MEDICINE GRAND ROUNDS

THE A B C

OF HLA

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GLOSSARY

ADCC -- Antibody dependent cellular cytotoxicity.

Bf -- Factor B of the properdin system.

C2° -- Gene for deficiency of the second component of complement.

Capping -- Movement of molecules of the cell surface by cross-linking to forma polar "cap" demonstrated by immuno-fluorescence.

CDC -- Complement dependent cytotoxicity.

CML -- Cell mediated lympholysis.

CTL -- Cytotoxic T lymphocyte.

CYNAP -- Cytotoxicity negative absorption positive.

GvH -- Graft versus host reaction.

HLA -- Region of the sixth chromosome containing genes of the human main histocompatibility complex.

HLA antigens -- Products of HLA genes.

HLA genotype -- HLA genes in each of two sixth chromosomes of a person.

HLA haplotype -- All the information coded by one HLA chromosome (paternal or maternal).

HLA phenotype -- HLA antigens detectable by testing of an individual.

HTC -- Homozygous typing cell.

Ia -- Immune response region associate antigens.

Ig -- Immunoglobulin.

Ir -- Immune response loci that control ability to respond to a variety of antigens.

LD -- Determinants that cause strong lymphocyte response (originally: "Lymphocyte defined").

MLC -- Mixed lymphocyte culture.

PFC -- Plaque forming cells.

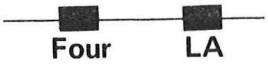
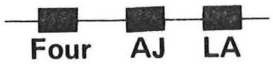
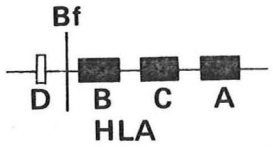
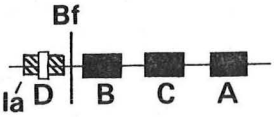
SD -- Determinants that were originally "serologically defined".

Stripping or lysostrip -- Development of resistance to cyto-lysis after treatment with antibody; used to demonstrate molecular independence of surface antigens.

HISTORICAL DEVELOPMENT OF HLA.

It is interesting to look back on how it developed, now that HLA has reached a stage of considerable maturity . Thirteen years ago when it became clear that leukocyte antigens were relevant to histocompatibility - a Workshop was organized (1). These Workshops were to be a major factor for progress in this field. The achievement in the first one (1964) was that a few specificities could be recognized with reagents developed in several different laboratories. By the time of the Third Workshop in 1967 (Table 1) the term HL-A was invented and used to describe what was then thought to be a single system controlling all the major human histocompatibility antigens. Three years

Table 1

Mapping of Loci	Data	Time of Publication
——— ? ———	Leukocyte antigens relevant to histocompatibility	1st Workshop, 1964
		2nd Workshop, 1965
	HL-A as a single system	3rd Workshop, 1967
	Two Segregant series of antigens	4th Workshop, 1970
	Third locus (AJ)	5th Workshop, 1972
	Serologic loci: HLA-A, B, C. Main locus: HLA-D. Also Bf, C2, C4 controlled by HLA.	6th Workshop, 1975
	Ia-like antigens linked to HLA-D. CML target antigens.	7th Workshop, 1977

later it became apparent that the known antigens could be divided into two segregant series. It was then thought that the system consists of two loci, which were called LA and Four or First and Second. For several years the Scandanavians had a serum AJ, that did not fit this simple scheme. By 1972, experiments were performed that demonstrated that there was indeed a third series of antigens and the Third locus was called AJ. The Fifth Workshop was devoted largely to anthropology. The inheritance of HLA genes in different populations around the world was mapped and many interesting differences were found. Then after a deceivingly quiet period of three years the 6th Workshop in 1975, brought about a revolution. The terminology was changed. The chromosomal region was to be named HLA (without the hyphen). The loci were designated using the letters of the alphabet. LA became A, Four became B, AJ became C. The newly discovered locus coding for determinants that produce strong stimulation in MLC became HLA-D. It became known that Bf, C2 and C4 are controlled by the HLA region, and that the HLA region is on the sixth chromosome. Further work has confirmed and consolidated these facts. Two important new developments that will constitute large part of the deliberations of the Seventh Workshop (1977) (Table 1) are the identification of Ia-like antigens in man many of which are linked to HLA-D, and the finding of specific CML target antigens probably coded by still another locus of the HLA chromosomal region.

PRESENT CONCEPT OF THE HLA CHROMOSOMAL REGION.

The human main histocompatibility complex appears to be in every respect similiar to the MHC in rodents and other species including rhesus monkeys (2). The mapping of the loci is based on the study of recombinants and is well established for HLA-A,B.C and D. It seems likely that there are other HLA linked antigens that produce weak MLC stimulation. An LD-2 locus close to HLA-A is reasonably well documented. The precise location of genes coding for some of the complement components is still uncertain. The Ia-like antigens associated with alleles of HLA-D are coded by genes close to HLA-D. Other B cell antigens appear to be coded in the region close to HLA-A (3).

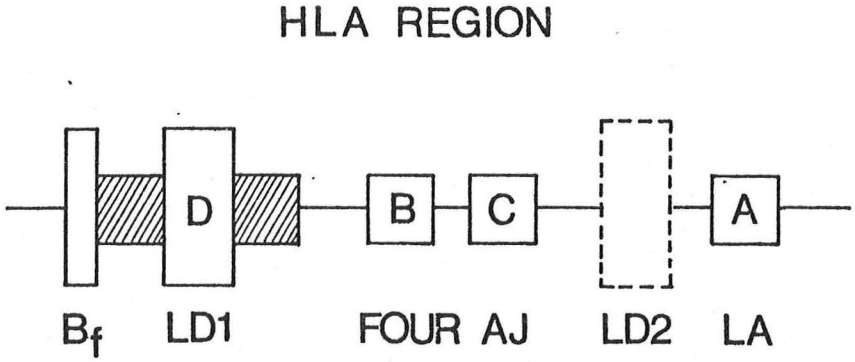


Fig. 1

HLA-A, B and C Specificities.

The presently accepted A, B and C specificities (4) are shown in Table 2. These are antigens that are detected in

Table 2

HLA-A, B and C Antigens

(WHO-IUIS, 1975)

New	Previous	New	Previous	New	Previous
HLA-A1	HL-A1	HLA-B5	HL-A 5	HLA-CW1	T1
HLA-A2	HL-A2	HLA-B7	HL-A 7	HLA-CW2	T2
HLA-A3	HL-A3	HLA-B8	HL-A8	HLA-CW3	T3
HLA-A9	HL-A9	HLA-B12	HL-A12	HLA-CW4	T4
HLA-A10	HL-A10	HLA-B13	HL-A13	HLA-CW5	T5
HLA-A11	HL-A11	HLA-B14	W14		
HLA-A28	W28	HLA-B18	W18		
HLA-A29	W29	HLA-B27	W27		
HLA-AW23	W23	HLA-BW15	W15		
HLA-AW24	W24	HLA-BW16	W16		
HLA-AW25	W25	HLA-BW17	W17		
HLA-AW26	W26	HLA-BW21	W21		
HLA-AW30	W30	HLA-BW22	W22		
HLA-AW31	W31	HLA-BW35	W5		
HLA-AW32	W32	HLA-BW37	TY		
HLA-AW33	W19.6	HLA-BW38	W16.1		
HLA-AW34	Malay 2	HLA-BW39	W16.2		
HLA-AW36	Mo*	HLA-BW40	W10		
HLA-AW43	BK	HLA-BW41	Sabell		
		HLA-BW42	MWA		

all nucleated cells using the complement dependent cytotoxicity test. The chemistry is not as advanced as the serology but it is already becoming clear that in the variable region of the heavy chain of the HLA molecule aminoacid differences correspond to the specific antigenic determinants recognized by the typing sera (5). The polymorphism of this system is remarkable.

The Basis of Mixed Lymphocyte Culture Typing (Table 3).

In one-way MLC, with irradiated stimulating cells, genetic differences result in a detectable reaction only in the responding cells. Their reaction to foreign stimulating determinants consists of blastic transformation and proliferation. The magnitude of the response is determined by radioactive thymidine incorporation and is maximal after 5

or 6 days. In this reaction the responding cells are mainly T lymphocytes (6). The activating determinants are known to be coded by the HLA chromosomal region. Strong stimulation is produced by products of HLA-D (7), which are expressed mainly in B lymphocytes, and can also be demonstrated in macrophages, epidermal cells and sperm and are probably also present in endothelial cells (8).

Table 3

The Basis of Mixed Lymphocyte Culture Typing

1. **One-way mixed lymphocyte culture – genetic difference stimulates transformation and proliferation**
2. **Responding cells are mainly T lymphocytes**
3. **Activating determinants are coded by genes of the HLA chromosomal region**
4. **Strong stimulation produced by products of HLA-D which are expressed mainly in B lymphocytes, epidermal cells, macrophages, endothelial cells and sperm**
5. **HLA-D homozygous cells used for typing – negative or very low reaction ("typing response") if responding cells have the antigen**

Cells from individuals who are homozygous at the HLA-D locus can be used for HLA-D typing. Most responders having the same antigen will produce a negative or a very low reaction to the homozygous stimulating cells. These low reactions are considered to represent a "typing response".

Some Practical Considerations Regarding Typing with Homozygous Typing Cells (HTC) (Table 4).

The most important technical consideration is that homozygous individuals must be found. Then, after appropriate testing their lymphocytes must be frozen in such a way that they can be used as a reagent for typing. In addition certain kinds of experiments can only be performed if one can also work with frozen responding lymphocytes. It is then possible to select groups of cells to be tested in various combinations and to perform large experiments including the necessary controls. Data handling, editing and double normalization of the results is performed by computer. In our laboratory typing responses using these methods tend to fall below 30%.

Table 4

Technique of Mixed Lymphocyte Culture Typing

1. **Frozen homozygous typing cells (HTC) — used as reagents**
2. **Frozen responding lymphocytes — permit large experiments needed for stable relative responses**
3. **Editing and double normalization by computer**
4. **Bimodal distribution of responses — typing responses usually <30%**
5. **Specificities defined by the 6th International Histocompatibility Workshop: DW1, DW2, DW3, DW4, DW5, DW6, LD-107, LD-108**
6. **New specificities: LD-17, LD-TMo, LD-BCo, LD-WFI**

The HLA-D specificities that were established during the 1975 International Workshop and several new specificities recognized using HTC developed in our laboratory are shown in Table 5. The phenotype frequencies of these antigens in a Caucasian population in Dallas, as well as the B locus associations are also given.

Table 5

Frequency of HLA-D Antigens in

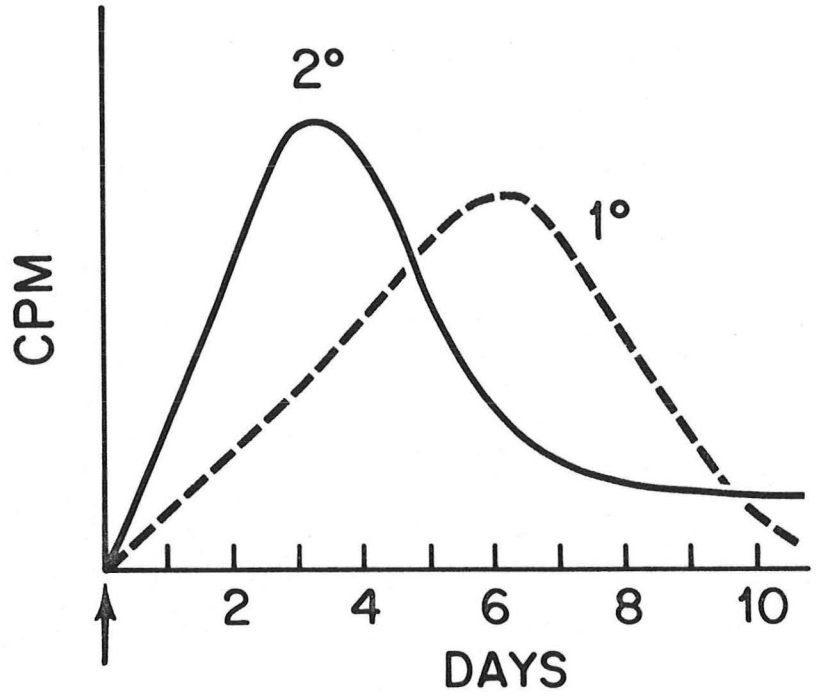
a Dallas Caucasian Population

Antigen HLA	Number Tested	Phenotype Frequency (%)	B-Locus Associations
DW1	56	18	BW35,(B27)*
DW2	59	25	B7,(B18)
DW3	61	10	B8
DW4	63	17	BW15
DW5	49	6	BW16,(BW21)
DW6	56	11	BW16,(B12)
LD-107	50	12	B12
LD-108	61	3	B27?
LD-17	63	8	BW17
LD-TMo	60	0	—
LD-BCo	33	18	—
LD-WFI	30	14	—

*Antigens given in parenthesis have weaker associations.

Primed Lymphocyte Typing (PLT).

Another method for typing for the determinants that stimulate in MLC is now available. It's basis is the difference in timing of the peak proliferative response in primary and secondary MLC reactions. When the antigen is offered for the first time the peak of responding lymphocyte proliferation occurs in 5-6 days (Figure 2). Ten to 14 days after the primary MLC, proliferation ceases and the responding cells revert to a resting stage. The population remaining contains memory T cells, or "primed lymphocytes".



Primed lymphocyte typing (PLT)

Fig. 2

When new stimulating cells are added to such cultures they respond in a primary or in a secondary fashion depending on the nature of the stimulating determinants presented.

Cells that have the same or similar determinants as those of the primary stimulator elicit a secondary response which occurs rapidly and peaks at 24-48 hours.

Cells that present entirely different determinants also generate a response, but it is slower, similar to a primary MLC with a peak of proliferation after 5-6 days.

The advantage of PLT is that recognition of the MLC determinants is based on a positive response rather than a negative response as in typing with HTC.

Table 6

Primed Lymphocyte Typing (PLT)

1. Ten to fourteen days after a primary MLC, proliferation of the responding cells ceases.
2. The remaining population contains memory T cells.
3. If new stimulating cells are added to such cultures they can respond in primary or secondary fashion depending on the stimulating determinants presented.
4. The response is rapid (24-48 hrs) if the restimulating cells have the same or similar determinants as the primary stimulator.
5. The response is slow (5 to 6 days) if the restimulating cells are different from the primary stimulator.
6. Primed lymphocytes recognize the HLA-D products and also certain weak MLC determinants. Therefore, PLT results can be more complex than typing with HTC.
7. One advantage of PLT is that it recognizes antigens by a positive response.

The same specifities that are defined with HTC are recognized by primed lymphocytes (9). In addition it is possible to obtain primed cells against weaker stimulating determinants. They seem to recognize some weak HLA associated MLC determinants (perhaps coded by LD-2) and perhaps even some non-HLA associated products (10). Because of the reactions to both strong and weak stimulating determinants interpretation of PLT results can be quite complex.

Ia-like Antigens in Man.

Recognition of Ia-like antigens in man is a relatively recent development. Immune response region associated (Ia) antigens were first discovered in mice (11). In man, certain alloantisera contain antibodies reacting with B lymphocytes and not with the corresponding T lymphocytes. Such antibodies are not absorbed by platelets. Platelet absorption can be used to remove undesirable antibodies against HLA-A, B or C frequently contaminating such sera. The reactions of these sera are usually not blocked by treatment of the B cells with anti-β2-microglobulin (12). Structurally the antigens involved are glycoproteins which appear to contain two polypeptide chains which are characteristic and different from those of other proteins of the cell surface Figure 3 (13).

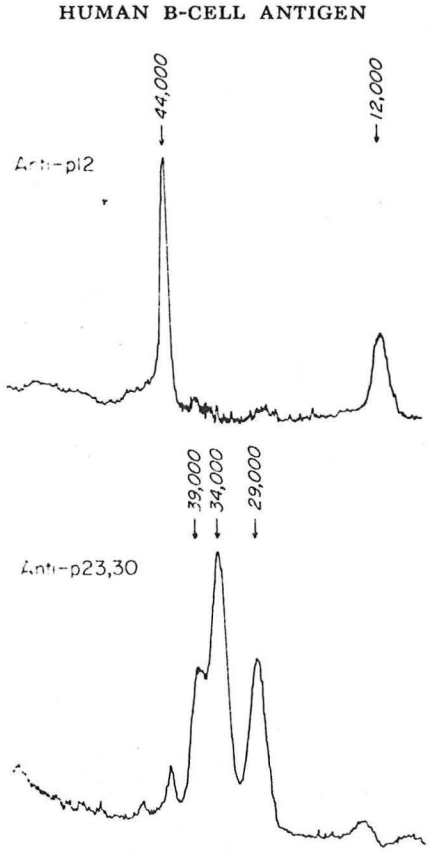


Fig. 3

Some Ia-like specificities detected in man are closely associated with alleles of HLA-D.

Table 7

Ia-like Antigens in Man

1. **Antibodies reactive with B lymphocytes (cultured, leukemic or normal) and not with the corresponding T lymphocytes.**
2. **Not absorbed by platelets.**
3. **Not blocked by anti-β2-microglobulin.**
4. **Antigen is a glycoprotein, apparently containing two polypeptide chains (30,000 and 25,000 daltons).**
5. **Some Ia specificities are closely associated with HLA-D types (I1, I2, I3 and I4).**

TISSUE DISTRIBUTION OF IA-LIKE ANTIGENS IN MAN.

These antigens have not been detected in human T lymphocytes either by cytotoxicity or by immunofluorescence (14). They are also absent from platelets, fibroblasts, erythrocytes, adult liver and brain (Table 8). They are expressed in B lymphocytes from normal persons, as well as in leukemic B cells and cultured B cell lines. They appear to be present in macrophages, endothelial cells, epidermal cells, sperm and fetal liver (15).

TABLE 8

Tissue distribution of Human Ia-type alloantigens

Tissue	Expression of Ia-type antigens
B lymphocytes	++
T lymphocytes	—
'Null' cells in PBL	+
Macrophages	+++
Epidermal cells	++
Endothelial cells (umbilical cord veins)	+
Spermatozoa	++
Fetal liver cells (14th week)	+
Platelets	—
Fibroblasts	—
Erythrocytes	—
Liver	—
Brain	—

FUNCTION OR PRODUCTS INHIBITED BY ANTI-IA SERA

Most of the information on the functions of Ia containing molecules comes from work performed in mice (Table 9).

Table 9 Functions or Products Inhibited by Anti-Ia Sera.

1. MLR stimulating factors
2. In vitro PFC responses
3. In vitro antigen-induced proliferation
4. Fc receptor
5. B cell response to LPS
6. In vivo graft rejection (enhancement)
7. T cell helper and suppressor factors
8. B cell acceptor sites for factors
9. Macrophage-T cell interaction factors

Antibodies to Ia antigens have been used as probes to investigate their role. It will be apparent from Table 9, that the impact of these molecules is enormous. Some of the listed experiments are now being confirmed in man. Human anti-Ia antibodies can be shown to block the MLC stimulating determinants. They inhibit the Fc receptors of B-lymphocytes and they appear to block the interaction of lymphocytes with macrophages.

COMPLEMENT COMPONENTS INHERITED IN LINKAGE WITH HLA.

In addition to the various components of cell membranes that have been discussed above, the HLA chromosomal region contains genes that control complement components which are secreted proteins, present in serum.

One of these is factor B of the properdin system. Genes coding for the different electrophoretic varieties of this protein are linked to HLA (16).

Similarly, electrophoretic polymorphism of C4, the fourth component of complement (Figure 4), is inherited in close linkage to HLA (17). A blank gene causing C4

deficiency is inherited with the HLA haplotypes. Interestingly patients with C4 deficiency have had a disease resembling systemic lupus erythematosus (SLE).

The most frequent complement deficiency in humans is the absence of C2 (the second component of complement). Subjects with absent C2 protein are found to be homozygotes that inherited two C2° genes. Heterozygotes have approximately one-half the normal amount of C2 protein in the serum. C2 deficiency has also been found to be associated with SLE-like disease, and other rheumatic conditions (18). C2° genes are inherited in families in linkage with HLA haplotypes. In many families with this condition the haplotype involved carried HLA-A10, B18 and DW2.

Table 10

Complement Components Inherited

in Linkage with HLA

1. **Electrophoretic polymorphism of factor B is linked to HLA. Bf locus probably located between HLA-B and D.**

2. **C2 deficiency frequently associated with SLE or other rheumatic conditions. In families C2° genes segregate in linkage with HLA. Most commonly associated with HLA-A10, B18, DW2 haplotypes.**

3. **C4 electrophoretic polymorphism inherited in close linkage to HLA. C4 deficiency also may be associated with SLE. C4° gene inherited with HLA haplotypes.**

4. **C8 deficiency has been reported in three patients (including one patient with SLE studied by Jasin). Linkage to HLA is at present uncertain.**

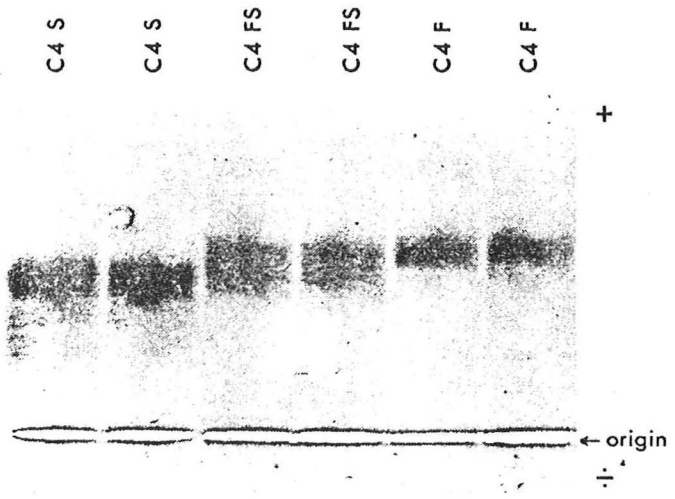


Fig. 4 The common C4 phenotypes revealed by immunofixation electrophoresis.

We have recently had the opportunity of studying a family of a patient with juvenile rheumatoid arthritis who is heterozygous C2° (Figure 5). The C2° gene was inherited from the mother with an HLA-AW25,B18 haplotype (Stastny, Jasin and Fink, unpublished). Two other children that have the same maternal haplotype are also C2 deficient.

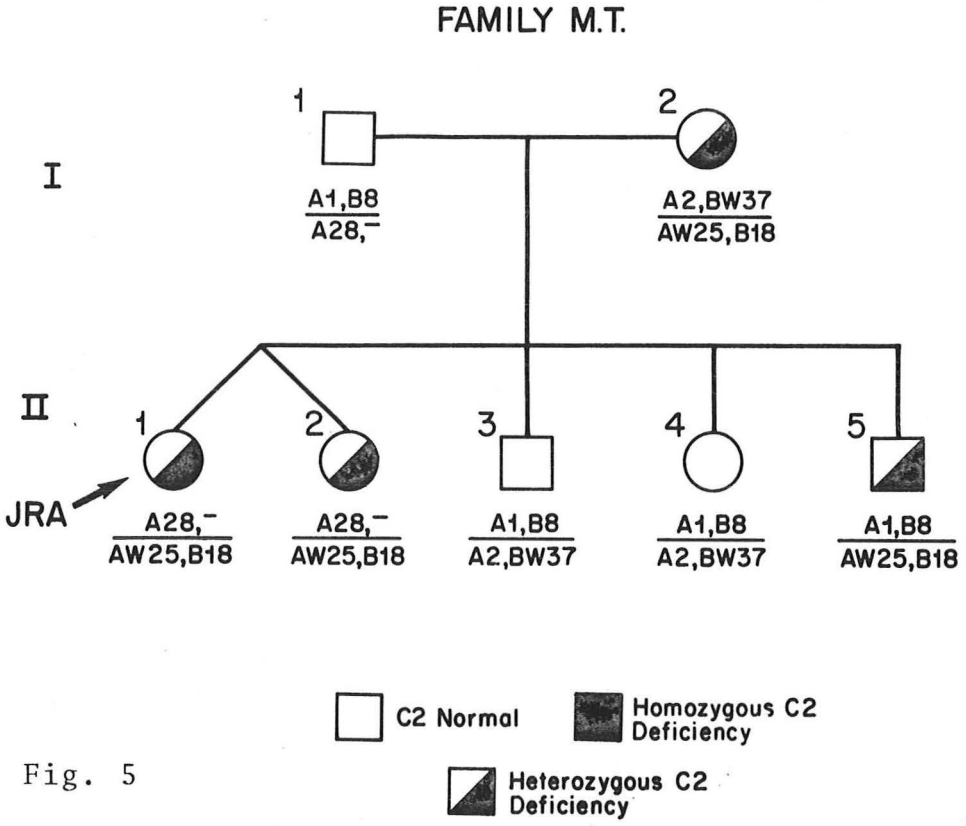


Fig. 5

LINKAGE BETWEEN HLA AND PAGET'S DISEASE

Although inheritance of a gene in linkage with HLA can be seen in population studies as an association with certain HLA types (as for example A10, B18, DW2 with C2 deficiency) in other conditions no particular HLA type is increased. An example of linkage without association was reported in families of patients with Paget's disease of the bone.

Fotino (19) has genotyped several families each containing several affected members. Interestingly development of Paget's disease was linked to HLA haplotypes but the antigens in these haplotypes were different in each family. In one family it was A2, B13; in another AW31, B14 and in a third A11, B18. It is possible that the immune response may be involved in Paget's disease since a slow virus infection in the osteoclasts is now thought to be the cause of this condition. It remains to be seen whether an I region marker may not show the association in future studies.

Table 11

Linkage Between HLA and Paget's Disease

1. Several families with multiple cases of Paget's disease of bone have been studied (Fotino, 1977).
2. Development of Paget's disease was linked to HLA haplotypes in each family.
3. The haplotypes concerned were different in each family: A2, B13; AW31, B14; and A11, B18.
4. The disease predisposition gene might be related to slow virus infection in osteoclasts now thought to be the cause of this condition.

RESULTS OF CLINICAL KIDNEY TRANSPLANTATION

Another example of HLA linkage with lack of evidence for any specific antigens involved in the general population, comes from the results of clinical kidney transplantation. The basic facts are summarized in Table 12. Clinical immunosuppression dampens the effect of the weaker transplantation antigens. If only irreversible rejection is taken as an endpoint it is clear that within each family the factor causing rejection is linked to the HLA haplotypes. When recipient and donor have the same haplotypes the kidney allografts survive for many years in the majority of patients (20). Everything else being equal, the transplantation antigen associated with one haplotype, in related transplants with

one haplotype being the same and the other different, produces irreversible loss of the grafted organ in about 25% of recipients in one year and in about 33% at two years. The success of HLA identical grafts is unequivocal evidence that the HLA chromosomal region is the main barrier to kidney transplantation. The results with random unrelated cadaver kidney allografts show that irreversible rejection occurs in about 50% of cases at one year. After two years about 40% of the organs are functioning. Matching for the HLA-A and B antigens alone produces a small improvement of about 10% (21). The conclusion to be drawn is that other genes of the 6th chromosome must be involved in rejection.

Table 12

Results of Clinical Kidney Transplantation

1. **HLA identical sib kidney allografts survive in 90% of the patients for many years.**
2. **One haplotype mismatched related grafts function in 75% of the recipients at one year and in about 2/3 after two years.**
3. **Cadaveric transplants survive in about 50% of cases after one year, about 40% after 2 years.**
4. **HLA-A, B and C matching of unrelated kidney allografts produces only a small improvement in the U.S. Results are somewhat better in some European centers where the population is more homogeneous. It is likely that other genes of the HLA region are involved in rejection.**

HLA CHROMOSOMAL REGION PRODUCTS THAT MIGHT BE INVOLVED IN KIDNEY ALLOGRAFT REJECTION.

As we broaden our understanding of the HLA chromosome new antigens can be tested to see whether they play the determining role in causing irreversible rejection. At this writing there is hope that matching for one of the

newer systems may produce the desired improvement in cadaveric kidney allograft survival. On the other hand if the current results are due to an additive effect of many antigens, matching alone may never make cadaveric transplants as successful as those from related donors.

Several HLA region products are now being considered for improving the matching of unrelated kidney allografts (Table 13).

Table 13

HLA Chromosomal Region Products That Might be Involved in Kidney Allograft Rejection

1. **The strong MLC stimulating determinants coded by HLA-D.**
2. **The human Ia-like antigens now detectable by cytotoxicity in B lymphocytes and monocytes.**
3. **The endothelial specific antigens also detectable in blood monocytes.**
4. **The CML target antigens probably coded by a new HLA locus.**

Several studies have claimed correlation between MLC and graft results (22). Unfortunately none of them is free of defects and the issue remains unresolved. Moreover, MLC typing even by the PLT technique is not fast enough to be available at the time a clinical decision has to be made.

The Ia-like antigens which are closely associated with the HLA-D alleles can now be detected using a cytotoxicity test with B-lymphocytes or monocytes as targets. Because results could be made available in about 5 hours, such a test would be practical. Preliminary results in rhesus monkeys are encouraging (23). Whether it will work in humans remains to be seen.

A new antigen system expressed in endothelial cells and monocytes has recently been described (24). Because the endothelium of the renal vasculature is the main site of both acute and chronic rejection of renal allografts endothelial specific antigens may well play an important role in rejection.

Finally, one must consider the target antigens for CML. Recent work in several laboratories suggests that it is possible to type by CML for antigens presumably coded by still another HLA locus. CML is considered to represent a good in vitro model of the rejection phonomenon. According to some reports results of CML against the donor correlate with clinical outcome of kidney allografts (25).

HLA AND DISEASE

Subtle genetic influences which do not appear to have an obvious Mendelian inheritance pattern and do not correlate in the usual sense with an enzymatic defect or an inborn error of metabolism appear to play a role in many diseases. Immunogenetics, a combination of immunology and genetics, is a relatively new and fertile discipline. Much of it's activity centers on the products of the MHC which appear to serve as markers of individuality. The spectrum of variation includes certain types that may predispose for the development of disease. This is not an entirely new approach to medicine. Research on the possible association of blood groups with various diseases has been going on for years. It is the success of HLA which is unprecedented. It is a testimony to the importance of the MHC. The mechanisms are stilll largely unknown. The researchers are still too busy mapping out the surface of this interesting new territory. New findings are constantly being reported. Exploration in depth is yet to come. Only a few of the salient points can be covered in this presentation.

COMMON FEATURE OF DISEASES SHOWING HLA ASSOCIATIONS

Most of the diseases in which HLA associations have been found have been known for years to have an increased frequency in multiple cases in families. In most cases, however, the genetic component is not well understood and the mode of inheritance not established.

Immunologic abnormalities are a common feature of many but not all of these diseases and pathogenetic mechanisms involving humoral or cellular components of the immune response are frequently considered.

These diseases have usually a chronic course, frequently continuing over a period of years. Their etiology is unknown, in most cases. A viral infection of an atypical variety that has until now eluded detection has been considered in a number of these diseases. A peculiar interaction of genetic, immunologic and viral causes has repeatedly been postulated.

Table 14

COMMON FEATURES OF DISEASES

SHOWING HLA ASSOCIATIONS

1. **Multiple cases in families - genetic mechanism usually not well established.**
2. **Immulogic abnormalities common.**
3. **Chronic course.**
4. **Unknown etiology.**

VARIOUS FORMS OF HLA AND DISEASE ASSOCIATIONS

It may be useful to look at a number of separate groups of diseases according to the region of the HLA chromosome with which they show the strongest associations. In Table 15, 5 groups of diseases are considered.

The first group has a strong association with an HLA-A locus antigen. Idiopathic hemochromatosis is at present the only example. It's association is with HLA-A3.

The next group includes diseases with a strong association with an allele of the HLA-B locus. The best example is ankylosing spondylitis associated with HLA-B27.

Another group of diseases are associated with certain of HLA-A and B antigens but have an even stronger association with HLA-D. Good examples are: multiple sclerosis, Graves' disease, juvenile diabetes and gluten sensitive enteropathy.

In the next group, there is a strong association with an HLA-D antigen, as well as perhaps with the corresponding Ia antigen, but distribution of HLA-A and B alleles is perfectly normal. Rheumatoid arthritis is the prototype of this group.

Finally, there are some diseases linked to HLA, but involving genes which are separate from HLA-A,B,C and D. Examples are the C2 or C4 difficiencies and perhaps Paget's disease of bone.

Another group may have to be added soon, as it appears that psoriasis may have its strongest association with a new allele of HLA-C.

Table 15

VARIOUS FORMS OF HLA AND DISEASE ASSOCIATIONS

1. Strong association with HLA-A locus antigen (hemochromatosis with HLA-A 3).

2. Strong association with an HLA-B locus antigen (ankylosing spondylitis with HLA-B27).

3. Association stronger with an HLA-D antigen but association with HLA-A and B also found (Graves' disease, juvenile diabetes, celiac disease, multiple sclerosis).

4. Strong association with an HLA-D antigen (and an Ia-like antigen?) without association with HLA-A or B (rheumatoid arthritis).

5. Linkage with an MHC gene other than HLA-A, B, C or D. (C2 deficiency, Paget's disease).

HLA ASSOCIATION IN IDIOPATHIC HEMOCHROMATOSIS

Because the chemical mechanism involved in the development of idiopathic hemochromatosis is not understood the genetic basis of this disease has remained controversial. This is one disease in which there is no evidence at present for an innunologic mechanism and yet an HLA association has been found in three separate studies. Interestingly, one antigen, HLA-A3 was found to be increased both in France and in Brittain. It is the only disease showing a strong A locus association. Therefore, an abnormal gene responsible for development of hemochromatosis may be located close to the HLA-A locus. Excess homozygosity of HLA-A3 has not been found in patients with hemochromatosis, suggesting that earlier papers stating that the condition is recessive and that homozygosity was required for development of full blown disease may have been in error (26).

Table 16

HLA Associations in Idiopathic Hemochromatosis

1. Idiopathic hemochromatosis appears to have a genetic basis. Little is known about the mode of inheritance and the chemical mechanism is not understood.
2. HLA-A3 was increased in three separate groups of patients with idiopathic hemochromatosis.
3. In France (Brittany) A3 was most often associated with B14 and linkage with an A3, B14 haplotype was observed.
4. In British studies the usual A3, B7 linkage was present.
5. It is postulated that the abnormal gene responsible for development of hemochromatosis may be close to the HLA-A locus on the 6th chromosome.

THE HLA-B27 DISEASES

The most remarkable association and the one that has already entered into medical practice is between the spondylitis group of diseases and HLA-B27. The salient points are summarized in Table 17.

Table 17

The HLA-B27 Diseases

1. Normal frequency of B27 in Caucasians is about 9 percent.
2. In patients with ankylosing spondylitis it is 90 percent (RR=120).
3. In patients developing spondylitis in association with Crohn's disease, ulcerative colitis or psoriasis B27 found in about 50 percent.
4. Patients with Reiter's disease have B27 in 80 percent of cases, this is true also of Reiter's developing after Yersinia, Shigella or Salmonella infections.
5. About 50 percent of patients with acute anterior uveitis have B27.
6. In juvenile rheumatoid arthritis B27 frequent in boys with pauciarticular onset after the age of 10, who tend to develop spondylitis.

In Figure 6, the shaded area represents the geographic distribution of populations having the antigen B27. The clear zones in the southern hemisphere represent the populations when B27 is absent and ankylosing spondylitis appears to be rare. In Japan and in American Blacks B27 is rare (1 or 2% compared to 9% in Caucasians) and ankylosing spondylitis occurs often in the absence of B27 (27).

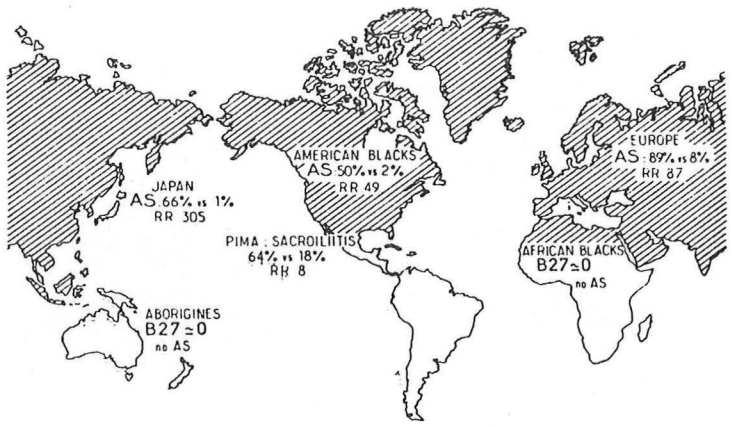


Fig. 6

THE HLA-B8-DW3 DISEASES

A number of diseases with "autoimmune" features belong to this group (Table 19). Although the same HLA haplotype is involved (A1,B8,DW3) different disease predisposition genes must be postulated to explain the organ specificity in different conditions. Some of these genes appear to be closer to the B locus and others more linked to HLA-D. Thus, chronic active hepatitis and myasthenia gravis have the strongest association with HLA-B8. The other diseases in the group show stronger associations with DW3 (Table 18) (28) An even stronger association with the DW3 associated Ia antigen exists in celiac diseases (29) and the same may be true in some of the other diseases of this group.

Table 18

The HLA-B8-DW3 Diseases

1. The normal frequency of B8 in Caucasian is 26 percent, that of DW3, 10 percent (Dallas material).

2. Diseases having strongest association with B8: chronic active hepatitis, myasthenia gravis.

3. Diseases having strongest association with DW3: celiac disease and dermatitis herpetiformis, Graves' disease, Addison's disease, Sjögren's syndrome, juvenile onset diabetes.

4. An even stronger association with DW3 associated Ia antigen in celiac disease and probably others of this group.

An interesting finding is that in Japan where HLA-B8 is not found (Figure 7), Graves' disease is associated with HLA-BW35 instead of B8 (30).

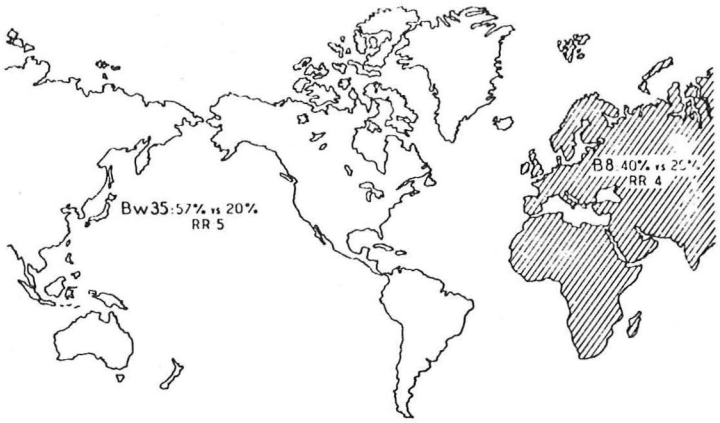


Fig. 7

ASSOCIATION OF HLA-DW4 WITH RHEUMATOID ARTHRITIS

In rheumatoid arthritis the main association is with an HLA-D locus antigen. The distribution of HLA-A and B antigens has been found to be normal. The antigen DW4 was defined with an HTC from a patient with RA.

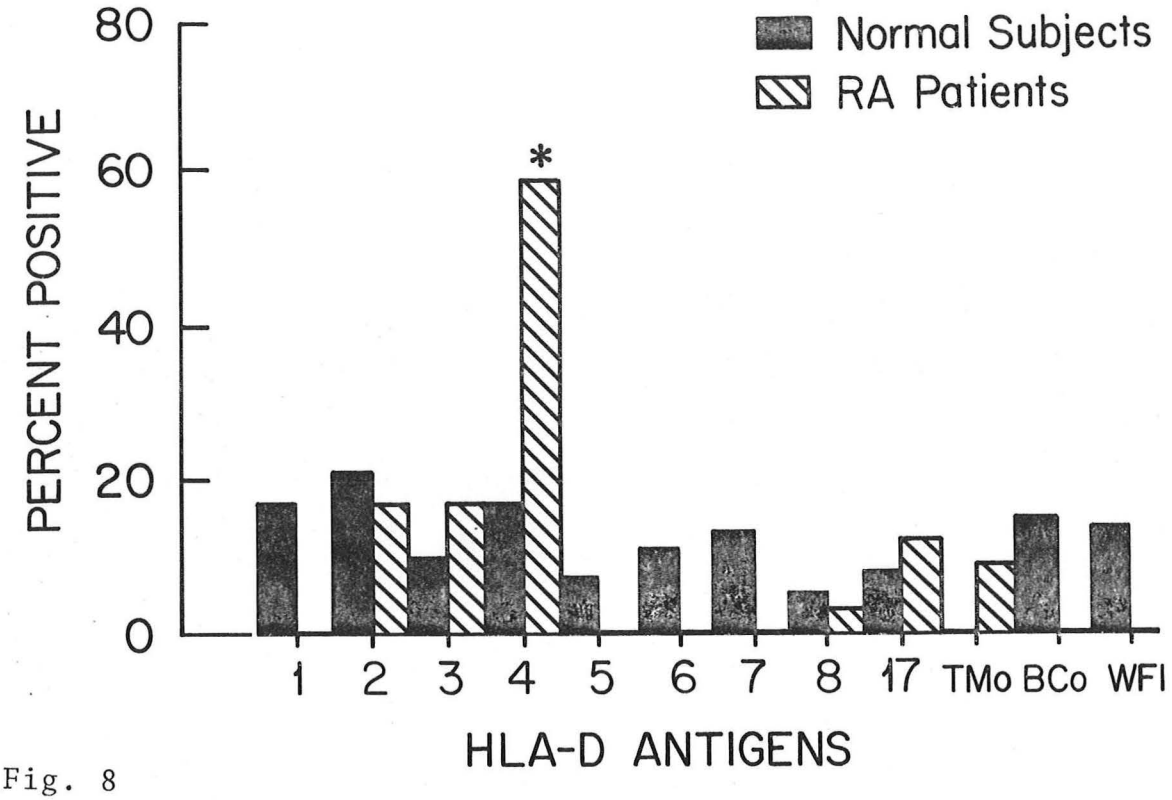


Fig. 8

Figure 8, shows the distribution of HLA-D locus antigens we currently type for in normal subjects and in RA patients. HLA-DW4 stands out. The normal frequency was 17 percent and the frequency in RA patients was 59 percent (31). Preliminary results suggest that an even stronger association exists with the DW4 associated Ia antigen I4.

Although patients with adult onset and juvenile onset RA develop similar erosive arthritis leading to destruction of their joints, other clinical features are quite different in the two groups (Table 19).

Table 19

Adult and Juvenile Onset

Rheumatoid Arthritis

Clinical Features	Adult	Juvenile
Erosive arthritis	+	+
Rheumatoid factor	frequent	rare
Subcutaneous nodules	frequent	rare
Fever	0	frequent
Skin rash	0	frequent
Lymph nodes, spleen	rare	frequent

When the distribution of HLA-D antigens was examined in patients with juvenile RA, it was found to be quite different from that in adults (Table 20). None of the juvenile RA groups showed an increase in HLA-DW4, but another antigen, LD-TMo was increased in the JRA patients with a pauciarticular onset. Thus it appears that different immunogenetic factors are involved in predisposition for development of juvenile and adult onset RA.

Table 20

Frequency of HLA-DW4 and LD-TMo in Patients with Different Clinical Onset of Juvenile Rheumatoid Arthritis

Clinical Type of Onset	Number Subjects	HLA-DW4 Positive	Percent	LD-TMo Positive	Percent
Controls	60	10	17	0	–
JRA					
Pauciarticular	56	3	5*	14	25**
Polyarticular	27	4	15	1	4
Systemic	23	4	17	3	13*

* $p<0.05$
** $p<0.001$

POSSIBLE MECHANISMS

Some of the recently described functions of the products of the MHC in experimental animals are shown in Table 21. It emphasizes the strong involvment of this chromosomal region in many aspects of immunity. On this background a number of possible mechanisms for disease associations come to mind. All of them are at present speculative.

Table 21

Functions of products of the main histocompatibility complex

1. Cell mediated lysis of virus infected cells.
2. Cell mediated lysis of chemically modified cells.
3. Macrophage–lymphocyte co-operation in antibody production.
4. T cell–B cell interaction in antibody production.
5. T cell helper and suppressor factors.
6. Immune response (*Ir*) genes.
7. Complement components.

A virus or a viral product might persist in the tissues if the Ir genes code for a defect in the immune response to such an infectious agent.

Another possibility is that given an antigen, the immune response (under control of disease predisposing genes) has itself a harmful effect. Injury might be due to either cellular or humoral immune mechanisms.

An attractive possibility is that I-region genes would enable the patient to develop high titer, high affinity, IgG antibodies capable of forming injurious antigen-antibody complexes. One would speculate that perhaps in RA, the antibodies are rheumatoid factors and in SLE, they might be high affinity, IgG anti-DNA.

Similarly, antibodies against certain receptors (myasthenia gravis, Graves' disease) might develop only if an individual possesed the appropriate Ir genes.

Finally, it is conceivable that injury to skin, as in DLE or scleroderma, or to muscle, as in polymyositis might be mediated through the activity of lymphocytes capable of becoming sensitized to specific autoantigens, in persons inheriting the corresponding I region genes.

Table 22

Possible Mechanisms of Immunogenetic Factors

1. Immunologic defect causing persistence of an infectious agent: slow virus or viral product.

2. Abnormal immune response with pathogenic consequences: cellular or humoral immunity (or both) causing tissue injury.

3. High affinity IgG autoantibodies: rheumatoid factors (RA) anti-DNA antibodies (SLE) - lesions due to "toxic" antigen-antibody complexes.

4. Antibodies against receptors as in myasthenia gravis and Graves' disease.

5. Delayed type hypersensitivity to organ specific antigens — perhaps in DLE, scleroderma, polymositis.

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