role of HLA molecules is to present peptides from invasive organisms, it is likely that this extreme polymorphism has

Table 2: The common HLA antigens and their molecular types expressed more frequently among the HLA-A, HLA-B, HLA-C, HLA-DR loci are presented.

HLA Antigens	*Broad Group*	*No. of molecular types**	*Most common alleles*
HLA A			
A1		9	A*0101
A2		58	A*0201, A*0202
A3		9	A*0301
A11		13	A*1101
A23	A9	9	A*2301
A24	A9	36	A*2402
A25	A10	4	A*2501
A26	A10	18	A*2601
A29	A19	6	A*2901, A*2902
A30	A19	12	
A31	A19	8	A*3101
A32	A19	7	A*3201
A33	A19	6	A*3301
A34	A10	4	A*3401
A36		3	A*3601
A43		1	A*4301
A66	A10	4	A*6601
A68	A28	22	A*6801
A69	A28	1	A*6901
A74		8	A*7401
A80		1	A*8001
HLA B			
B7		31	B*0702
B8		16	B*0801
B13		10	B*1301
B14		6	B*1401(64), B*1402(65)
B15		73	B*1501
B18		18	B*1801, B*1802
B27		24	B*2701, B*2702
B35		44	B*3501, B*3502
B37		5	B*3701
B38	B16	8	B*3801
B39	B16	26	B*3901
B40		44	B*4001
B41		6	B*4101
B42		4	B*4201
B44	B12	32	B*4402
B45	B12	6	B*4501
B46		2	B*4601
B47		4	B*4701
B48		7	B*4801
B49	B21	3	B*4901
B50	B21	3	B*5001
B51	B5	29	B*5101
B52	B5	4	B*5201
B53	B5	9	B*5301
B54	B22	2	B*5401
B55	B22	12	B*5501, B*5502
B56	B22	8	B*5601
B57	B17	9	B*5701
B58	B17	6	B*5801
B59		1	B*5901
B67		2	B*6701
B73		1	B*7301
B78		5	B*1517
B81		1	B*8101

Table 2: Contd...

HLA Antigens	*Broad Group*	*No. of molecular types**	*Most common alleles*
B82		2	B*8201
B83		1	B*8301
HLA C			
Cw1		6	Cw*0101
Cw2		5	Cw*0202
Cw3		15	Cw*0303
Cw4		10	Cw*0401
Cw5		5	Cw*0501
Cw6		7	Cw*0602
Cw7		16	Cw*0701, Cw*0702
Cw8		9	Cw*0802
Cw12		8	Cw*1203
Cw14		5	Cw*1401
Cw15		11	Cw*1502
Cw16		3	Cw*1601
Cw17		3	Cw*1701
Cw18		2	Cw*1801
HLA DR			
DR1		8	DRB1*0101, 0103
DR15	DR2	13	DRB1*1501, 1502
DR16	DR2	8	DRB1*1601, 1602
DR3		23	DRB1*0301
DR4		44	DRB1*0401, 0404
DR11	DR5	43	DRB1*1101
DR12	DR5	8	DRB1*1201
DR13	DR6	52	DRB1*1301, 1302
DR14	DR6	43	DRB1*1401, 1402
DR7		6	DRB1*0701
DR8		24	DRB1*0801, 0802, 0803
DR9		2	DRB1*0901
DR10		2	DRB1*1001

* The number of variants is approximate, as there will be more reported regularly

evolved as a mechanism for coping with all of the different peptides that will be encountered. That is to say, each HLA molecule differs slightly from each other in its amino acid sequence - this is what we see as different HLA antigens. This difference causes a slightly different 3-dimensional structure in the peptide binding cleft. Since different peptides have different shapes and charge characteristics, it is important that the human race has a large array of different HLA antigens, each with different shaped peptide binding areas (clefts) to cope with all of these peptides. However that is not all, as the polymorphism is population specific. The frequent HLA antigens in different populations are clearly different. For example, HLA-A34, which is present in 78% of Australian Aborigines, has a frequency of less than 1% in both Australian Caucasoid and Chinese. Several workers have reported HLA studies from various populations of World (Imanishi et al. 1992; Clayton and Lonjou 1997) and India (Shankarkumar et al. 1999, 2000, 2001, 2002, 2003; Mehra et al. 1984; Pitchappan et al. 1984). Thus HLA antigens are of great significance in anthropological studies. Populations with very similar HLA antigen frequencies are clearly derived from common stock. Conversely, from the point of view of transplantation, which will be discussed later, it is very difficult to match HLA types between populations.

Inheritance of HLA

The normal way to present a tissue type is to list the HLA antigens as they have been detected. There is no attempt to show which parent has passed on which antigen. This way of presenting the HLA type is referred to as a Phenotype (Thomas et al. 1998). HLA PHENOTYPE example: HLA - A1, A3; B7, B8; Cw2, Cw4; DR15, DR4, When family data is available, it is possible to assign one each of the antigens at each locus to a specific grouping known as a haplotype. An haplotype is the set of HLA antigens inherited from one parent (Fig. 4). For example, the mother of the person whose HLA type is given above may be typed as HLA-A3, A69; B7, B45; Cw4, Cw9; DR15, DR17; Now it is evident that the A3, B7, Cw4 and DR15 were all passed on from the mother to the child above. This group of antigens is a haplotype.

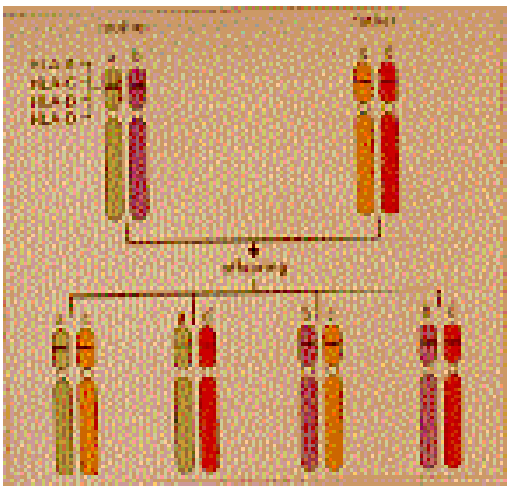

Fig. 4. The segregation of HLA antigens in a family.

In the absence of genetic crossing over, 2 siblings who inherit the same two HLA chromosomes (haplotypes) from their parents will be HLA identical. There is a one in four chance that this will occur and therefore in any family with more than four children at least two of them will be HLA identical. This is because there are only two possible haplotypes in each parent.

Linkage Disequilibrium

Basic Mendelian genetics states that the frequency of alleles at one locus does not influence the frequency of alleles at another locus (Law of independent segregation). However in HLA genetics this is not true. There are a number of examples from within the HLA system of alleles at different loci occurring together at very much higher frequencies than would be expected from their respective gene frequencies. This is termed linkage disequilibrium. The most extreme example is in Caucasians where the HLA-A1, B8, DR3 (DRB1*0301), DQ2 (DQB1*0201) haplotype is so conserved that even the alleles at the complement genes (Class III) can be predicted with great accuracy. Similar haplotypes are observed in selected caste groups and tribal groups of India (Shankarkumar et al. 1999). Also, at HLA Class II, this phenomenon is so pronounced, that the presence of specific HLA-DR alleles can be used to predict the HLA-DQ allele with a high degree of accuracy before testing. Because of linkage disequilibrium, a certain combination of HLA Class I antigen, HLA Class II antigen and Class III products will be inherited together more frequently than would normally be expected. It is possible that these "sets" of alleles may be advantageous in some immunological sense, so that they have a positive selective advantage.

Cross-Reactivity

Cross-reactivity is the phenomenon whereby one antibody reacts with several different antigens, usually at the one locus (as opposed to a mixture of antibodies in the one serum) (Shankarkumar et al. 1998). This is not a surprising event as it has been demonstrated that different HLA antigens share exactly the same amino acid sequence for most of their molecular structure. Antibodies bind to specific sites on these molecules and it would be expected that many different antigens would share a site (or epitope) for which a specific antibody will bind. Thus cross-reactivity is the sharing of epitopes between antigens.

The term **CREG** is often used to describe "**C**ross **Re**acting **G**roups" of antigens. It is useful to think in terms of CREG's when screening sera for antibodies, as most sera found are "multi-specific" and it is rare to find operationally monospecific sera. The rarity of monospecific sera means that most serological tissue typing is done using sera detecting more than one specificity and a typing is deduced by subtraction. For example, a cell may react with a serum containing antibodies to HLA-A25, A26, and A34 and be negative for pure A26 and pure A25 antisera. In this case, HLA-A34 can be assigned, even in the absence of pure HLA-A34 antisera

METHODS OF TESTING FOR HLA ANTIGENS

Lymphocytotoxicity (Serological Testing)

In the lymphocytotoxicity test (Terasaki and McClleland 1964), lymphocytes are added to sera, which may or may not have antibodies directed to HLA antigens. If the serum contains an antibody specific to an HLA (Class I or Class II) antigen on the lymphocytes, the antibody will bind to this HLA antigen. Complement is then added. The complement binds only to positive cells (i.e. where the antibody has bound) and in doing so, causes membrane damage. The damaged cells are not completely lysed but suffer sufficient membrane damage to allow uptake of vital stains such as eosin or fluorescent stains such as Ethidium Bromide. Microscopic identification of the stained cells, indicates the presence of a specific HLA antibody. The cells used for the test are lymphocytes because of their excellent expression of HLA antigens and ease of isolation compared to most other tissue. The most important use of this test is to detect specific donor-reactive antibodies present in a potential recipient prior to transplantation.

Historically, this test has long been used to type for HLA Class I and Class II antigens, using antisera of known specificity. However, the problems of cross-reactivity and non-availability of certain antibodies has led to the introduction of DNA based methods. Currently, many

laboratories have changed to molecular genetic methods for HLA Class typing.

Mixed Lymphocyte Culture (MLC)

When lymphocytes from two individuals are cultured together, each cell population is able to recognize the "foreign" HLA class II antigens of the other. As a response to these differences, the lymphocytes transform into blast cells, with associated DNA synthesis. Radio-labelled thymidine, added to the culture, will be used in this DNA synthesis. Therefore, radioactive uptake is a measure of DNA synthesis and the difference between the HLA Class II types of the two people. This technique can be refined by treating the lymphocytes from one of the individuals to prevent cell division, for example by irradiation. It is thus possible to measure the response of T lymphocytes from one individual to a range of foreign lymphocytes. It has thus proved possible by using the mixed lymphocyte culture (MLC) test to use T lymphocytes to define what were previously called HLA-D antigens. The "HLA-D" defined in this way is actually a combination of HLA-DR,DQ and -DP.

An important use of the MLC is in its use as a "cellular crossmatch" prior to transplantation - especially bone marrow. By testing the prospective donor and recipient, an *in-vitro* transplant model is established which is an extremely significant indicator of possible rejection or Graft-Versus-Host reaction.

Molecular Genetic Techniques

RFLP (Restriction Fragment Length Polymorphism)

Restriction Fragment Length Polymorphism (RFLP) methods (Dupont 1989) rely on the ability of certain enzymes to recognize exact DNA nucleotide sequences and to cut the DNA at each of these points. Thus the frequency of a particular sequence will determine the lengths of DNA produced by cutting with a particular enzyme.

The DNA for one HLA (Class II) antigen, e.g. DR15, will have these particular enzyme cutting sites (or "restriction sites") at different positions to another antigen, e.g. DR17. So the lengths of DNA seen when DR15 is cut by a particular enzyme, are characteristic of DR15 and different to the sizes of the fragments seen when DR17 is cut by the same enzyme (Bidwell 1988).

Polymerase Chain Reaction

The Polymerase Chain Reaction (PCR) (Michael et al. 1995) is a recently developed and revolutionary new system for investigating the DNA nucleotide sequence of a particular region of interest in any individual. Very small amounts of DNA can be used as a starting point, such that it is theoretically possible to tissue type using a single hair root. Sequencing DNA has been transformed from a long and laborious exercise to a technique that is essentially automatable in the not too distant future.

The first step in this technique is to obtain DNA from the nuclei of an individual. The double stranded DNA is then denatured by heat into single stranded DNA. Oligonucleotide primer sequences are then chosen to flank a region of interest. The oligo- nucleotide primer is a short segment of complementary DNA, which will associate with the single stranded DNA to act as a starting point for reconstruction of double stranded DNA at that site.

If the oligonucleotide is chosen to be close to a region of special interest like a hypervariable region of HLA-DRB then the part of the DNA, and only that part, will become double stranded DNA, when DNA polymerase and deoxy-ribonucleotide triphosphates are added. From one copy of DNA it is thus possible to make two. Those two copies can then, in turn, be denatured, reassociate with primers and produce four copies. This cycle can then be repeated until there is sufficient copies of the selected portion of DNA to isolate on a gel and then sequence or type.

There are a number of PCR based methods in use. For example:

Sequence Specific Priming (SSP)

In this test, the oligonucleotide primers used to start the PCR have sequences complimentary to known sequences which are characteristic to certain HLA specificities. The primers, which are specific to HLA-DR15, for example, will not be able to instigate the PCR for HLA-DR17. Typing is done by using a set of different PCR's, each with primers specific for different HLA antigens.

Sequence Specific Oligonucleotide (SSO) Typing

By this method, the DNA for a whole region

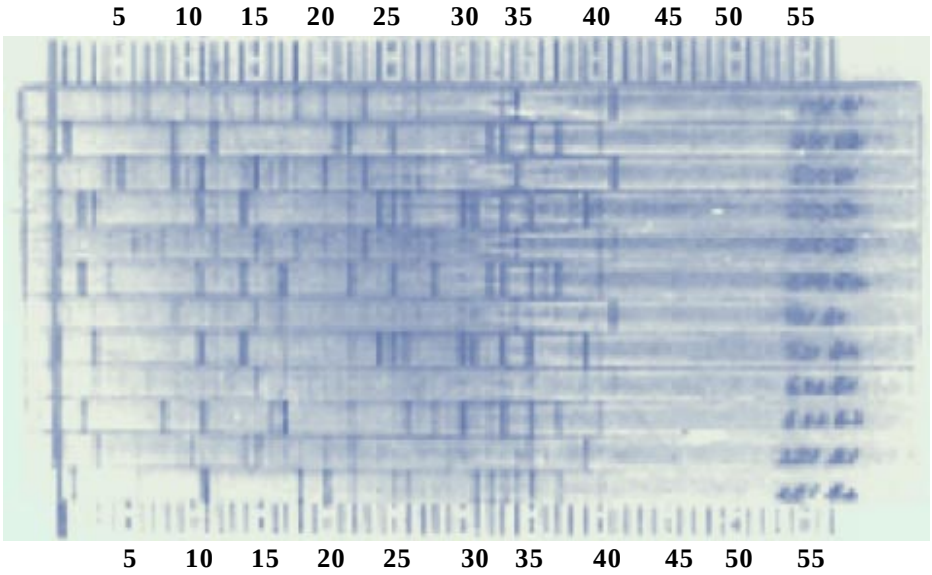

Fig. 5. The SSOP hybridized bands of the HLA gene

(e.g. the HLA DR gene region) is amplified in the PCR. The amplified DNA is then tested by adding labeled (e.g. Radioactive) oligonucleotide probes, which are complementary for DNA sequences, characteristic for certain HLA antigens. These probes will then "type" for the presence of specific DNA sequences of HLA genes (Fig. 5).

CLINICAL RELEVANCE OF THE HLA SYSTEM

Despite the temptation to think of them as "transplantation antigens", HLA antigens are not present on tissues simply to confound transplant surgeons. The most important function of MHC molecule is in the induction and regulation of immune responses. T-lymphocytes recognize foreign antigen in combination with HLA molecules.

In an immune response, foreign antigen is processed by and presented on the surface of a cell (e.g.. macrophage). The presentation is made by way of an HLA molecule. The HLA molecule has a section, called its antigen (or peptide) binding cleft, in which it has these antigens inserted. T-lymphocytes interact with the foreign antigen/HLA complex and are activated. Upon activation, the T cells multiply and by the release of cytokines, are able to set up an immune response that will recognize and destroy cells with this same foreign antigen/HLA complex, when next encountered. The exact mode of action of HLA Class I and HLA Class II antigens is different in this process. HLA Class I molecules, by virtue of their presence on all nucleated cells, present antigens that are peptides produced by invading viruses. These are specifically presented to cytotoxic T cells (CD8) which will then act directly to kill the virally infected cell. HLA Class II molecules, have an intracellular chaperone network which prevents endogenous peptide from being inserted into its antigen binding cleft. They instead bind antigens (peptides) which are derived from outside of the cell (and have been engulfed). Such peptides would be from a bacterial infection. The HLA Class II molecule presents this "exogenous" peptide to helper T cells (CD4) which then set up a generalized immune response to this bacterial invasion. Thus it is apparent that MHC products are an integral part of immunological health and therefore it is no surprise to see a wide variety of areas of clinical and genetic implications. The following is a general overview of some of the important functional aspects of HLA antigens.

HLA and Transfusion

The HLA Class I antigens are carried in high

concentrations by leucocytes and platelets, but only in trace amounts on erythrocytes. Each transfusion of either platelets or leucocytes therefore carries a risk of immunizing the patient. Patients, with an intact immune system, who require multiple transfusions of whole blood, platelets or leucocyte concentrates will therefore usually develop antibodies to HLA antigens (Rudmann 1995). This risk can be minimized by washing or filtering the red cell preparations and by reducing leucocyte contamination as far as practicable.

In multi-transfused patients, such as those with leukemia, anti-HLA antibodies may lead to two problems. Firstly, these patients become refractory to platelet transfusions, which they destroy rapidly, and secondly non-haemolytic transfusion reactions may occur in response to HLA antigens. Both these problems can be circumvented with some difficulty. Family donors, especially HLA identical siblings, provide one source of platelets, which may not be consumed. It is possible to use platelet or lymphocyte crossmatching techniques to confirm the suitability of an individual donor, but there is a limit to the frequency with which a single individual can provide platelets. An alternative source, is to HLA phenotype a bank of potential platelet volunteers for use in the appropriate patients. The disadvantage of this approach is that the polymorphism of each of the HLA class I allelic systems gives rise to low chances of finding HLA matched donors for patients with any tissue type other than an extremely common one. The volunteer banks thus have to be large to offer any chance of success.

HLA and Transplantation

Renal Transplants

HLA typing was applied to kidney transplantation very soon after the first HLA determinants were characterized (Terasaki 1992; Opelz 1985; Sanfilipo et al. 1984). The importance of reducing mismatched antigens in donor kidneys was immediately apparent with superior survival of grafts from HLA identical siblings compared to one haplotype matches or unrelated donors. It is apparent that the effect of HLA matching is significant, even with the highly efficient immunosuppression used today. In renal transplantation there are two major priorities that reduce the (already low) chance of obtaining good HLA matching. These are the need for ABO compatibility and the need for a negative T-lymphocyte crossmatch (using cytotoxicity). Anti-HLA Class I antibodies present at the time of transplant will cause "hyperacute rejection" of the graft (i.e. when the T cell crossmatch is positive).

Liver Transplantation

Patients awaiting liver transplantation can seldom afford to wait for a well matched graft. Therefore, liver transplantation is more involved with problems such as physical size rather than HLA. Also with the effects of Cyclosporin-A and the action of the liver itself as a form of "immunological sponge" (to mop up immune complexes) the effect of HLA matching is difficult to determine. The lymphocytotoxic T cell crossmatch is an important factor in liver transplantation. Transplants, which are, through urgency, carried out despite a positive T cell crossmatch, have a significantly lower success rate.

Heart Transplants

There has only recently been sufficient accumulated experience to show the effect of HLA antigens on cardiac allograft survival. It is now clear that HLA-DR antigens exert a powerful effect, analogous to that seen in renal transplantation. However, the problem of applying that knowledge to clinical practice is more analogous to liver transplantation. Cardiac size match and availability at the right time, are of more pressing importance than matching HLA antigens.

Corneal Transplantation

There is evidence that the cornea may survive slightly better if the HLA class I antigens is matched (Mayer et al. 1983). In the low risk, first corneal graft recipient, the efforts to HLA match and the hindrance to routines of corneal procurement and transplantation do not appear to warrant tissue typing. The situation is however somewhat different when the recipient's cornea has become vascularised from previous inflammation or they have rejected one or more previous grafts. In those patients, matching for HLA class I antigens does seem to be worthwhile.

Bone Marrow Transplantation (BMT) or Haematopoitic Stem Cell Transplantation (HSCT)

Complete HLA matching of bone marrow donor and recipient is crucial to the success of allogenic BMT (Shankarkumar and Undevia 1999; Ghosh 1999; Shankarkumar 2001). Incompatibility may not only lead to rejection but also to the greater problem of graft-versus-host-disease (GVHD) in which the immunologically compromised recipient is "attacked" by the grafted bone marrow. Most bone marrow transplants involve HLA-identical siblings with the HLA identity confirmed by family study and MLC or highly definitive molecular genetic techniques. Failing an HLA identical sibling being available, a close relative with very similar (e.g. one HLA antigen mismatch) may be considered. However since 60% to 70% of potential candidates do not have a suitable family member to act as a donor, there has been interest in developing lists of tissue typed volunteers prepared to donate bone marrow or peripheral blood stem cells. There are now a number of such Registries established, which by International collaboration now able to find an HLA-A, B, C, DR, and DQ matched donor, for a further 30% or 40% of candidates of Caucasian patients. The success rates of these transplants was initially dismal, but better conditioning of both the patient and bone marrow are now resulting in much better results.

HLA and Paternity Testing

The vast polymorphism of the HLA system makes it a most valuable tool in the field of paternity testing (Bryant 1988). There are two possible roles of paternity testing depending upon the situation. Probability of exclusion of paternity, and probability of paternity require different mathematical formulae. Excluding paternity may in some cases be straightforward, for example when a putative father does not have any of the HLA antigens that a child must have inherited from its father, this is first order exclusion. When the father is homozygous or the child appears to be homozygous it is possible that unidentified antigens explain the differences.

The probability of paternity on the other hand has to consider, even when the HLA antigens are fully compatible with paternity, that an alternate father may have possessed those same antigens. The probability of exclusion for the HLA test alone is 93% which means that out of every 100 falsely accused fathers 93 would be excluded from paternity. If the alleged father does express the requisite HLA tissue type, the match between the child and alleged father is a significant piece of evidence consistent with paternity (Rudmann 1995). However it is uncommon due to the diverse nature of the HLA system that the requisite HLA tissue type is found in the alleged father's population group. Here one could analyze the haplotype frequency data for HLA A, B and DR and would be very probative with a power of exclusion increasing to 99%. Most laboratories now combine red cell and HLA testing of the mother, child and putative father, together with further genetic analysis, such as "DNA fingerprinting" - where direct comparison of DNA fragments yields excellent results.

HLA and Disease Susceptibility

In the 1960's, it was discovered that the mouse MHC (called H-2) controlled both the genetic susceptibility to certain leukemia's and the immune response to certain antigens. Since then innumerable reports have been published aimed at discovering the role of the human MHC in the control of responsiveness and disease susceptibility (Tiwari and Terasaki 1985).

There are two general explanations for HLA and disease associations (McDevitt 1985).

Firstly, there may be a linkage disequilibrium between alleles at a particular disease associated locus and the HLA antigen associated with that disease - this is so for HLA-A3 and Idiopathic Haemochromatosis.

Another possible explanation for these associations is that the HLA antigen itself plays a role in disease, by a method similar to one of the following models:

a) By being a poor presenter of a certain viral or bacterial antigen
b) By providing a binding site on the surface of the cell for a disease provoking virus or bacterium
c) By providing a transport piece for the virus to allow it to enter the cell
d) By having such a close molecular similarity to the pathogen, that the immune system fails to recognize the pathogen as foreign and so

fails to mount an immune response against it.

It is most likely that all these mechanisms are involved, but to a varying extent in different diseases (Trosby 1997). In multiple sclerosis (Kankonkar et al. 2003) and ankylosing spondylitis (Shankarkumar et al. 2002), cell mediated immunity is often depressed, not only in the patients but also in their parents and siblings. Complement (C2) levels are known to be low in systemic lupus erythematosus, Pulmonary Tuberculosis, Leprosy, a disease associated with HLA DR2 and DR3 (Shankarkumar et al. 2003a,b; Rajalingham et al. 1996; Shanmugalashmi and Pitchappan 2002). In gluten enteropathy, which shows a high association with HLA-DR3, a specific gene product is thought to act as an abnormal receptor for gliadin, the wheat protein, and present it as an imunogen to the body. Whatever the explanation for the long list of HLA and disease associations, it is clear that the HLA system, collaborating with other non-linked genes has an influence on our response to environmental factors which provoke disease.

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