Histocompatibility: An Historical Perspective

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For the past two decades intensive study of the major histocompatibility complex (MHC) has involved almost every branch of clinical medicine. This gene complex (known as the HLA system) is situated on the human sixth chromosome and governs the acceptance or rejection of foreign tissue. Furthermore, products of this system regulate the immune response and this may explain the predisposition of those of a given HLA tissue type to certain diseases.

The development of this field began in the latter half of the last century, stimulated by interest in the evolution of man. At that time biochemical methods were not sufficiently sophisticated to study protein structure in detail and serological techniques led to the discovery that individual proteins in various animals are different and unique to each species.

In 1875, Landois[1] reported that animals generally died when they were transfused with blood from an animal of a different species. Later Bordet[2] showed that when red cells from another species were injected into a rabbit the cells lysed, whereas red cells from another rabbit did not. In a study of over 500 species Nuttall[3] showed that the intensity of the lytic reaction between the red cells of one animal and the serum of another was related to the distance that the animals were apart on the phylogenetic scale.

Landois also noted that even within a single species a recipient's erythrocytes could be haemolysed by serum from certain blood donors. This observation was not developed further until agglutination reactions were used by Landsteiner to define the human 'ABO' red blood group system[4]. Later in his Nobel lecture, Landsteiner[5] suggested that an analogous system might exist for tissue cells—compatibility within this system might govern the acceptance or rejection of a tissue transplant. He also predicted that serological reactions might be employed to determine these antigens.

Early Animal Studies

Early work on transplantation showed that mice were resistant or susceptible to inoculated tumours according to their strain[6,7] but that this predisposition was not related to any visibly recognisable factor such as colour of eyes or coat[8].

The first attempt at genetic analysis showed that the closer the genetic relationship between the donor of tumour cells and the recipient, the more likely the tumour was to survive[9]. When well established inbred strains of mice became available for study around 1930, more accurate statistical analysis suggested that if simple Mendelian genetics were applied, between four and 19 genes were responsible for the acceptance or rejection of foreign tissue[10,11]. Sir Peter Gorer[12,13], showed that one genetic locus was particularly important. Unlike the human, the mouse possesses antigens coded for by this locus on its red cells. Gorer, therefore, immunised rabbits with red cells from inbred mouse strains in an attempt to define blood groups and identified three red cell surface antigens (antigens I, II and III). By backcross experiments he showed that the presence or absence of antigen II on murine red cells determined whether a tumour was accepted or rejected.

Further work on transplanted tumours showed that while an animal which had rejected one tumour rejected a second tumour from the same donor more rapidly, a transplanted tumour from an unrelated donor was unaffected[14]. Sir Peter Medawar[15] confirmed this work by showing that the second of two successive skin grafts from the same donor was rejected more rapidly than the first. Taken in conjunction with Gorer's work, these results suggested an immunological basis for graft rejection which was under the control of specific genes, coding for antigens such as antigen II. These genes were named 'histocompatibility' genes[16]. The prefix 'histo' was used, as Snell[16] felt that the same genes which governed susceptibility to transplanted tumours also controlled the ability to accept or reject transplanted normal tissue. The gene locus coding for antigen II was named 'Histocompatibility-2' or 'H-2' and was shown to be carried on the seventeenth mouse chromosome[17].

The existence of other histocompatibility loci, as predicted by earlier workers, was confirmed, but their effect on tissue transplantation was shown to be minimal[18]. The H-2 system was subsequently found to comprise several genes[19], each of which has many possible alleles[20] and became known as the mouse major histocompatibility complex (MHC).

The genes of the MHC coded for alloantigens expressed on tissue cells and they could be detected by a complement dependent lymphocytotoxicity assay[21]. In this test lymphocytes carrying a given alloantigen on their surface were killed, in the presence of complement, by serum containing alloantibody to that antigen. A modification of this technique is currently used in human tissue typing. Thus, as predicted by Landsteiner[5], a serological test is currently used to define the antigens of the major histocompatibility complexes of all species.

The Human Major Histocompatibility Complex: HLA

The human major histocompatibility complex was defined in a different way, as human red blood cells do not express histocompatibility antigens. Research in this field developed from a search in the sera of leucopenic patients for antibodies directed against white blood cells. Jean Dausset[22] in 1952 was the first to find such antibodies in the serum of a patient with agranulocytosis. The patient's serum agglutinated leucocytes from normal individuals and those from patients with chronic myeloid and chronic lymphatic leukaemia. Similar activity was found in sera from individuals who had received multiple blood transfusions[23]. In a further study Dausset[24] showed that leucoagglutinins were almost exclusively present in the sera of leucopenic patients who had received blood transfusions from several donors. This, taken in conjunction with the finding that sera from leucopenic patients had no activity against syngeneic cells[25], indicated that these leucoagglutinins were isoantibodies which had developed as a result of 'immunisation' by blood transfusion rather than autoantibodies occurring as a feature of the underlying leucopenic disease.

The first leucocyte antigen was defined after the sera from patients who had received multiple blood transfusions were tested against leucocytes from a panel of normal donors[26]. Certain sera showed activity against leucocytes of the same 11 panel members but not against a further three. These sera, therefore, contained antibody to a common antigen present in the 11 'positive' panel cells but absent from the three 'negative' panel cells. This first human histocompatibility antigen, present in 60 per cent of the population, is now known as HLA-A2. All the HLA antigens determined since have been defined by this method of testing sera against lymphocyte panels.

A further advance was made when leucocyte antibodies were found in sera from parous women[27,28]. These sera agglutinated the leucocytes of fewer cell panel members than the sera from transfused patients. They were, therefore, more useful in determining whether leucocyte surface antigens segregated into genetic groups analogous to the ABO red blood cell groups. By computer analyses of these serological reactions two independent allelic antigen systems emerged and they were named 'Group 4'[29] and 'LA'[30].

Over the next few years work in this field accelerated and many antigens in the new systems were defined. This expansion was largely brought about by the establishment in 1964, by Dr D. B. Amos, of International Histocompatibility Workshops which resulted in the exchange of sera between a large number of participating laboratories and analysis of the extensive data produced.

As the number of new antigen specificities increased, the need for agreement on nomenclature became apparent. In 1967 the World Health Organisation set up a Nomenclature Committee which named the human major histocompatibility complex HLA (H for human, L for leucocyte and A for the first system defined in man). Payne's 'LA' system[29] was named HLA-A and van Rood's 'Group 4' system[30] HLA-B. In 1971 a third locus was suggested[31] which has subsequently been named HLA-C.

The HLA-A, B and C gene loci code for antigens expressed on most of the body's nucleated cells. As the genes are co-dominant, each cell expresses on its surface two antigens from each series, i.e. one inherited from each parent. The combination of genes that codes for antigens inherited from one parent is known as a haplotype. This term, derived from haploid phenotype, was introduced by Cepellini in 1967[32]. The HLA gene complex is markedly polymorphic; at the 1984 Histocompatibility Workshop 23 alleles were recognised at the A locus, 49 at B, and eight at C[33].

The location of the HLA gene complex was defined in 1974[34]. In six members of a large family the centromere of one of the sixth chromosomes occurred in an abnormal position (pericentric inversion). These six family members all shared the same HLA haplotype (HLA-A2, B12) whereas this haplotype was not present in any of the seven family members possessing a normal sixth chromosome. The strong linkage of the HLA haplotype with this chromosomal abnormality implied that the HLA gene complex was situated on the human sixth chromosome.

Immune Response Genes and the HLA-D Locus

A fourth rather different locus was defined in a more complex manner. In 1961 it was shown inadvertently that when lymphocytes from two patients were incubated together stimulation occurred[35], i.e. they underwent DNA and RNA synthesis, blast cell formation and cell division. This mixed lymphocyte reaction (MLR) was thought to be the *in vitro* homologue of allograft rejection. In the mouse the MLR was indeed shown to be governed by the major histocompatibility complex. Strain pairs, identical except for a difference at H-2, displayed strong stimulation whereas those H-2 identical but with multiple non-H-2 differences gave weak stimulation[36]. Strong stimulation between donor and recipient lymphocytes in the MLR was shown to correlate with poor murine heart transplant[37] and skin graft[38] survival. Using animal strains differing at only a certain segment of H-2, MLR control was localised to one end of the H-2 segment[39].

In initially unrelated studies it was shown that the genes controlling the immune response to simple synthetic polypeptide antigens (Ir genes) were linked to H-2[40]. More recently the Ir genes have assumed increasing immunological importance and have been shown to play a major part in regulating cellular immunity to virus infected cells[41] and the response to tumour inducing viruses[42]. Around this time it was shown that these Ir genes also controlled the activity of the mixed lymphocyte reactions[43]. The region of the H-2 complex in which the Ir genes were located was named the I-region[44] and, unlike the other regions of the mouse H-2 complex, it has not been shown to code for any serologically defined antigens. However, David[45] reported the development of cytotoxic antibodies in one strain of mouse directed towards lymphocytes of a strain differing only at the I-region. These antibodies were cytotoxic to only 35 or 40

per cent of splenic lymphocyte population and were shown to be directed against antigens present mainly on B lymphocytes[38]. The antigens were named I-region-associated (Ia) antigens[46] and were shown in the mouse[47] and rat[48] to be extremely strong transplantation antigens.

Investigation of the human equivalents of the I-region followed a similar path. Bach and Amos[49] observed that MLRs between the lymphocytes of HLA identical siblings were negative and thus showed that the mixed lymphocyte reaction was governed by the MHC in the human. Yunis and Amos[50] showed, however, that MLRs between unrelated subjects who shared the same four HLA-A and B antigens and thus appeared HLA identical, were commonly positive. They therefore postulated that the MLR was controlled by a gene separate from the known HLA-A and B loci. This was confirmed by similar studies on several recombinant families and the MLR was shown to map outside the previously recognised HLA region near the B locus[51]. It was named the D locus at the Sixth International Histocompatibility Workshop[52]. D locus typing was performed by incubating test cells with cells homozygous for known D locus antigens; a negative response showed that the test cells shared the D antigen carried by these homozygous typing cells.

Cepellini *et al.* [53] first observed that some HLA-A and B typing sera could inhibit the MLR. This activity could not be absorbed out by platelets[54], which carry HLA-A, B and C antigens, suggesting that other antigens were present. A higher frequency of reactions was also noted against cultured lymphoblastoid cell lines[55] and cells from patients with chronic lymphatic leukaemia, most of whose lymphocytes are B lymphocytes, and it was suggested that such antigens were probably the human equivalent of the mouse Ia antigens[56] and were expressed solely on B lymphocytes. Over 100 sera with this presumed anti-Ia activity but without anti-HLA-A or B activity were tested internationally against a variety of B lymphocytes. The results defined eight main specificities at this new serologically defined locus which was named HLA-DR (D-related)[57].

HLA and Organ Transplantation

The evidence that HLA is the particular gene system which governs acceptance or rejection of a human tissue allograft came from experiments performed in the 1960s. Intrafamilial skin grafts survived longer if donor and recipient were HLA identical than if they shared only one haplotype. The shortest skin graft survival occurred when donor and recipient had no haplotype in common[58].

Successful renal transplantation was first performed in 1954 when a kidney from one identical twin was transplanted to the other[59]. Over the next 10 years several transplants between HLA identical siblings were carried out and very few failures were reported[60,61]. An analysis of the role of HLA in renal transplantation was not possible until sufficient transplants had been performed between different family members. Large studies have now confirmed that a significant graded improvement in allograft survival occurs when groups of donors and recipients, matched for 0, 1 and 2 haplotypes, are compared[62]. Family studies thus established the dominant role of the HLA system in clinical transplantation.

HLA and Cadaver Donor Renal Transplantation

The results from the numerically more important group of cadaver donor transplants are, unfortunately, more difficult to interpret. Early studies showed that those recipients well matched with the donor for HLA-A and B antigens had significantly superior graft survival to those poorly matched[63,64]. Dausset[65] was the first to study sufficient patients to show a progressive improvement in transplant survival as the number of HLA-A and B antigens common to donor and recipient increased from none to four. Thereafter Paul Terasaki's laboratory in Los Angeles analysed at intervals transplant survival data from most of North America and also on certain occasions from Europe and Australasia. Its analyses of several thousand transplants[66,67] showed an overall effect of HLA-A and B matching on graft survival but significant differences between the matching subgroups were not always demonstrated.

Similar results were found in other multicentre studies[68,69]. In a recent analysis of worldwide data[70], however, neither a graded improvement nor an overall statistically significant correlation between the degree of HLA-A and B matching and allograft survival was found.

These reports have been criticised because they may conceal differences in patient selection and therapeutic policies practised by the individual transplant centres submitting data. When single centres analysed their own data a beneficial effect of matching for HLA-A and B antigens was generally shown[71-74]. In several cases, however, the small numbers dictated that the data be analysed in groups, and statistical significance was not always reached.

Thus, the data on HLA-A and B matching in cadaver donor renal transplantation appear sufficiently strong to support the concept that the HLA system is the human major histocompatibility complex. However, the lack of a consistent response between the degree of HLA-A and B matching and graft survival diminishes the usefulness of such matching as a means of selecting a kidney donor for a given individual recipient.

The MLR takes six days to perform and therefore HLA-D typing and MLR between donor and recipient lymphocytes are not of value as predictive tests in a cadaver donor transplantation. It is practical, however, to perform HLA-DR typing prior to a cadaver donor renal transplant although the preparation of the necessary B lymphocyte suspension is time-consuming. The gene frequencies for the eight well characterised HLA-DR antigens account for 80 per cent of the Caucasian population[58]. The HLA-DR locus is thus less polymorphic than the HLA-A and B loci, and consequently if matching for HLA-DR antigens also could be shown to improve graft survival it would become easier to find a well-matched donor for a given recipient.

Several studies have now shown that HLA-DR matching has a strong influence on graft outcome[75-79], although one group has failed to confirm this. The beneficial effect was shown by one group[77] to be independent of any influence of HLA-A and B matching and by another[81] to occur regardless of whether the recipients had received blood transfusions prior to transplantation or not. Traditionally most transplant centres selected recipients for transplantation on the basis of HLA-A and B matching. Recently, however, the United Kingdom Transplant Service has altered its policy so that donor kidneys may be requested on the basis of the HLA-DR antigens shared with a prospective recipient.

Therefore, although the results of living related transplantation have established the HLA complex as the major influence on allograft survival, evidence from the larger group of cadaver transplants is less convincing. This implies that other areas of the HLA chromosome remain to be defined. At the 1984 Histocompatibility Workshop[33] recent data on the D region were critically evaluated and it now appears that several sub-divisions exist, analogous to those of the I-region in the mouse. In addition to DR the Workshop named three further subregions DP, DQ and DZ. Their role in transplantation has not yet been evaluated.

Understanding of the human MHC has developed rapidly in the last 20 years and the main stimulus to this has been the need to improve matching of donor and recipient for renal transplantation. More detailed evaluation of the HLA-D region may not only be of further benefit in the selection of transplant recipients but may also establish that this region codes for genes controlling the human immune response. If this can be confirmed, studies of the HLA system and its products may help to determine the pathophysiological basis of many disease states, including immunologically related conditions and malignancy.

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Quinine, Willow Bark and Thomas Bewick

Peruvian bark was brought to Spain in 1636 and its ingredient, quinine, was later named after the fourth Countess of Cinchona, wife of the Viceroy of Peru. The fame of the medicinal properties of the powder derived from the bark spread rapidly, but its price was so high that worthless astringent substitutes became common[1]. Pomet comments that when introduced to France it was 'in such vogue as to be sold weight for weight at the price of gold'[2]. In England it was not included in the *Pharmacopoeia Londinensis* until 1677; at a time of intense anti-Catholic feelings its popular name of Jesuits' powder had inevitably aroused suspicion. The common claim, however, that Cromwell refused treatment with the 'tainted source of the Popish powder'[3], is now thought to have no foundation. After doubts and setbacks it became clearer that quinine was a specific drug for the true 'marsh agues or intermittents', rather than for all febrile illnesses.

During the eighteenth century the imported bark remained costly, and an indigenous substitute was sought. In 1763, the Reverend Edmund Stone presented 'An Account of the Success of the Bark of the Willow in the Cure of Agues', to the Royal Society[4]. He described how he 'accidentally tasted it and was surprised by its extraordinary bitterness, which raised the suspicion of its having the properties of Peruvian bark. As this tree delights in a moist and wet soil where agues abound, the general maxim that natural maladies carry their cures with them was so very appropriate that I could not help applying it; and that this was the intention of Providence I must own had some little weight with me'. Stone had special success in treating rheumatic fever. Many years later salicin was isolated from willow bark, and eventually aspirin was synthesised.

There are many varieties of willow. In 1792 'the modest and candid Mr James, surgeon of Hoddesdon'[5], introduced *Salix latifolia* into practice, and 11 years later another provincial surgeon, Wilkinson from Sunderland, published a treatise on the broad-leafed willow[6]. As a surgeon he explained that his interest was included in 'that branch of the healing art termed medical surgery'.

Lest there should be any doubt about his subject he employed a local engraver in Newcastle-upon-Tyne to illustrate a branch of the tree 'sufficiently accurate to enable anyone to ascertain this species from others of the same genus'. The engraving was hand-coloured and forms the beautiful frontispiece of this otherwise modest book. The engraver was Thomas Bewick. Bewick's ledger records that the work was undertaken during the week ending 28th August 1802, and was charged at 15 shillings[7].

The engravings of Thomas Bewick and his workshop apprentices in Newcastle changed the face of illustration, bringing a new richness and subtlety to the art. His delicate wood-engravings, worked on the end-grain of box-wood, are best known from illustrations in his books on quadrupeds, British birds, and the fables of Aesop. But he executed more work on copper and silver than on wood, mostly for commercial purposes in making bank notes and bills. The frontispiece of the broad-leafed willow was made from a metal plate and then hand-coloured.

Though Bewick had doctors as friends and thought

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