The HLA system and immunological defence against cancer: a review[1]

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Human lymphocyte antigen (HLA) refers to a group of antigens coded for by genes on a single segment of chromosome 6 (Francke & Pellegrino 1977). At present four separate loci have been demonstrated within this region, each coding for a series of from 8 to 20 alternate alleles (Table 1) (*see* Bach & Van Rood 1976). The antigens of the A, B and C loci are detected using immune sera and complement in a lymphocytotoxicity test, whereas the D locus antigens are defined by the mixed lymphocyte culture technique. Family studies have shown that every individual inherits from both parents a segment of chromosome, known as a haplotype, coding for one antigen at each locus. In most families, the genes on a particular haplotype are always inherited together. However, on rare occasions, during meiosis, recombination occurs (less than 1 in 100) and this has enabled the exact sequence for the four loci to be established.

The extraordinary thing about this complicated system is why it should ever have evolved.

Table 1. Antigens of the HLA complex● (Reproduced from Oliver 1977, by kind permission)

A locus	C locus	B locus	D locus
A1	CW1	B5	DW1
A2	CW2	B7	DW2
A3	CW3	B8	DW3
A9–AW23	CW4	B12	DW4
A9–AW24	CW5	B13	DW5
A11		B14	DW6
AW19–A29		BW15	DW7
AW19–AW30		BW16–BW38	DW8
AW19–AW31		BW16–BW39	
AW19–AW32		BW17	
AW19–AW33		B18	
A28		BW21	
AW34		BW22	
AW36		B27	
AW43		BW35	
		BW40	
		BW41	
		BW42	

● W antigens are those which have received only provisional WHO ratification

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All animal species that have been studied, including the frog, chicken, mouse, rat, guinea-pig, dog, pig, sheep, Rhesus monkey and chimpanzee, have a complex lymphocyte antigen system like the HLA system. There is some evidence for close biochemical similarity between lymphocyte antigens of species as far removed phylogenetically as mouse, rat and man. This comes from both chemical purification and sequencing studies and the demonstration of immunological cross reactivity between the lymphocyte antigens of different species. The techniques used to study this gene complex have been the same for all species and three classes of gene product have been demonstrated. The antigens defined by antibodies are known as serologically defined antigens and exemplified by the A, B and C locus antigens of the HLA complex. The antigens defined by the mixed lymphocyte culture reaction, the lymphocyte activating antigens or determinants (LAD) – e.g. the HLA-D locus antigens – also have been demonstrated in all the species studied. The third class of gene, the immune response (Ir) gene, has so far been demonstrated only in experimental animals (Katz & Benacerraf 1975), though there is a possibility that there are Ir genes in man situated close to the HLA-D locus. These genes regulate immune response, usually at the level of determining whether antigen recognition occurs.

HLA and transplantation rejection

In all species where this type of lymphocyte antigen has been studied, it can be detected on all tissues of the body and plays a significant role in initiating graft rejection. Since 1969 (Singal *et al.* 1969), it has been well recognized in man, when using standard immunosuppression, that identity for HLA between siblings leads to almost indefinite renal graft survival, almost as often as when grafting between genetically identical twins. Matching for the HLA-A and B loci between unrelated individuals does produce some improvement in graft survival (Oliver *et al.* 1972), though only when they are matched for the HLA-A, B and D loci does survival approach that of HLA identical siblings (Festenstein *et al.* 1976).

The long evolutionary history of the major histocompatibility system and the occurrence of HLA and disease associations, make it highly likely that the real biological importance of the HLA system is in determining resistance/susceptibility to infectious and malignant disease. To understand the relevance of the HLA associations with malignancy, it will be necessary first to review the more important HLA associations with nonmalignant disease.

Table 2. HLA-B27 associated diseases.
(Reproduced from Oliver 1977, by kind permission)

Disease	Antigen frequency
Ankylosing spondylitis	95%
Reiter's disease	65%
Juvenile rheumatoid arthritis	25%
Acute uveitis	27%
Control	5–10%

HLA-B27 and ankylosing spondylitis

More than 90% of patients with ankylosing spondylitis are B27 positive, though the frequency of this antigen in a normal population is about 10% (Table 2) (Ryder & Svejgaard 1976). The frequency of B27 in patients with ankylosing spondylitis approaches that of a positive rheumatoid factor in classical rheumatoid arthritis. However, more than 90% of normals with B27 would not be expected to have overt symptoms of the disease (Table 3). It is possible that this disease is under-diagnosed as the symptomatology could be confused with other conditions, but in a survey of B27-positive blood donors, Calin & Fries (1976) found only 18%

Table 3. Risk of ankylosing spondylitis in an individual with HLA-B27. (Reproduced from Oliver 1977, by kind permission)

	Males	Females
Incidence of ankylosing spondylitis	0.1–0.4%	0.025%
Frequency of B27	10%	10%
Frequency of B27 in patients with ankylosing spondylitis	90%	87%
Incidence of ankylosing spondylitis in individuals with B27	0.8–3.3%	0.2%

with symptoms and X-ray changes of ankylosing spondylitis. Even amongst relatives of patients with the disease, less than half the B27-positive individuals had overt disease.

Studies of patients with a reactive arthropathy after salmonella, shigella and yersinia enteritis provide a possible explanation as to why there is not an absolute association between B27 and disease. The frequency of B27 is the same in these patients as in ankylosing spondylitis suggesting that there might be immunological cross reactivity between B27 and an antigen in gram negative bacteria (Table 4) (*see* Aho *et al.* 1973). Supportive evidence for this hypothesis has come from Ebringer *et al.* (1976) who immunized rabbits with B27 positive lymphocytes and produced antibodies against a species of klebsiella, and vice versa. Subsequently, an increased incidence of klebsiella organisms in patients with active disease was found. Confirmation of these results will be very important as they open completely new approaches to the treatment of these patients.

Table 4. HLA-B27 in patients who develop arthritis after bacterial infection of the gastrointestinal tract. (Reproduced from Oliver 1977, by kind permission)

Type of infection	Incidence of B27 in patients with arthritis
Yersinia	44/49
Salmonella	14/15
Shigella	5/5

Association of HLA antigens with abnormalities of immune response and resistance to viral disease

Long before there was any knowledge of HLA antigen and disease associations, there were indications from studies of HLA antigen frequencies in different racial populations that HLA may have been of importance in terms of evolutionary selection (Bodmer 1972). It was observed that amongst unrelated individuals certain pairs of A and B-locus antigens occurred more frequently together on the same chromosome (or haplotype) than would have been expected given the frequency of either antigen in the population under study. An example of this phenomena, known as linkage disequilibrium, is the association of A1 and B8. More than 50% of European Caucasians who were positive for A1 were also B8 positive, and more than 65% of B8-positive individuals were also A1 positive. When methods for detecting D-locus antigens became available, the majority of A1/B8-positive individuals were also found to be DW3 positive. In contrast, in Asian Indians A1-positive individuals were never B8 positive, but more commonly than expected B17, while in Africa, Zambian Negro B8-positive individuals were rarely A1 positive, but more frequently than expected AW30. These studies suggest that there may have been selective pressure in each particular area, which has encouraged survival of individuals with the genes within the specifically selected haplotype.

When HLA and disease studies first started, it was surprising to discover that the haplotype most frequently associated with disease in Caucasians was also the most frequent in normal populations. However, all the diseases associated with A1/B8, and DW3 when it was tested for, were chronic diseases which had negligible incidence at the time that infectious disease was a dominant cause of death in Europeans (*see* Table 5) (Ryder & Svejgaard 1976). All of them were found to be associated with abnormalities of immune response and were in the main autoimmune diseases. Studies of immune response of A1/B8-positive individuals showed stronger responsiveness in mixed lymphocyte culture, higher antibody titres against bacterial antigens, higher frequency of autoantibodies and more likelihood of kidney graft rejection, than A1/B8-negative controls (*see* Oliver 1977). The importance of this enhanced responsiveness in recovery from viral disease was demonstrated by Jungers *et al.* (1976) in a study of patients on chronic haemodialysis who developed hepatitis B antigenaemia. There was a significantly higher incidence of A1/B8 in patients who cleared the virus from the blood within three months than in those who became carriers of hepatitis B antigen.

HLA and malignant disease

The demonstration (Lilly *et al.* 1964) that genes within the complex coding for the H2 antigens (murine equivalent of the HLA system) influenced survival of mice with Gross virus-induced leukaemia, provided the impetus for study of HLA antigens in human disease. Though malignant diseases, specifically Hodgkin's disease and acute leukaemia, were the first diseases studied, none of the associations reported in malignant disease have approached the level of significance found in the nonmalignant disease studies. Nevertheless, when the large numbers of series reported in the literature are analysed together, significant associations such as that of Hodgkin's disease with B8 (Table 5) have emerged. This slight excess of B8 is consistently found in most of the series reported in the literature though in only a few was the difference significant (Ryder & Svejgaard 1976). Similar weak associations of A2 with acute lymphoblastic leukaemia and A10 with breast carcinoma have also been reported (Ryder & Svejgaard 1976). Most of these reports did not take account of the clinical stage of the patient at the time that HLA typing was performed. An important insight into the nature of the HLA association with malignant disease came when Falk & Osoba (1971) reported that A1/B8 had a significantly higher incidence in patients with Hodgkin's disease tested more than 5 years after diagnosis than in those patients who had been treated for less than one year. It was postulated that this might be due to HLA associated resistance factors which influenced survival. There have been several reports since, associating certain HLA antigens with survival of patients with other types of malignant disease (Rogentine *et al.* 1973, Lawler *et al.* 1974, Dellon *et al.* 1975, Oliver *et al.* 1977). Acute myeloid leukaemia was the only disease other than Hodgkin's disease

Table 5. HLA-B8 associated diseases.
(Reproduced from Oliver 1977, by kind permission)

Disease	Antigen frequency
Coeliac disease	83%
Dermatitis herpetiformis	85%
Myasthenia gravis: females	80%
males	30%
Chronic active hepatitis	58%
Juvenile diabetes	36%
Addisonian adrenalitis	62%
Graves' disease	44%
Hodgkin's disease	29%
Control	18–25%

Table 6. HLA and survival in acute myelogenous leukaemia

	No. of cases	Survival at twelve months (per cent)
A2+/B12+	36	58
A2+/B12−	37	35
A2−/B12+	17	41
A1+/B8+	26	60
A1+/B8−	19	26
A1−/B8+	9	33
A1−;A2−/B8−;B12−	18	39

where resistance was associated with the A1/B8 haplotype (Table 6). The A1/B8 haplotype is the one which has been associated with enhanced immunoresponsiveness in the studies of HLA antigens in nonmalignant disease, and it is possible that the same mechanism is responsible for its influence on survival in Hodgkin's disease and acute myeloid leukaemia.

The possibility that there may be other genes within the HLA region which determine susceptibility to malignant disease has been suggested by Lynch *et al.* (1975) who found that in a family with a very high incidence of malignant disease the haplotype A2/B12 showed a strong association with this occurrence. Our own findings in the families of patients with Hodgkin's disease and acute myeloid leukaemia, where a second malignancy has occurred, do not support this, suggesting that it is only relevant in families where the susceptibility gene is well expressed.

Summary

In nonmalignant disease, there have been two mechanisms implicated in the association of HLA antigens with disease. In ankylosing spondylitis, evidence is accumulating for cross tolerance between a bacterial antigen and the HLA-B27 antigen; while in the autoimmune diseases, the involvement of an abnormal immune response gene, associated with A1/B8 haplotype, is strongly suspected. The same haplotype has also been associated with recovery from hepatitis B infection and survival of patients with Hodgkin's disease and acute myeloid leukaemia. At present, there are no techniques to study directly immune response genes in man and so these observations are still strictly academic. However, with increasing interest in the use of immunotherapy in cancer and the demonstration in mice that the major histocompatibility system may be the site of action of soluble mediators of immune memory, understanding the mechanisms of action of the HLA associated resistance factors may enable a more rational approach to immunotherapy in man.

References

Aho K, Ahnonen P, Lassus A, Siever S K & Tilikainen A (1973) *Lancet* ii, 157
Bach F H & Van Rood J J (1976) *New England Journal of Medicine* **295**, 806
Bodmer W F (1972) *Nature* **237**, 139
Calin A & Fries J F (1975) *New England Journal of Medicine* **293**, 835
Dellon A, Rogentine G N & Chretian P B (1975) *Journal of the National Cancer Institute* **54**, 1238
Ebringer A, Cowling P, Ngwa Sun N, James D C O & Ebringer R W (1976) In: Abstract of International Congress of HLA and Disease. Eds. J Dausset and A Svejgaard. Publication INSRM, Paris; p 27
Falk J & Osoba D (1971) *Lancet* ii, 1118
Festenstein H, Sachs J, Paris A M I, Pegrum D & Moorhead J F (1976) *Lancet* i, 157
Francke U & Pellegrino M A (1977) *Proceedings of the National Academy of Sciences of the USA* **74**, 1147
Jungers P, Descamps B, Naret C, Zingraff J & Bach J F (1976) In: Abstract of International Congress of HLA and Disease. Eds. J Dausset and A Svejgaard. Publications INSRM, Paris; p 160
Katz D H & Benacerraf B (1975) *Transplantation Reviews* **22**, 175
Lawler S D, Klouda P T, Smith P G, Till M M & Hardisty R M (1974) *British Medical Journal* i, 547
Lilly F, Boyse E A & Old L J (1964) *Lancet* ii, 1207
Lynch H T, Thomas R J & Tersaki P I (1975) *Cancer* **36**, 1315
Oliver R T D (1977) *British Journal of Hospital Medicine* **18**, 449
Oliver R T D, Pillai A, Klouda P T & Lawler S D (1977) *Cancer* **39**, 2337
Oliver R T D, Sachs J A, Festenstein H, Pegrum J D & Moorhead J F (1972) *Lancet* ii, 1381
Rogentine G N, Tepani R J, Yankee R A & Henderson E S (1973) *Tissue Antigens* **3**, 470
Ryder L P & Svejgaard A (1976) Report from HLA and Disease Registry. Compactes, Copenhagen; p 4
Singal D P, Mickey M R & Terasaki P I (1969) *Transplantation* **7**, 246