THE CLINICAL AND BIOLOGICAL SIGNIFICANCE OF THE HLA SYSTEM

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Introduction

When the first human leucocyte blood group antigen was discovered by Dausset in 1958 [1], nobody could anticipate that this was the beginning of an entirely new and exceedingly fruitful research field in human biology and medicine. Firstly, it became clear that this and a large number of other leucocyte antigens belong to the same genetic system (HLA) and that this system is the major human histocompatibility system which is the main barrier in organ transplantation. Later, and even more surprising, it was discovered that HLA is deeply involved in the development of a group of diseases so diverse as ankylosing spondylitis, multiple sclerosis, and juvenile diabetes among others. Finally, as the coping stone, it now appears that the same genetic system plays a fundamental role in the immune response towards a variety of foreign antigens.

The purpose of this short survey is to summarise the most pertinent knowledge of the HLA system, to discuss its clinical significance with main emphasis on the relationships to disease susceptibility, and finally to outline its biological significance as it seems to emerge now. No attempt to cover the original literature will be made, but recent review articles are given preference.

The HLA System

While most of the presently known genetic systems in higher animals each controls only one character, the HLA system controls at least three different properties: (transplantation) antigens, some complement components, and various immune responses [2,3,4].

The HLA antigens belong to two classes, HLA-ABC and HLA-D/DR which differ in terms of biochemistry (although they are both glycopeptides composed of two different polypeptide chains), cellular distribution and function. The HLA-A, B and C antigens are controlled by genes at three closely linked loci (HLA-A, B, and C) and are present on all nucleated cells and on blood
platelets as well. The HLA-D/DR antigens are mainly present on macrophages, B lymphocytes and a few other cell types. While the HLA-ABC antigens are readily detectable by serological methods, the HLA-D antigens are recognised by in vitro cell cultures (MLC = mixed leucocyte culture), and the HLA-DR antigens by special serological techniques, HLA-DR and DR antigens are controlled by alleles on at least one locus and may be present on the same molecules.

The complement components controlled by HLA involve both some from the classical activation pathway, C2 and C4 and properdin factor Bf, from the alternative pathway. Several of the other complement components seem not to be controlled by genes in the HLA region, but information is lacking on some.

Strictly speaking, Immune response (Ir) determinants have not yet formally been proven to be present within the HLA system, but as they have been found in the HLA analogues of all other vertebrates studied and as there is striking homology between all these systems, there is little doubt that HLA contains such determinants. In animals, Ir determinants control some specific immune responses (cell-mediated immunity and IgG antibody production) to various foreign antigens [5]. The Ir genes have been mapped close to those controlling the HLA-D and DR analogues in animals, and thus human Ir genes can be anticipated to be very close, if not identical with the HLA-D/DR genes.

The HLA gene complex is located on the short arm of chromosome No. 6 and the order of loci as revealed by the consequences of occasional crossing-overs is shown in Figure 1. The basis for considering all the above characters to belong to one and the same genetic system is the fact that HLA factors controlled by genes at different loci are non-randomly associated in the population. For example, the HLA-A1, B8 and Dw3 antigens occur together much more frequently in the same individual than would be expected from the frequencies of each of these antigens. This phenomenon reflects linkage disequilibrium between the HLA genes, i.e. certain HLA genes (e.g. A1, B8 and Dw3) tend to be present in the same HLA haplotype, a haplotype being genes found on one and the same of the two homologous chromosomes. This linkage disequilibrium must be born in mind in relation to the HLA and disease associations: e.g. a primary increase of Dw3 in a group of patients will automatically lead to secondary increases of B8 and A1.

![Figure 1](image-url)
Clinical Significance of the HLA System

Although its role as the major transplantation system in man was the first impetus to study the HLA system, it is now clear that this system also plays a considerable role in blood transfusion therapy and for the genetic susceptibility to a variety of diseases.

The major role of the HLA system in organ transplantation is exemplified by the high success rate of kidney allografting with HLA-identical sibling donors compared with other donors. When similar good results have not been obtained with unrelated donors, it may be attributed partly to the incomplete knowledge of the HLA system and partly to the difficulties in matching for all known HLA factors. Presently, it is hoped that the inclusion of HLA-D/DR matching in cadaver kidney transplantation will improve the results and preliminary observations from Oxford [6], Oslo [7] and Geneva [17] seem to justify this hope. Table I shows the combined data from these three studies. It appears that the 3 months graft survival is 100, 78, and 56 per cent for grafts sharing 2, 1 and 0 HLA-DR antigens, respectively. Nevertheless, these figures should be taken with reservation because the numbers of patients are small and the observation period is short.

<table>
<thead>
<tr>
<th>No. of Shared DR Antigens</th>
<th>No. of Grafts</th>
<th>Graft Survival at 3 Months</th>
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<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Per Cent</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>6 100 (100)</td>
</tr>
<tr>
<td>1</td>
<td>89</td>
<td>69 78 (63–88)</td>
</tr>
<tr>
<td>0</td>
<td>94</td>
<td>53 56 (45–68)</td>
</tr>
<tr>
<td>Total</td>
<td>189</td>
<td>128 68 (54–75)</td>
</tr>
</tbody>
</table>

Data from refs 6, 7 and 17 have been pooled. Figures in brackets are range of percentages in the three studies. The difference between the three groups is significant \( x^2 = 12.30 \) with 2 d.f., \( p < .005 \).

The most frequent - though rarely severe - complication seen in blood transfusion therapy is the febrile reaction caused by recipient HLA antibodies directed against donor HLA antigens. As this complication is largely prevented by the use of leucocyte-poor blood, matching for HLA is not indicated in ordinary blood transfusion therapy. However, when attempts are made to substitute platelets and/or granulocytes in thrombocytopenic and granulocytopenic patients, prior HLA immunisations of the recipient necessitate the use of HLA compatible blood products. In such cases, cell separators make it possible to obtain large numbers of cells from the few HLA compatible donors who are available.

One of the most unexpected and exciting developments in the exploration of the HLA system was the discovery that it is deeply involved in the development of a variety of diseases [8]. The primary importance of these relation-
ships is that the HLA system provides genetic markers which make it possible to study the inheritance of disease susceptibility and that they give new information about the aetiologies and/or pathogenesis of the diseases in question. This latter possibility is becoming more feasible with the increasing knowledge about the biological significance of the HLA system as discussed in the next section of this survey.

Some of the most important relationships between HLA and disease are listed in Table II which gives the frequencies in controls and patients of the HLA antigens showing the strongest associations with the diseases in question [9,10]. In addition we have given the relative risk which is a measure of the

<table>
<thead>
<tr>
<th>Disease</th>
<th>Associated HLA antigen</th>
<th>Frequency (%) of antigen</th>
<th>Relative risk</th>
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<tbody>
<tr>
<td>Ankylosing spondylitis</td>
<td>B27</td>
<td>8.6</td>
<td>89</td>
</tr>
<tr>
<td>Reiter's syndrome</td>
<td>B27</td>
<td>8.6</td>
<td>77</td>
</tr>
<tr>
<td>Acute anterior uveitis</td>
<td>B27</td>
<td>8.6</td>
<td>47</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>Dw4</td>
<td>19.4</td>
<td>48</td>
</tr>
<tr>
<td>Multiple sclerosis</td>
<td>Dw2</td>
<td>25.8</td>
<td>60</td>
</tr>
<tr>
<td>Myasthenia gravis</td>
<td>B8</td>
<td>23.7</td>
<td>56</td>
</tr>
<tr>
<td>Coeliac disease</td>
<td>Dw3</td>
<td>26.3</td>
<td>91</td>
</tr>
<tr>
<td>Dermatitis herpetiformis</td>
<td>Dw3</td>
<td>26.3</td>
<td>83</td>
</tr>
<tr>
<td>Chronic autimmune hepatitis</td>
<td>Dw3</td>
<td>26.3</td>
<td>71</td>
</tr>
<tr>
<td>Sicca syndrome</td>
<td>Dw3</td>
<td>26.3</td>
<td>87</td>
</tr>
<tr>
<td>Idiopathic Addison's disease</td>
<td>Dw3</td>
<td>26.3</td>
<td>76</td>
</tr>
<tr>
<td>Graves' disease</td>
<td>Dw3</td>
<td>26.3</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td>Dw2</td>
<td>25.8</td>
<td>0</td>
</tr>
<tr>
<td>Insulin-dependent diabetes</td>
<td>Dw3</td>
<td>26.3</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>Dw4</td>
<td>19.4</td>
<td>49</td>
</tr>
<tr>
<td>Subacute thyroiditis</td>
<td>Bw35</td>
<td>13.1</td>
<td>72</td>
</tr>
<tr>
<td>Psoriasis vulgaris</td>
<td>Cw6</td>
<td>33.1</td>
<td>88</td>
</tr>
<tr>
<td>Idiopathic haemochromatosis</td>
<td>A3</td>
<td>26.9</td>
<td>73</td>
</tr>
<tr>
<td>C2-deficiency</td>
<td>Strongly associated with Dw2, in particular the A25, B18, Dw2 haplotype</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Congenital adrenal hyperplasia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(21-hydroxylase deficiency)</td>
<td>Closey linked to HLA, but probably not associated with HLA factors</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Most of the above data are extracted from refs. 8 and 9, some are from the International HLA and Disease Registry in Copenhagen, and the linkage between HLA and congenital adrenal hyperplasia was reported by Dupont et al. [16]. The frequencies in controls refer to a Danish population. The relative risk is explained in the text.

The strength of the association and indicates how many times more frequently the disease develops in the individuals carrying the disease associated factor as compared with individuals lacking it. All of the relationships in Table II are highly significant even when taking into account that a considerable number...
of antigens was studied in each case.

Most of the diseases related to HLA appear to show the strongest association with HLA-D antigens, HLA-Dw3 being the antigen encountered most often in Table II, but there are some notable exceptions such as the associations between B27 and ankylosing spondylitis and between A3 and idiopathic haemochromatosis. One of the current arguments is whether the associations are caused directly by the antigens listed in Table II or whether they are due to as yet unknown HLA factors occurring in linkage disequilibrium with those known to be associated. As there are most probably more loci within the HLA system than we know today, the latter possibility is a real one and may be exemplified by the strong association between the recessive C2 deficiency and Dw2: this deficiency is not due to the Dw2 gene but to an abnormal C2 gene closely linked to the D locus. Apart from this, it is, in general, an unsettled question which HLA factors are responsible for the associations although there is a case for suspecting the B27 antigen itself to be a causative agent in ankylosing spondylitis and the related disorders.

The strong association between B27 and ankylosing spondylitis has given HLA typing a certain place as a diagnostic tool in suspected cases of this disorder, but otherwise HLA typing has little or no diagnostic value.

Most of the diseases listed in Table II have previously been known to have a genetic background, but the precise mode of inheritance has, with few exceptions, been entirely unknown. Now, the associations with HLA make it possible to clarify the genetics of these diseases. For example, the HLA controlled susceptibility to ankylosing spondylitis is due to a dominant gene with incomplete penetrance. Moreover, studies of ‘healthy’ B27 positive individuals have shown that B27 associated diseases - sacroiliitis in particular - is much more frequent than assumed previously, and surprisingly that the incidence in females approaches that in males although ankylosing spondylitis has mainly been considered a disease of males.

The observation that only the juvenile form of diabetes is associated with HLA whereas the maturity-onset form is not, finally proves that these two disorders are different. Moreover, it has become clear that insulin-dependency is a better criterion than the age-at-onset when distinguishing between the two forms of diabetes. The genetic background of the susceptibility to insulin-dependent diabetes appears to be rather complex: while the HLA-Dw2 antigen (or another HLA factor closely associated with Dw2) ensures an extraordinary protection, the HLA-Dw3 and Dw4 antigens are both associated with a strong susceptibility to this disease. These possibilities of clarifying the inheritance of disease susceptibility on the basis of HLA associations are far from exhausted.

Another important aspect of the relationships between HLA and disease is that they open new ways for exploring the aetiologies of these disorders. Again, these possibilities have hardly begun to be realised, but with the increasing knowledge of the biological function of the HLA system the prospects become better. In general, it seems reasonable to suggest that the diseases showing the strongest associations with HLA-D antigens are due to the action of specific Ir determinants and in this context, it is striking to note that most
of the HLA-D associated disorders in Table II have an element of ‘abnormal’ immunity. For example, various autoantibodies are frequently found in juvenile diabetes, Graves’ disease, Addison’s disease, and the sicca syndrome, and hypersensitivity to gluten is a major element in coeliac disease. However, aberrant immunity may not explain all the associations; in particular there is no evidence that the relationship between HLA-A3 and haemochromatosis be explained in this way.

The Biological Function of the HLA System

The recent fascinating developments in our knowledge concerning the biological functions of the major histocompatibility complexes (MHC) in vertebrates are mainly due to two important sets of observations in animal experiments. Firstly, the discovery by Benacerraf and coworkers [11] of immune response (Ir) determinants which control the development of cell-mediated immunity and IgG antibody production, and the mapping of the corresponding genes within the MHC of the species in question [12]. Secondly, the ‘altered self’ or ‘dual recognition’ hypotheses advanced by Doherty and Zinkernagel [13] to explain their observation that virus-specific killer lymphocytes show specificity not against the virus as such but against virus infected cells carrying the same MHC antigens as were present in the animal from which the killer cells were derived, i.e. the killer cells react both against the foreign antigen and against the MHC antigens of the individual. In contrast to the immune response determinants which are closely associated with the HLA-DR analogues of mice, the MHC antigens involved in the lysis of virus infected cells appear to be the analogues of the HLA-ABC antigens.

Based on these and other observations, a common concept of the role of MHC antigens in the body’s immune reaction now seems to emerge. A large body of literature already exists about this topic, and although there are still several points to be clarified, the scheme outlined below is gaining increasing support [14,15]. It should be emphasised that most of the knowledge is derived from animal studies, but there is increasing evidence that the same mechanisms operate in man. A major basis for the scheme is the involvement of MHC in the cooperation between various cells of the immune system. The cells cooperating are the macrophages, the T lymphocytes, and the B lymphocytes. The macrophages have been known for some time to play a role in the ‘processing’ of foreign antigen. As illustrated in Figure 2, the macrophages cooperate with so-called T helper lymphocytes which both assist another subset of T lymphocytes, effector T lymphocytes, to become killer cells and help B lymphocytes to develop into plasma cells producing large amounts of IgG antibodies. Macrophages and B lymphocytes contain large amounts of HLA-D/DR antigens and it now seems as if the role of these antigens on macrophages is to combine with foreign antigens and present them to T helper lymphocytes which have receptors for ‘altered’ HLA-D antigens. This presentation leads to a proliferation of T helper lymphocytes which then help B lymphocytes, also carrying HLA-D/DR, in their transformation to IgG antibody-producing plasma cells. The same or another set of T helper lympho-
cytes reacts with precursor cells of T effector lymphocytes which in turn transform into active killer cells and/or cells releasing lymphokines upon contact with foreign antigen. These T effector cells must have receptors to ‘altered’ HLA-ABC antigens as they only react with target cells, e.g. virus-infected cells, carrying the adequate ABC antigens.

According to this scheme, the role of both HLA-D/DR antigens and HLA-ABC antigens is to present foreign antigen to various immuno-competent cells. The difference between these two classes of antigens lies in the cells to which they present the foreign antigen: while the HLA-D/DR antigens present foreign antigen to T helper lymphocytes, the HLA-ABC antigens present foreign antigens to T effector lymphocytes.

In Figure 2, we have chosen to illustrate the ‘altered self’ or ‘presentation’ model, but it should be noted that the ‘dual recognition’ theory may also explain the restriction imposed by HLA. According to this theory, HLA antigen and foreign antigen are recognised by two different receptors on the T lymphocyte. In any case, it appears that HLA antigens are deeply involved in some very important pathways of the immune reaction, both in cell-mediated immunity and in most, but not all, cases of humoral immunity.

As the T lymphocytes are thus predestined to recognise and react to ‘altered self’ HLA antigens, it becomes more easy to understand the strong immune response elicited by HLA incompatibility, e.g. in the case of transplantation: this strong reaction to foreign HLA antigens may be considered a reaction to ‘altered’ self.

Apart from the above mechanisms, the existence within the HLA system
of genes coding for some complement components indicates another important role of this system in the body's defences against microbial invasion.

A fundamental difference between the HLA controlled complement components and the HLA antigens is the degree of genetic polymorphism: while the complement components show very little variation from one individual to another, there are so many different HLA antigens in the population that it is difficult to find two unrelated individuals with identical phenotype. For example, one of the most common HLA-A,B,D phenotypes (HLA-A1,3,B7,B8, Dw2,Dw3) in Caucasians has a frequency of only about 0.2 per cent. A possible explanation for this extraordinary polymorphism now emerges from the knowledge about the biological function of the HLA system discussed above: HLA heterozygous individuals possess at least two (probably more) different Ir determinants and consequently they can respond to more foreign antigens than can HLA homozygotes. Moreover, it seems as if the number of killer lymphocytes reacting with virus-infected cells is higher in heterozygous than in homozygous individuals which would make it easier for the former to eliminate virus. A selective advantage of heterozygous over homozygous individuals is well-known as one of the mechanisms which can ensure the maintenance of genetic polymorphism, and it is possible that this mechanism is a major basis for the polymorphism of HLA. Conceivably, the associations between HLA and various diseases may be considered untoward effects of HLA factors which otherwise may confer resistance to infectious diseases.

Acknowledgments

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Open Discussion

BAILEY (Christchurch, New Zealand) I have been interested for some time in the familial and genetic aspects of reflux nephropathy, and was wondering if you have any data on the HLA typing of patients with reflux nephropathy? The reason I ask is that we have some preliminary data, from our patients coming on to the dialysis-transplant programme, that HLA B12 is significantly more frequent in patients with reflux nephropathy when compared with those with all other causes of renal failure combined. I wonder if you have any information on possible genetic markers in this renal lesion?

SVEJGAARD No, we do not have any information. That is an original observation. The only renal disease in which there seems to be, to my knowledge, an association with HLA is Goodpasture's syndrome which may be associated with BW2, but that still needs confirmation. There is some evidence that Japanese Streptococcal glomerulonephritis may be associated with B12, but I have not heard of an association with reflux nephropathy.

YAZICI (Istanbul) I would like your comment on the different antigen associations with the same disease reported from different parts of the world. I refer to multiple sclerosis and Behcet's disease.

SVEJGAARD That is a very important point. Some diseases are associated with different antigens in different populations. For example, this is true of almost all of the Dw3-associated disorders. They hold only for Caucasians, not for Japanese. And it is true that coming from the northern part of Europe and southward the association with Dw2 in multiple sclerosis tends to disappear. Now the two main possible explanations are either that there are different infectious agents involved in multiple sclerosis in the Northern part of Europe and in the Southern part of Europe and in Japan, or that, in fact, it is not the Dw2 in multiple sclerosis itself which is responsible for the association but another HLA factor closely linked to Dw2, a factor which we do not know today. As I said, we know there is more than one D-locus in mice, and these probably also exist in man.

YAZICI Ehringer is evidently growing Klebsiella from the guts of people with ankylosing spondylitis who are B27 positive, and he is finding a very close antigenic association between the Klebsiella surface antigens and the B27 antigens. Do you have any comments?

SVEJGAARD Ankylosing spondylitis shows association with the same antigen all over the world; for Japanese, Caucasians, and Blacks. And so you would therefore think it could be B27 itself which is involved and there could be some kind of cross-reactivity between B27 and a microbial antigen.

HAWKINS (Birmingham) I have heard rumours that women having severe and repeated pre-eclamptic toxaemia with a very high foetal loss rate have been
found in an unexpectedly high number of cases to have only three typeable antigens, suggesting that they have two antigens in common from their parents. Do you think that there is a mechanism operating against this loss of polymorphism of the HLA system?

SVJGAARD It could be. These data still need confirmation, but it is a very interesting point and certainly that would be one of the mechanisms. The other would be that individuals who are heterozygotes seem to have a higher chance of presenting the different foreign antigens in the system, as I recollect.

STRUYVENBERG (Chairman) I would like to ask a question which has been asked many times before. For years we have been waiting for a breakthrough in improving the success rate of renal transplantation. Do you expect in the near future serological typing for HLA-DR will markedly improve the success rate and it will be clinically possible to improve it?

SVJGAARD I am convinced that the inclusion of HLA-DR matching will improve the results, but it is extremely difficult to say how much it will improve them. I am somewhat optimistic because these factors are the ones responsible for the primary act in the immune response: the triggering of cell division. The one problem which remains is how important is the influence of the classical antigens going to be in addition to the DR antigen.

HAYRY (Helsinki) Is there any correlation between HLA and cancer? I think especially the virus-induced cancers, namely Burkitt’s tumour, nasopharyngeal carcinoma, and possibly testicular seminoma?

SVJGAARD There have been many studies of HLA and malignancies because the H2 system of mice seems to be involved in susceptibility to leukaemia viruses. What remains is that Hodgkin’s disease seems to show a weak association with HLA and in Chinese, one specific Chinese antigen seems to be associated with nasopharyngeal carcinoma. But this has not been found in other populations, and studies with Burkitt’s lymphoma have been entirely negative. So it has been very disappointing in malignancies, I must say.