Significance of human leukocyte antigens in immunobiology of renal transplantation

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Most of the time, transplantation rejection is immunologically mediated. Both T cells and circulating antibodies are induced against allografts and xenografts. Antibodies produced are responsible for hyperacute rejections, T cells are mainly responsible for rejection of most other tissues. The most important transplantation antigens, which cause rapid rejection of the allograft, are found on cell membranes and are encoded by genes in the major histocompatibility complex (MHC) which is known as HLA in humans and H-2 in mice. HLA helps in discriminating between self and non-self. The approaches to enhance graft survival are gaining acceptance and wide use in human tissue and organ transplantation. Various mechanisms involved in allograft rejection are discussed in detail in this review.

THE clinical application of our knowledge of the immune barriers to transplantation has advanced allo-organ replacement therapy to the level of routine practice. The success of an organ transplant is the function of several variables. However, the major determinant of acceptance or rejection of a technically perfect graft is the magnitude of the immunologically mediated responses against graft. The delineation and application of recent discoveries in cell co-stimulatory events, antigen presentation and differential T lymphocytes are opening pathways towards the development of tolerogenic protocols for clinical transplantation. The genetic differences between recipient and donor elicit immune response that could be prevented by genetic compatibility, which is determined on the basis of human leukocyte antigens (HLA). These antigens play an important role in immune discrimination between self and non-self (foreign) and effectively promote detection and eradication of foreign molecules. Similarly, immune mechanisms are associated with the recognition of alloantigens in allogenic transplantation. In this review an attempt has been made to deal with the role of major histocompatibility complex (MHC) antigens in renal transplantation.

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Immunogenetics of the HLA system

Classical HLA antigens

The classical MHC or HLA molecules are encoded by two highly polymorphic gene families located in a 3600-kb region of chromosome 6p (6p21.3) (Figure 1). HLA molecules are polymorphic in nature. They are membrane-bound glycoproteins that bind processed

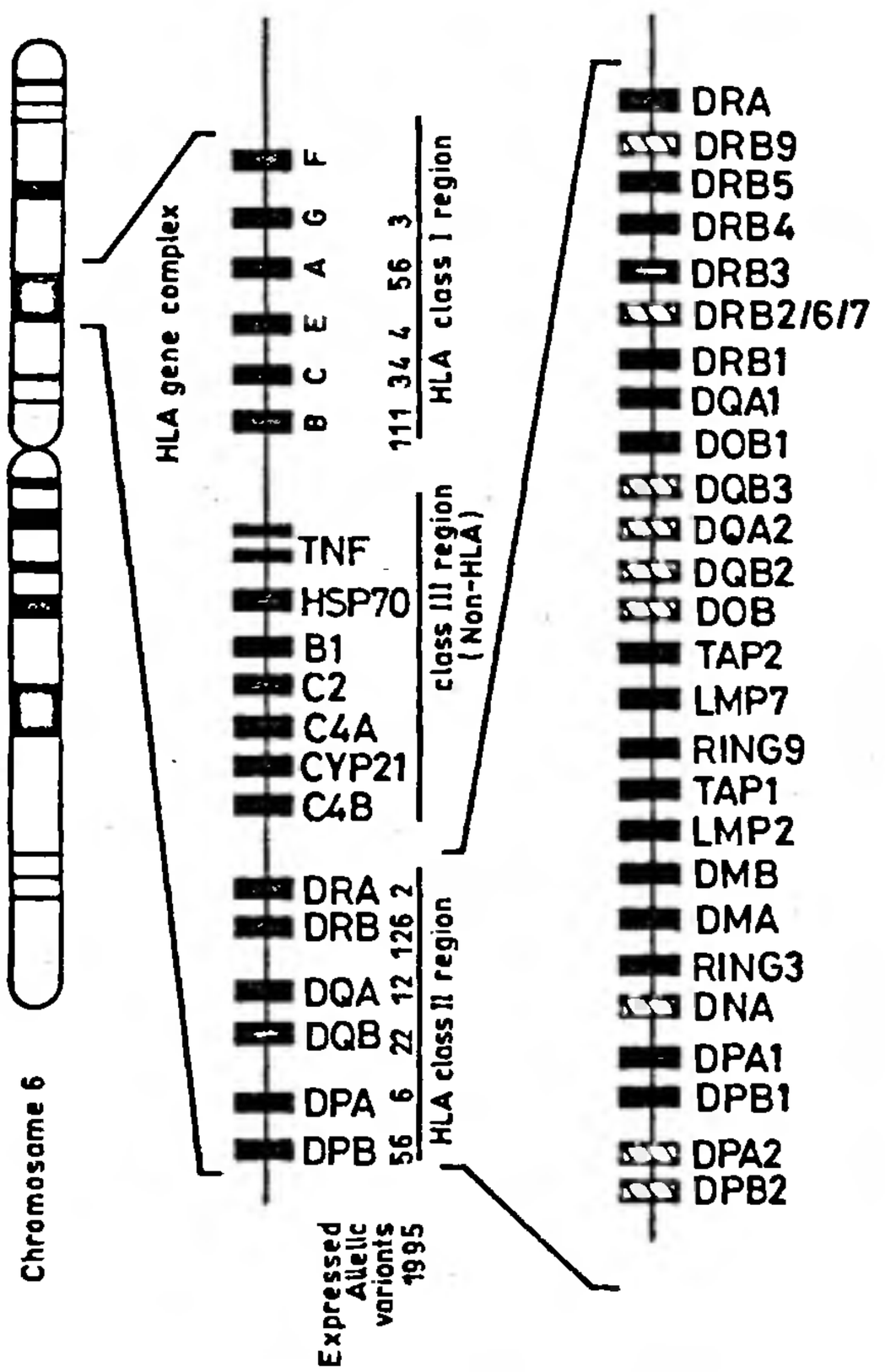

Figure 1. *HLA* gene complex on the short arm of chromosomes 6. Loci encompassing gene whose products are known to be expressed are marked with filled squares.

antigenic peptides and present them to T cells. The HLA class I A, B and C molecules are composed of an MHC-encoded heavy chain (MW 45 kD), non-covalently associated with a nonpolymorphic polypeptide, β_2-microglobulin (MW 12 kD), encoded on chromosome 15 (Figure 2). There are now known to be 56 different expressed *HLA-A* alleles, 111 *HLA-B* alleles and 34 *HLA-C* alleles, excluding silent substitutions and null alleles[1]. These class I antigens are expressed on all nucleated cells (except foetal trophoblast cells) and platelets and function to present peptides of largely endogenous (viral) origin to CD8+ T cells, which mainly function as the cytotoxic cells. The bound peptides are highly circumscribed in length, usually 8–9 amino acids, and are held in a peptide-binding groove. X-ray crystallography has shown that this groove has allele-specific conformation[2]. The polymorphic residues that distinguish between the different alleles of a particular HLA class I locus are found, mainly within the peptide-binding groove[3].

In contrast to class I molecules, HLA class II molecules, comprising three main subclasses – DR, DQ and DP – are found on a more restricted range of cell types, including B cells, activated T cells, the monocyte/macrophage lineage and are also interferon-γ inducible. An expressed class II molecule consists of a α chain (MW 31–34 kD) encoded by an *A* gene, noncovalently associated with a β chain (MW 26–29 kD), encoded by a *B* gene (Figure 2). Each DR, DQ or DP subregion consists of at least one expressed *A* and one expressed *B* gene. Both *A* and *B* genes may be polymorphic, but most polymorphism resides in the *B* genes. There are now known to be 2 *DRA*, 126 *DRB*, 12 *DQA*, 22 *DQB*, 6 *DPA* and 56 different expressed *DPB* alleles excluding silent substitutions[1]. Both α and β chains combine to form a peptide-binding groove shown by X-ray crystallography to be very similar to the class I groove[4]. However, class II molecules present peptides of largely exogenous origin to CD4+ T cells of largely 'helper' phenotype. These bound peptides are generally longer and more variable in length than peptides bound to class I molecules (i.e. 14–21 amino acids), due to the more open ends of the peptide-binding groove.

Both classes of HLA molecule function to present self-antigens in the thymus and so induce tolerance, while foreign antigens are presented in the context of self-HLA molecules in the periphery, invoking an immune response.

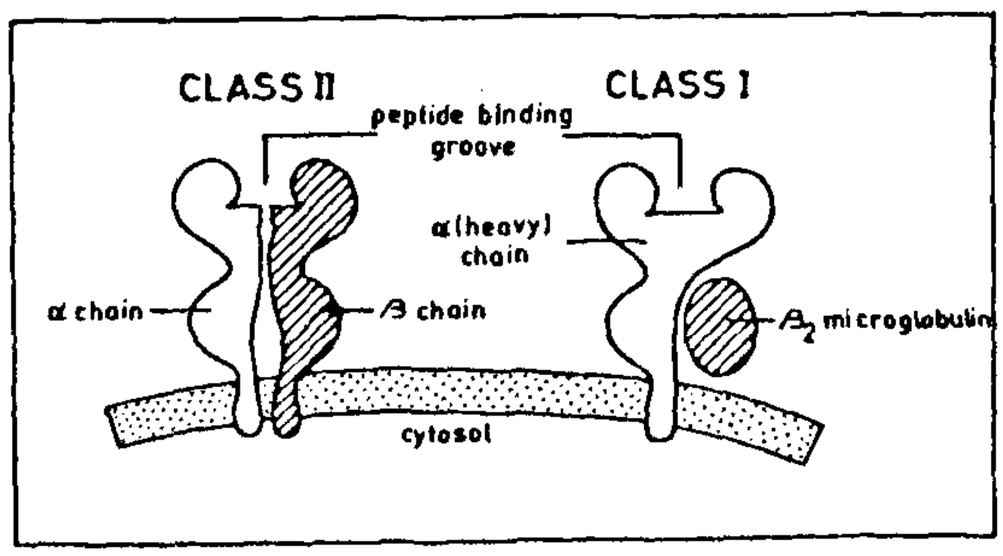

Figure 2. Basic structure of HLA class I and class II molecules.

Non-classical HLA and non-HLA genes in the HLA class I/II regions

The application of molecular techniques like cloning, sequencing and gene mapping has also revealed a number of additional HLA and non-HLA genes in the class I/II regions. In the class I region, there are known to be 17 'nonclassical' genes or gene fragments, although only 3 of these – *HLA-E*, *HLA-F* and *HLA-G* – are known to be transcribed[5]. Little is yet known of the possible function of *HLA-E* and *HLA-F*, more is known about *HLA-G*, which is closely homologous to other class I gene sequences and was thought to show little polymorphism, although this may not be so[6]. *HLA-G* is primarily, although not exclusively, expressed on foetal cytotrophoblast cells. These are the only foetally derived cells in contact with maternal cells and lack expression of classical class I genes. In consequence, it is thought that the *HLA-G* gene product may function as a foetal antigen presenting/recognition molecule and hence in the absence of classical, highly polymorphic class I molecules, may permit maternal tolerance of the placenta[7].

A series of gene mapping studies carried out independently in the laboratories of John Trowsdale (ICRF, London) and Thomas Spies (Harvard), and similar studies of the mouse MHC by John Monaco (Virginia) have revealed a series of novel genes in the class II region, located between the DQ and DP subregions[8–12]. Gene sequencing, deletion, mutant and transfection studies have now demonstrated a role for many of these genes in pathways of antigen processing and presentation. While HLA class I and II molecules are synthesized and assembled in the endoplasmic reticulum and peptides binding to class I molecules also occur here, it has been a conundrum as to how these peptides are generated from proteins present in the cytosol and are transported into the endoplasmic reticulum. The proteasome complex consisting of at least 16 polypeptides (each of MW 15–30 kDa), catalyses the degradation of the vast majority of cytosolic proteins and generate most peptides presented by class I molecules[13]. Two subunits of the proteasome are encoded by two genes located between DQ and DP – *LMP2* and *LMP7* (LMP, low molecular-mass polypeptide) (Figure 1). Deletion of these *LMP2*/*LMP7* genes alters the nature of the peptides generated by the proteasome, so that they no

longer have optimal characteristics for class I binding[14]. Two additional genes, *TAP*1 and *TAP*2 (TAP, transporter of antigen peptides) also located in the DQ–DP interval (Figure 1) encode separate chains of a trans-endoplasmic reticulum membrane heterodimer which functions as a peptide pump, transporting peptides generated by the proteasome into the endoplasmic reticulum. The *TAP* genes show some polymorphism and this may influence the nature of the peptides transported, with results suggesting that the TAP transporter molecule preselects peptides according to sequence and length in a manner compatible with subsequent presentation by class I molecules[15].

The DQ–DP region is still richer in what were once termed RING (really interesting new genes) by John Trowsdale's group. Two further genes – *DMA* and *DMB* – map to this region and have sequences intermediate between those of classical class I and II genes, but may encode a class II-like heterodimer with a modified (more rigid) peptide-binding groove[16]. Recent transfection experiments in mutant B lymphoblastoid cell lines suggest that *HLA-DM* is expressed and appears to function at an intracellular site to promote peptide binding to classical class II molecules. Peptide binding to class II molecules in the endoplasmic reticulum is prevented by co-assembly of the α and β chains with a third chain, the so-called invariant chain (Ii, MW 35 kD, encoded by a gene on chromosome 5). The Ii chain also acts as an 'address label' and directs the class II-like complex to an intracellular endosomal compartment[17,18]. It is currently thought that *HLA-DM* acts as a 'sink' for the removal of Ii chain-derived 'CLIP' (class II-associated invariant chain peptides) in this compartment, hence freeing classical class II molecules for peptide binding.

Taken together, these discoveries have overthrown earlier concepts of the MHC class I and II regions as solely containing genes encoding for molecules which present antigenic peptides to T cells. Rather, the current view is a genetic region encoding many different types of molecules collectively involved in pathways of antigen processing and presentation to helper and cytotoxic T cells. All of these gene products may have a role in immunologically-mediated immune rejections.

Allograft rejection

Allograft rejection remains the single largest impediment to success in the field of transplantation. Graft rejection is different from other immune responses as two different sets of antigen-presenting cells are involved, one from the donor and the other from the recipient. The exact mechanism by which allograft rejection can occur is still not fully understood because of the complex immune mechanisms involved in the graft rejection. Rejection episodes lead to adverse immune response and affect the allograft survival. The immune response following an allograft is primarily against MHC molecules of the donor which are different from those of the recipient. About 8–10% of the normal adult T cell repertoire is capable of recognizing and responding to the foreign MHC molecules. This response is not to the host's benefit but occurs due to cross reactivity of some of the host T cells whose TCR were selected to recognize MHC plus foreign peptide during thymic education and recognize foreign MHC antigens in the context of self MHC and get activated. T cells recognize MHC antigens in a transplantation by two different pathways, i.e. direct pathway and indirect pathway[19]. The three evidences that support the direct recognition pathway in allograft rejection are: (i) Stimulation is very high in primary allogenic mixed lymphocyte culture (MLR); (ii) The depletion of donor antigen presenting cells (APCs) can sometimes prolong the allograft survival; (iii) Donor MHC are more important than minor antigens in causing graft rejection. Hornick *et al.*[20] have shown in cardiac transplant rejection that two populations of T cells with direct allospecificity are activated after recognition of intact MHC alloantigens displayed at the surface of the donor passenger, i.e. leukocytes carried within the graft. The direct recognition pathway involves T cells that recognize intact allogenic MHC/peptide complexes on the surface of donor target cells. This form of recognition does not require processing and presentation by host APCs. Because the frequency of T cells that are able to recognize alloantigen directly is very high, even in non-immunized responders it is believed that this process reflects T cells' recognition of allogenic MHC/peptide complex via molecular mimicry with other antigenic structures[21]. Although the majority of T cells infiltrating the graft during early acute rejection exhibit direct recognition ability, it is unlikely that these cells can mediate late or chronic rejection because their stimulation requires the presence of passenger APC of the donor in the graft. The absence of costimulatory molecules on the surface of the graft endothelial and parenchymal cells renders such putative targets more likely to induce anergy rather than stimulate the recipient's T lymphocytes[22].

In contrast to the direct recognition pathway, T cells that react against peptides derived from the processing of allogenic MHC and proteins mediates indirect alloimmune responses by host APCs[23]. Peptides resulting from the proteolysis of allogenic MHC molecules bind to MHC-class II antigens of host APC and trigger T cell alloimmune responses. This form of alloreactivity is restricted by host HLA-DR antigens and is carried out by an oligoclonal population of T cells, which are capable of recognizing the dominant epitope of the allogenic MHC molecule. Because the stimulatory peptide can be generated continuously from soluble MHC alloantigens released from the graft and processed by host

APC, the indirect pathway may be responsible both for initiation and perpetuation of allograft rejection.

The exact mechanism by which T cells destroy the graft is not yet clear. Both CD4 and CD8 subclass of effector cells probably destroy graft cells by classical cytotoxic T cell mechanisms. Another important consequence of T cell activation is the release of other lymphokines, especially interferon (IFN-γ). IFN-γ induces increased expression of *HLA-A*, *HLA-B* and *HLA-DR* on graft tissues, thus potentially making the graft more vulnerable to effector mechanisms[24,25]. IFN-γ also activates monocytes to mediate a destructive delayed hypersensitivity response against the graft.

In addition to IL-2 and IFN-γ released from activated T cells, IL-4 and IL-5 play a role in directing B cell production of antibodies. Antibody-mediated damage may then take place directly through complement activation or recruitment of antibody dependent cell mediated cytotoxic (ADCC) effector cells. Most of the cells that arrive in the graft early after transplantation are lymphocytes, which migrate out of the capillary beds; after 7 days a remarkably heterogenous collection of cell types appears. Those of the lymphocytic series predominate over the monocytes/macrophages although few polymorphonuclear neutrophils are also present.

Mechanisms involved in allograft rejection

Immunological mechanisms involved in rejection could be mediated by (i) cell, (ii) antibody, (iii) delayed type hypersensitivity (DTH), and (iv) natural killer (NK) cell.

T cell-mediated rejection

The requirement for T cells in acute graft rejection has been shown conclusively in athymic mice, which fail to produce mature T cells. These mice accept grafts from either syngenic or allogenic donors, or even xenogenic donors, without evidence of rejection. Furthermore, the passive transfer of T cells into athymic mice leads to vigorous graft rejection. In clinical transplantation, the role of T cells has been confirmed by the dramatic effects of anti-T cell antibodies, including monoclonal anti-CD3 antibody (OKT3), antithymocyte globulin and antilymphocyte globulin, the effectiveness of which is often limited by the side-effects of non-specific immunosuppression. The allografts differ from the host at class I and class II loci. Both CD8+ and CD4+ T cells are activated by recognition of alloantigens of the grafts; the CD8+ T cells recognize foreign MHC class I molecules, which are expressed by all the cells in the graft[26,27]. The differentiation of cytotoxic T lymphocytes (CTLs) is largely dependent on CD4+ T helper cells being stimulated by allogenic class II molecules present on APCs in the allograft[28]. Therefore, one can predict that tissue allografts that stimulate strong rejection, contain class II bearing APCs. It has been appreciated that some CD8+ T cells can also provide sufficient help to allow cytotoxic T lymphocytes to differentiate independent of CD4+ T cells. However, these CD8+ T cells appear to depend upon the same professional APCs, as those required by conventional CD8+ T cells[29]. The most important APCs stimulating an antigraft response may be dendritic cells residing in the interstitium of the graft. The key features of these APCs are the presence of co-stimulators that contribute to the activation of CD8+ as well as CD4+ T cells. The importance of professional APCs in stimulating an alloantigenic immune response has most clearly been demonstrated *in vitro* by experiments in rodents.

Antibody-mediated rejection

The role of antibody in hyperacute rejection has been clearly established[30]. A direct correlation is seen between positive pre-transplant cross-match which detects anti-MHC class I antibodies and the development of hyperacute rejection[31]. Antigraft antibodies can be eluted from donor kidneys after hyperacute rejection. The passive transfer of antigraft antibodies in experimental models can provoke hyperacute rejection. It is likely that antibodies also play a role in other types of rejection; however, their mechanisms remain incompletely understood and controversial especially in chronic rejection[32]. The scanty cellular infiltrate in most cases of chronic rejection has led to the suggestion that antibodies[33] mediate the process of rejection. However, there is no direct evidence for antibody-mediated damage in chronic dysfunction. The antibodies causing hyperacute rejection may be preformed[34] or they may develop under the influence of immunosuppressive drugs, which could modulate their rate of production. Antibodies can bind to the graft, making the detection of soluble antigraft antibody difficult. Thus the role of antibody in the pathogenesis of chronic dysfunction remains undetermined.

Delayed type hypersensitivity mediated rejection

The CD4+ T cell regulates the DTH-mediated response. However, the effector cells are most likely macrophages and possibly CD8+ positive cytotoxic T cells. Consequently, the effector mechanisms may involve immunologically non-specific mediators including IFN-γ and TNF-α (ref. 35). In a DTH response the activated CD4+ T cell recruits other cells, including macrophages and CD8+ T cells, by secreting lymphokines (previously termed macrophage inhibition factor) and other unchar-

acterized substances. The CD8+ cells also secrete IFN-γ and may substantially increase the recruitment of macrophages. The CD4+ cells which induce a DTH response belong to the Th1-T cells and cause a DTH reaction. Evidence that the DTH response is involved in acute graft rejection is based on a correlation between graft rejection and the ability to generate DTH responses to the same antigenic challenge[36].

Natural killer cell-mediated graft rejection

NK cells are frequently identified in the infiltrating cells during acute graft rejection[37]; however, the role of graft cell lysis by NK cells remains unknown[38]. NK cells are CD4– and CD8– and do not express T cell receptors; the mechanism of target cell lysis is clearly different from that of cytotoxic T cells. In addition, target cell susceptibility to lysis by NK cells has been shown to be reduced by MHC class I expression. However, because not all cells are susceptible to NK cell lysis, it is likely that some form of antigen-specific recognition is involved. The precise role of NK cells in graft rejection remains to be determined.

Molecular basis of allograft rejection: Allogenic recognition

Alloreactivity is the fundamental mechanism underlying graft rejection. During graft rejection, T lymphocytes of the recipient recognize MHC molecules expressed by the engrafted tissue (allo MHC). Each foreign MHC molecule is recognized by multiple clones of T cells whose receptors are specific for different foreign peptides in association with self-MHC molecules. For example, foreign MHC molecule X (with some bound self-peptide A) may be recognized by T cell I, which is specific for self-MHC molecule Y and a foreign peptide I and by T cell 2, which is specific for self-MHC molecule Y and a foreign peptide II.

Multiple bound peptides in combination with one foreign *MHC* gene product may produce determinants recognized by different cross-reactive T cells. Any single foreign MHC molecule can bind only with one peptide at a time, but on each foreign cell surface there are many copies of each foreign MHC molecule, and each copy can form a complex with different peptides. A different T cell may recognize each different complex. For example T cell 3, which is specific for self-MHC and foreign peptide 3, may recognize foreign MHC plus self-peptide B. However, T cell 4 which is specific for self-MHC molecule Y and foreign peptide Y, may recognize foreign MHC molecule X and self-peptide C. Peptides B and C could also be foreign peptides (Figure 3). The key point is that self-peptides can contribute to T cell recognition when bound to foreign MHC molecules because the TCRs that recognize determinants formed by foreign MHC and self-peptides were not eliminated during negative selection in the thymus. Because many different self-peptides form determinants with foreign MHC molecules that are recognized by different T cell clones, each allogenic cell may be recognized by many different T cell clones, each with a distinct specificity for a different foreign peptide.

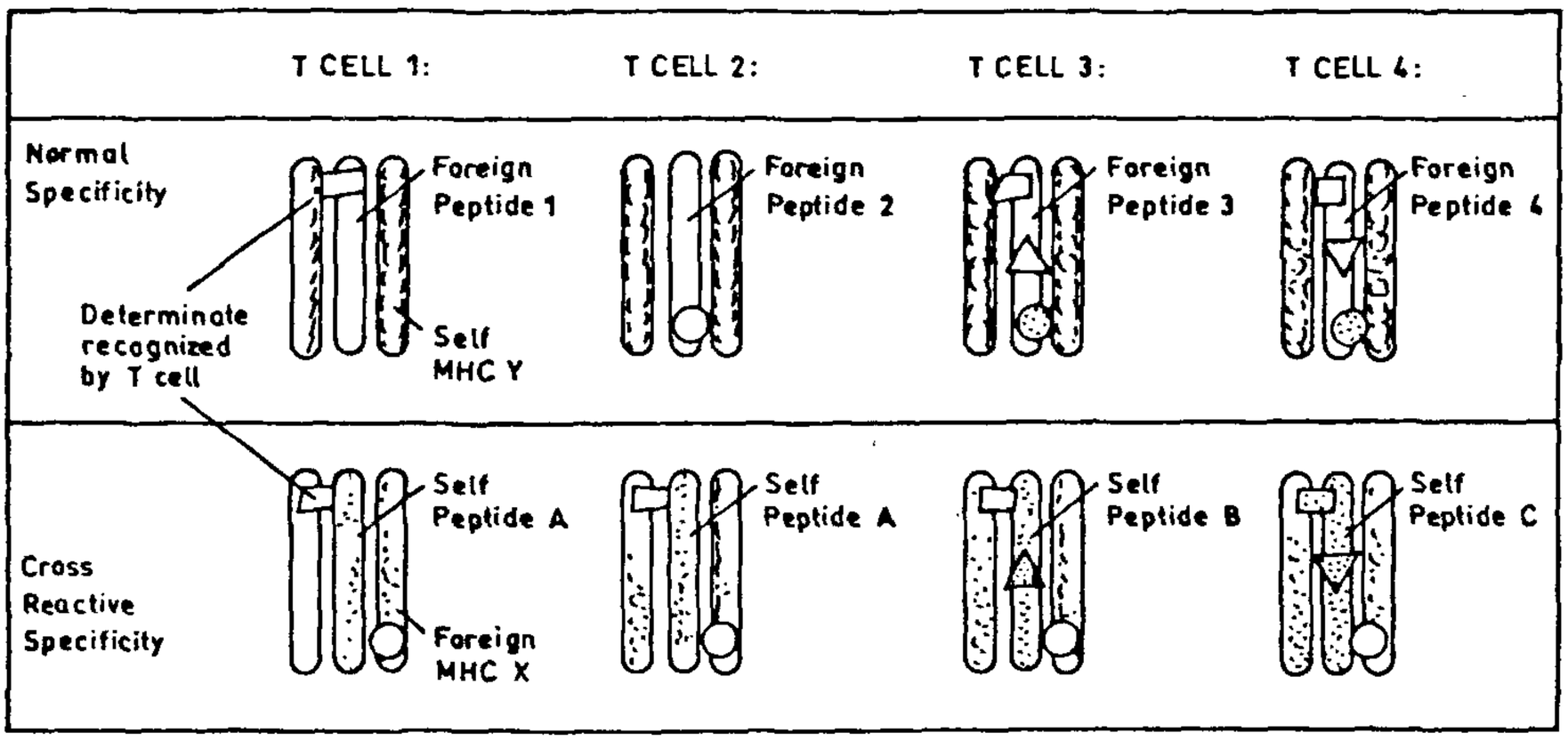

Figure 3. Molecular bases of alloantigen recognition, T cells bearing TCRs selected to recognize self-MHC Y molecules complexed with foreign peptides (1–4) may cross react with foreign MHC X molecules complexed with self-peptides (A–C). In this example, T cells 1 and 2, specific for amino acid residue determinant S □ and ○ on foreign peptides 1 and 2, respectively, cross react with the same determinants formed by polymorphic amino acids of the foreign MHC molecules, the amino acid determinants recognized by T cells 3 and 4 on foreign peptides 3 and 4 are formed by amino acid side chains contributed by both the foreign MHC molecule and bound self-peptides B and C.

A foreign peptide does not occupy more than 1% of the total MHC molecules expressed by an APC. The high density of these allogenic determinants on foreign APCs may allow activation of T cells with low specificity for the determinants increasing the number of T cells that can respond. Minor histocompatibility antigens also cause a weak rejection episode[39]. Vigorous rejection reactions of allografts generally result from the recognition of the transplanted tissue by both CD4+ and CD8+ T cells. In graft rejection alloreactive cells recruit and activate macrophages, initiating graft injury by a DTH response, where alloreactive cytotoxic T lymphocytes (CD8+) directly lyse graft endothelial and parenchymal cells. However, CD4+ cells release cytokine to activate the B-lymphocytes to produce the alloantibodies. Alloantibodies bind to the complement system and injure graft blood vessels.

Role of HLA matching in renal transplantation

It has been established that the graft immunogenicity plays a key role during the allograft rejection, which is determined by HLA antigens. These antigens are highly polymorphic and are present on the nucleated cells. Because of the high degree of polymorphism, no two individuals in an outbred population are identical with respect to their *MHC* gene products and hence there is rejection even when the graft is from the same species. If the graft is between 100% matched or compatible donor and recipients at both classes, i.e. HLA class II and class I loci, the chances of graft rejection are negligible. Terasaki *et al.*[40] reported that one-year graft survival was better in HLA identical siblings (90%) when compared to one haplotype shared or matched (70%). Literature suggests that the association between HLA compatibility and graft survival has a chequered history. Some investigators have reported an association between recipient's and donor's HLA compatibility[41,42] while others have not found any relationship. Better-matched graft has superior allograft survival. In live related donors, HLA matching should be done. Both broad specificities (routine serology) and split antigens should be matched[43–47]. For this purpose molecular typing, both low resolution and high resolution, has been suggested. Those who are not in favour of 100% HLA matching advocate that if we go for a complete matching then chances of finding a suitable donor become remote for end stage organ disease. Hence, in the immunosuppression era, mismatched donors could be considered for renal transplantation.

Bucin *et al.*[48] have shown the combined effect of CsA and HLA mismatch on kidney graft survival in 1085 patients[48]. They have reported that the HLA-A mismatching has beneficial effect on graft survival in cyclosporin and prednisolone treated (high and medium dose of immunosuppression) patients. They have illustrated long-term renal graft survival in HLA-A mismatch when compared to HLA-B and HLA-DR mismatched transplants.

Mendez *et al.*[49] analysed 1000 patients transplanted during 1978–84 in the pre-cyclosporin era and during 1984–89 in the cyclosporin era. They have reported that HLA matching is the way for better graft survival even if the patients are on cyclosporin. However, in CsA era the HLA-A, HLA-B and HLA-DR mismatches for 0, 1 and 2 showed a significantly better graft survival than found in the pre-cyclosporin era at all time intervals. In Table 1, the graft and number of HLA-A, HLA-B and HLA-DR mismatches and renal allograft survival rates at 1, 3 and 5 years are shown from 1981 to 1997. The cumulative experience of various studies from Table 1 (refs 50–57) reveals that if there are no mismatches at the A, B and DR loci, the graft survival is found to be better. However, the beneficial effect of cyclosporin cannot be ruled out.

Klehr *et al.*[58] compared the results of ten-year kidney transplantation with and without HLA typing in cadaveric transplants. A total of 236 kidneys were transplanted in 234 recipients. Out of these, 40 kidneys were obtained from Eurotransplant. However, the remaining kidneys were locally obtained and transplanted into recipients in the local transplant waiting list according to strict criteria of ischemia time, same blood group, waiting period and negative current cross-match in recipient serum and donor lymphocytes. Transplantation results were analysed retrospectively according to ischemia time, HLA mismatch, post-operative renal failure and renal function, rejection rate, and graft survival. The mean observation period was 55 months for local and 50 months for eurotransplant kidneys. The number of HLA matches was greater in eurotransplant groups. However, cold ischemia time was greater for this period 20.2 h vs 15.7 h, which was significant ($P < 0.0001$). No significant difference with regard to 1-year and 5-year graft survival was found. However, it has been seen that the 1-year and 5-year allograft survival was 90.2% and 88.3% for recipients who had received kidneys from the local donor pool compared to 81% and 62% for recipients who had received the graft from the eurotransplant group. Acute renal failure was less common with locally assigned kidneys (33% vs 53%, $P < 0.02$).

Baltzan *et al.*[59] reported that HLA matching enhances the long-term graft survival but did not correlate this with acute rejection episodes. They postulated that acute rejection episodes are independent of HLA matching while chronic rejection is HLA dependent. Hyperacute and chronic rejection are related and are part of humoral immunity.

Opelz[60] discussed the influence of mismatching for HLA-A, HLA-B and HLA-DR splits and reported that the difference in survival at 3 years between grafts with

Table 1. Graft survival and half lives by number of HLA-A + B + DR mismatches and post-transplantation period

Investigators	Center and no. of transplant	MM	Pre CsA era graft survival			CsA era graft survival		
			1 yr	2 yr	3 yr	1 yr	2 yr	3 yr
Persijn *et al.*[50]	Euro-transplant	0	81.0	81.0	68.0	89.0	76.0	75.0
	n = 9348	1	77.5	64.0	56.0	89.5	80.0	73.0
	1992–1998	2	76.0	59.0	55.0	87.0	78.0	66.0
		3	74.5	64.0	56.0	86.5	76.5	64.0
		4	63.0	64.0	50.0	82.0	68.0	61.0
		5	78.0	66.0	58.0	82.0	72.0	68.0
		6	57.0	46.0	58.0	74.0	59.0	43.0
Gjertson[51]	UCLA	0				87.2		
	registry 1985–1988	1				86.5		
	n = 12,886	2				82.4		
		3				83.1		
		4				80.9		
		5				80.5		
		6				76.0		
Ciaccrelli *et al.*[52]	UNOS renal	0				89.0	84.0	
	Tx registry	1				89.0	79.0	
	1987–91	2				82.0	75.0	
	n = 9689	3				84.8	75.1	
		4				81.5	69.1	
		5				80.5	69.0	
		6				81.0	68.0	
Takemoto *et al.*[53]	UNOS renal	0				87.5	82.5	
	transplant	1				85.0	74.5	
	registry	2				83.0	72.5	
	n = 24269	3				82.5	69.0	
	1987–92	4				79.0	66.0	
		5				77.5	64.5	
		6				77.0	62.5	

0–6 mismatches for HLA-A, HLA-B and HLA-DR was 31% when antigen splits were also taken into consideration. However, this difference was 6% when broad antigens were considered. Hence, the conclusion was that split specificities or sub specificities should also be seriously considered at the time of donor selection for first transplant and especially for retransplantation. Various authors have discussed *DQ* and *DP* alleles matching and Opelz *et al.*[57] have indicated that the HLA-DPB is a clinically relevant histocompatibility locus in cadaveric kidney retransplantation. This is suggestive of prospective matching for HLA-DPB in cadaver for retransplant. Rosenberg *et al.*[61] have shown that there is no clear benefit for matching for *HLA-DPB* allele. Tong *et al.*[62] indicated the importance of determining *HLA-DRβ1* molecular alleles instead of *DPB1* for assessing graft survival and has shown that graft survival is better if matched at *HLA-DRB1* alleles. However, Fukuda *et al.*[63] have shown that there is a negative effect of HLA-DQ compatabilities on the survival of renal allograft. Cumulative data on molecular and serological typing show that DR antigens are the most important immunogenic factors during the graft rejection in kidney transplantation.

Table 2. Acceptable and unacceptable mismatch antigens

HLA combination	Category	Reference
HLA-A3-B60	Unacceptable mismatch	64
HLA-A1-B7	Unacceptable mismatch	64
HLA-DR1-DR2	Unacceptable mismatch	64
HLA-A24-B44	Uncceptable mismatch	65

It has been reported that in spite of mismatche substantial number of transplants do well[64,65]. On basis of this concept acceptable and unacceptable matches ('taboo mismatches') have evolved. Som these combinations are shown in Table 2.

Acceptable mismatches are defined as those matches that lead to no immunological failure or than 15% immunological failure. Unacceptable matches or non-permissible mismatches are those w the chances of immunological failure is greater 15%. This cut-off point (15%) was taken on the basi the failure rate. Eurotransplant data have also sh that the survival rate of graft with acceptable mism was similar to zero mismatched graft. Unaccept mismatch leads to significantly poor graft surviv

There was a clear and significant difference in the graft survival of acceptable mismatch and unacceptable mismatch. Some of the HLA class II antigen mismatches in donor–recipient combination have also been identified as unacceptable mismatches, e.g. HLA-DR1-DR2. Hence donors with taboo mismatches at both HLA class I and class II antigens should be avoided to decrease the chance of rejection. Shintaku *et al.*[43] have stressed that even for the renal transplantation low resolution typing at HLA-A, HLA-B and HLA-DR should be done and they have further suggested that HLA comptibilities show a beneficial long-term effect on graft survival. Beckingham *et al.*[67] examined 181 renal transplant patients receiving cadaver kidney and have shown that with HLA-DR and HLA-B matching there is significantly lower rejection rate than with less-matched grafts on these loci (rejection rate was 25%, 62% and 82% for 0,1 and 2 DR mismatches respectively). Significant rejection episodes occur earlier in mismatched grafts, superior matching was associated with improved graft function at one year after transplant. No association was demonstrated between degree of match and graft survival. Good matching reduces the number of rejection episodes and produces significant reduction in the cost and duration at the hospital. Long-term graft function is improved and minimizes the acute rejection episodes and prevents the development of chronic rejection.

It has been shown that there may exist ethnic differences that affect the long-term graft survival of kidney transplantation. Koyama, Cecka and Terasaki[68] projected half-life for HLA-identical sibling donor in blacks and have shown that there is a significant difference in the half-lives of blacks (15 years) when compared to 29 years for whites. This holds true for live related donors. However, for cadaveric transplant, the half-life was 5 years for blacks and 10 years for whites. Graft survival rate improves with better HLA matching in both blacks and whites. But the two-fold differences in long-term survival rates persisted even among recipients of well-matched grafts with zero HLA-A and HLA-B mismatched black donors who had 8 years half-life when compared with 17 years of white donors. The racial difference is more marked in young adults with a 15–20% disparity at 3 years between black and whites with ages ranging from 16 to 30 years. Paediatric and older patients had 3 years graft survival rate, which is similar to those of whites. In contrast to these findings in USA, 63 transplanted blacks in Canada had the same short- and long-term graft survival as whites, suggesting an important influence of health care systems and socio-economic factors. In addition to improved access to health care and improved HLA typing of blacks, more black donors are required with better-matched transplants for blacks awaiting transplantation.

A large number of single and multicentric studies have also examined the effect of HLA matching in live related donors and found a significant association between best matched and better graft survival. In live related donors, histocompatibility is graded by the number of haplotype matching (0, 1 and 2) and the degree of stimulation index in mixed lymphocyte culture. Opelz and Terasaki[69] first reported that the two haplotype-matched living related pairs have best early and long-term prognosis. Other investigators have also demonstrated that the HLA-identical siblings' transplants show excellent 2 years patient and allograft survival in the range of 95–98% and 87–90%, respectively[70,71]. Despite the excellent prognosis of the graft based on HLA-identical siblings, some allografts fail because of recurrent disease, technical problems and delayed hyperacute rejection[72–74]. Accelerated rejections have also been reported in HLA-identical kidneys. Such recipients were categorized as high or low responders depending upon the mixed lymphocyte culture (MLC) stimulation index (SI); those with low SI; (< 6.5–10%) had nearly double allograft survival to approximately 80%. The degree of stimulation in MLC has been used in non-identical live related donor recipient pairs in order to distinguish high and low responders[75–77]. Harmon *et al.*[78] reported that MLR is associated with acute rejection episodes and graft loss in HLA-identical recipient–donor pairs. Numerous other studies have also shown that patients with low MLC reactivity have better survival in living related allografts.

Due to the lack of live related donors various centres have tried spouses as the probable donors. In USA there is a constant increase in the number of persons donating their kidney to their spouses[40]. Despite the greater HLA incompatibilities, survival rate of these kidneys is higher than that of cadaveric kidneys. Terasaki *et al.*[40] examined the kidney transplant data from UNOS registry. They calculated graft survival using Kaplan Meier analysis and found that 3-year survival rate was 85% when kidneys were from 368 spouses, 81% for kidneys from 129 living related donors who were not married to the recipients, 82% for kidneys from 3368 parents and 70% for 43341 cadaveric kidneys. The three-year graft survival rate for transplant from wife to husband was 87%, it was same for transplant from husband to grafts if the wife had never been pregnant. If the wife had previously been pregnant then the 3-year graft survival rate was 76%. The three-year graft survival rate for the spousal graft that did not receive transfusion preoperatively was 81% compared with 90% for recipients who received transfusion 1–10 preoperatively. The superior graft survival rates from unrelated donors could not be attributed to HLA matching, racial origin or age of the donor. Terasaki *et al.*[40] emphasized that spouses could be another important source for kidney grafts despite poor HLA matching. Gjertson *et al.*[79] have also confirmed the above finding.

Multivariate and univariate analysis have shown that HLA mismatching had no independent effect on the

graft survival but HLA-A and HLA-B antigen mismatching was detrimental for six months after transplantation[80,81]. Connolly *et al.*[82] in a multivariate analysis have also reported that HLA-DR mismatching leads to early acute rejection episodes and poor graft survival.

Role of minor histocompatibility antigens

Minor histocompatibility antigens (mi-HAgs) may play an important role in the graft rejection and are defined as cell surface antigens other than the MHC antigens. These antigens may not be universally present on all the cells and they do not interact functionally with MHC antigens. However, the role of these antigens is not well defined in humans. Experimental data obtained from studies of congenic strains of mice suggest that polymorphism of mi-HAgs may be similar to that of the MHC antigens. The important difference being that mi-HAgs are less potent and immunogenic and they do not initiate the immune response independently, while MHC antigens are more immunogenic and can trigger the antibody production against incompatible alloantigens. mi-HAgs account for comparatively slower and more chronic rejections. Goulmy *et al.*[83] have first reported the possible involvement of mi-HAgs in human transplantation with a clinical observation in a female patient who received the bone marrow of a male HLA-identical sibling after ATG pre-treatment. *In vitro* analysis of the post-transplant peripheral blood lymphocytes of the female patient showed unambiguously that there were strong cytotoxic lymphocyte (CTL) responses that were specific for the male donor HLA matched target cells[84]. Naturally the impact of mi-HAgs on the outcome of an organ and bone marrow graft is dependent on other factors including cord endothelial cells and kidney proximal tubular epithelial cells or restricted to hematopoietic cell lineage including epidermal-derived Langerhans cells[85].

Linkage studies in congenic strains of mice have shown that mi-HAgs loci are scattered throughout the genome[86]. The total number of mi-HAgs is not known but theoretical estimates based on breeding and transplantation studies in mice suggest that there may be several hundred of them[87]. So far, forty mi-HAgs have been found in C57BL/6 and BALB/c strains of mice. Recently, from the genetic analysis of HLA-A2.1 CTLs restricted mi-HAgs have been characterized and categorized into the HA-4 and HA-5 antigen[88]. The immune response to the mi-HAgs is T cell-mediated[89–92], predominantly by cytotoxic T lymphocytes.

Role of tissue-specific antigens

Tissue-specific antigens are defined as a system of antigens that are expressed only on one type of organ, tissue or cell. These tissue-specific antigens are independent from the systemic antigens such as HLA antigens, which have a wide distribution throughout the body. In 1969, Calne *et al.*[93] first described the phenomenon of differential allograft survival between organs from the same donor. Whereas skin and kidneys were acutely rejected, liver allograft survival seemed to be prolonged in unrelated pigs. Several cases of multiple organ transplants have been reported in which one organ is rejected while the other continues to function. One possible explanation for this observation maybe the presence of tissue-specific antigens. Poindexter *et al.*[94] have characterized a kidney-specific peptide which recognizes kidney cell lines but not MHC identical B-lymphoblastoid cell lines. These peptides are nanomer residues with proline and lysine residues are presented on the allograft kidney and may be target of CTL recognition. This may further result into acute or chronic rejections. If all relevant transplant antigens were ubiquitous, graft survival would be fairly confirmed. HLA compatibility between recipient and donor may prevent sensitization to the tissue-specific antigens. However, HLA identity and negative MLC do not prevent immune response to the increasingly well studied VEL-specific antigens.

Vascular endothelial cells (VECs) of transplanted organs are at the interface between the graft and the recipient's blood containing immuno-competent cells. The VEC antigens are expressed in abundance throughout the renal vasculature. Baldwin and coworkers had reported high concentration of VEC antigens along with the peritubular capillaries and veins. In extra renal vasculature, the VEC antigens are expressed on the endothelial cells of major abdominal vessels, i.e. on both arterial and venous. VEC plays an important role in the rejection process. VECs serve as antigen presenting cells and are also able to phagocytosize and are partly responsible for the normal functioning of the platelets[95] antibody to antigen specific to VEC is the most commonly encountered antibody in patients rejecting a renal allograft. Ninety-six per cent of the patients who experienced a chronic rejection developed anti-VEC antibody[96,97]. Antibody to VEC is rarely encountered in normal control and only in low frequency in patients experiencing a benign clinical post-transplantation course. Most of these patients had anti VEC antibody present in the absence of any anti-HLA antibody to the donors. The VEC antigen appears to be an important immunogen in non-HLA identical combination.

Role of anti-HLA antibodies

Among transplant workers it is a known fact that pre existing alloantibodies directed against HLA antigens are contraindication for the kidney transplantation. These antibodies are present due to the humoral immune

response and play an important role in allo-responsiveness and hence cause rejection or allo-immune reactions. The prevalence of these antibodies among kidney recipients may vary from 20 to 40% (refs 98, 99). When antibodies are present in the recipients against HLA class I antigens then graft may be rejected as a result of hyperacute reaction after transplantation. These antibodies may be formed because of blood transfusion, failed previous graft or previous pregnancies in females. These antibodies are being detected in patient serum against donor lymphocytes in a cross-match test. The cross-match may be positive against T or B cells. Robert *et al.*[100] have reported that positive cross-matches are not an absolute contraindication for transplantation. Hence, it is important to characterize the antibody for its antigenic specificity, avidity, class, subclass, etc. A number of studies have demonstrated that high affinity and complement activity of antibodies to MHC class I are most injurious to the transplanted organ[101,102].

The different classes of antibodies that have been described in literature are IgM and IgG. IgM antibodies are autoantibodies circulating in the blood or they can be produced because of other mechanisms. These autoantibodies may produce false positive results, however, they are not injurious to the graft[103]. IgM antibodies are involved in primary immune response, hence are not harmful and are also not important to the renal graft function. IgM antibodies account for 10% of circulating immunoglobulin pool with half-life of 10 days and are usually present in low titre and have high avidity but low specificity to foreign antigens. There is no affinity maturation in IgM immune response even though their titre may increase the primary immune response. In addition, IgM synthesis decreases with immunosuppressive therapy.

Cardella *et al.*[104] suggested that peak positive and current negative cross-match may not be clinically relevant[104]. Earlier, if past serum (peak serum) showed a positive cross-match, even with current negative cross-match a patient was denied transplantation because of anti-HLA antibodies. Even with a positive T cell cross-match some of patients lose their antibody with time. Subsequent studies by Matas *et al.*[105] substantiated this observation. Early graft failure were also described by Sanfilippo *et al.*[106] under the above situation. Further studies by Reed *et al.*[107] described predictive factors of graft outcome, in this situation, which is related to immunoglobulin class of peak antibody, IgM had a better prognosis than IgG. Presence of antiidiotypic antibodies in this situation is favourable to the allograft. Minimum interval period recommended after the last positive cross-match and transplant has varied from investigator to investigator, e.g. 8 weeks or 2 months, 4 months, 6 months and one year[108,109].

The IgG antibodies which are produced against HLA antigens are injurious to donor vascular endothelium, they activate complement and other acute inflammatory mediators and initiate antibody-dependent cellular cytotoxicity (ADCC). The antibodies produced against donor-specific HLA antigens can be further sub classified into the antibodies against T and B cells. It has been reported that antibodies against T cells are more harmful than against B cells. However, recently Bittencourt *et al.*[110] have shown that antibodies to class II molecules are significantly associated with poor graft survival. HLA class II antibodies are not associated with acute rejection, however, these antibodies are important when they are present in high titre[111,112] and may effect the long-term survival of the graft. An isolated positive B cell cross-match may be due to antibodies against non-HLA molecules or class II molecules. Another possibility is the presence of low titre antibodies against donor class II molecules. This heterogeneity explains much of the controversy surrounding the clinical and immunological relevance of isolated B cell positive cross-match in transplantation. Ghasemian *et al.*[113] have proposed that the antibodies, which are directed against HLA-DR, are composed of IgM and are harmless. However, when they are of IgG type they cause acute or chronic rejection. They do not necessarily cause hyperacute rejection because class II is not readily expressed on the endothelial and tubular epithelial cells.

Role of panel reactive antibodies

Frequently it has been seen that there may be cytotoxic antibodies which are not only donor specific but against the lymphocytes taken from any individual. Such type of antibodies, which are reactive against a panel of lymphocytes, are known as panel reactive antibodies (PRA) and individuals that possess PRA antibodies are defined as sensitized patients. Sensitized patients show a poor graft survival and this sensitization may be because of blood transfusion, previously failed graft and previous pregnancies in females. It has also been reported that sensitization is dose dependent by whole blood or packed cells. Four per cent patients get sensitized after 5 transfusions, seven per cent after 10 blood transfusions. However, a wide range of patients may require more than 10 transfusions to be sensitized. Bucin *et al.*[48] have also reported the adverse effect of blood transfusion on long-term outcome of kidney transplantation. Two-year graft survival was significantly lower ($P < 0.05$) in transfused patients than in non-transfused patients, i.e. 81% vs 97%. It has also been seen that multiparous females constitute the major group at risk than male and nulliparous patients. However, they get sensitized after blood transfusion but rarely become highly sensitized. In an extensive examination of the role of sensitization in the UCLA renal transplant registry, less than 2% of the males developed cytotoxic antibodies 10% random

and only 10% developed PRA despite 10 units of blood transfusion. In female patients sensitization rate was higher (14%) which represented a severe increased risk of the predominant risk, cause of this extra risk was shown to be pregnancies. However, nulliparous females had an equivalent risk to males, while those with 1–3 or more than 3 pregnancies were at considerable risk of becoming sensitized approximately 18% and 30%, respectively[114] (Table 3).

The incidence of broadly-sensitized recipients (PRA > 50%) ranged from 2.4% (non-transfused males) to 29.3% (multiply transfused females with a history of pregnancy). Initial experiments of allograft rejection in presensitized recipients by blood transfusion in animals showed direct clinical relevance[115]. The first report of Opelz *et al.*[116] and others has contradicted the earlier findings and has shown a beneficial influence of blood transfusion in combination with immunosupression drugs or X-ray irradiation prior to engraftment[116–119]. The hypotheses put forward are pre-operative blood transfusion random or donor specific may preselect the population of high responder, induced clonal deletion of T cells with anti-donor reactivity and activate suppressor mechanism. Blood transfusion may lead to sensitization. But this sensitization may be nullified because of subsequent immunosuppression, which may induce clonal deletion of activated memory cells. However, experiments done in murine models do not support the above hypothesis.

Busson *et al.*[120] have shown that there is less significance of anti-HLA immunization after one transfusion in a population of dialysis patients who had no previous allogenic contact. Only 2 patients out of 282 (0.71%) developed background level (5%) anti T-IgG antibodies in both the groups transfused with a one DR matched blood units. A number of patients had developed B cell antibodies. Patients transfused with full HLA-DR mismatch blood have no risk of alloimmunization. The percentage of transfused patients who developed antibodies varies considerably in literature. This may be due to the differences in selection criteria and the techniques used by different investigators. Sensitized recipients (PRA > 50%) range from 2.4% of non-transfused males to 29.3% of multiple transfused female patients with a history of pregnancy.

Monteiro *et al.*[121] have reported that patients who are allosensitized are at high risk. However, due to lack of standardization of results of lymphocytotoxicity, the detection of alloantibody using current methodologies may not correlate with post-transplant events. They have used ELISA and tested 124 renal, allograft recipients with 18 months follow-up time and have found that pre transplant ELISA-PRA > 10% had more than 3-times graft loss compared to patients with negative ELISA-PRA. They observed a significant correlation between positive ELISA and early graft dysfunction. Almost all patients (88%) with a pre-transplant ELISA-PRA > 50% required post-transplant dialysis compared to 45% patients with a pre-transplant ELISA-PRA of 10–15% and 27% patients with a pre-transplant ELISA-PRA < 10%. These results suggest that allosensitization plays an important role and more sensitive methods should be used for it. Nelson *et al.*[122] have evaluated the role of flow cytometry cross-matching on graft survival in patients with cadaveric donors. They have shown that the flow cytometry cross-matching improves graft survival in cadaveric transplantation by identifying a subset of patients with donor HLA antibodies which were not detected by antihuman globulin cross-match. Bittencourt *et al.*[110] have also evaluated the influence of flow cytometry on B cell cross-match on renal transplant and suggested that positive B cell cross-match has deleterious long-term graft survival in renal allotransplantation.

Table 3. Percentage of sensitized patients after blood transfusion

UCLA UNOS Data	No. of recipients	Transfusions 0 PRA 11–50/> 50	1–4 11–50/> 50	> 4 11–50/ > 50
Male	12523	14/2	18/4	22/12
Female without Pregnancy	3466	17/9	20/12	24/21
With one or > more pregnancies	3508	20/10	26/2	25/29

Role of anti-anti alloantibody

Multiple mechanisms of autoregulation of antibody mediated immune responses have been demonstrated in animal models. These include antiidiotypic antibody network and development of suppressor cells. Anti-anti HLA antibodies are idiotypic antibodies against antibodies to HLA antigens, which are determinant of the antibody and cellular receptors produced by syngenic, allogenic and xenogenic immunizations. The molecular mechanism of antiidiotypic antibody responses is not well established till date. It has been argued that the antiidiotypic antibodies may be produced as a result of blood transfusion and may have beneficial effects[123–125]. Antiidiotypic antibodies (AB2) are believed to act by binding to the determinant within the variable region of combining sites of the HLA antibodies (AB1). In animal models it has been suggested that AB2 inhibit the binding of antibody to HLA antigens. Another immunosuppressive property of antiidiotypic antibodies is mediated by their ability to bind to the variable region of receptors on activated T lymphocytes and to the suppression

of the proliferation of B lymphocyte producing antibody[126,127]. Jerne[128] has proposed the existence of anti-anti-idiotypic antibodies. Reed and coworkers have proposed that non-cytotoxic sera might contain anti-antiidiotypic antibodies (AB3) which could mediate the release of alloantibody from AB1–AB2 complexes present in antibody positive sera, thus potentiating cytotoxicity activity[129–130]. Reed *et al.*[107] and others have also proposed that antiidiotypic antibodies against autologus anti MHC antibodies play a role in down-regulation of the immune response during transplantation. Sachs[131] has tried to produce cloned cytotoxic T cell receptors of cloned cytotoxic T cells and has proposed the utility of his work in transplant immunology. Suciu-Foca *et al.*[132] have reported that antiidiotypic antibodies block the cytotoxic activity of alloantibody and hence cause prolonged tolerance to the graft. There is a balance between cytotoxic antibodies and antiidiotypic antibodies to HLA, which may account for the length of the quiescence period that may be long or short in presensitized or in the patient with mismatched antigens, respectively. During sensitization it has been seen that it is not necessary that cytotoxic antibodies be developed always against the entire mismatched antigen but certain antigens may be blocked by antiidiotypic antibodies. Reed *et al.*[133] have also reported that the 5-year graft survival was significantly better (90%) in renal transplant recipients who had antiidiotypic antibodies compared to those who had no antiidiotypic antibodies (41%). Terness *et al.*[134] have characterized the antiidiotypic antibodies and have shown that physiologically active domains immobilize the F (ab)2 arm and thereby can be expected to show immunoregulatory function. Shoker *et al.*[135] have shown that antiidiotypic antibodies and blocking antibodies are two different identities. A fraction of IgG, which has blocking activity, is of type IgG3. However, this fraction is absent in the sera positive for antiidiotypic antibodies.

Role of mixed lymphocyte reaction blocking factor and transplantation

Immune activation by kidney allograft is a well-known phenomenon and specific and non-specific antibodies are produced against donor alloantigens[136–138]. Shoker *et al.*[138] have reported the existence of allotypic antibodies dependent mixed lymphocyte reaction blocking factor (MLR-BF) antibody activity from the serum of patients with known chronic rejection who had lymphocytotoxic activity in their serum at the time of transplantation. Burlingham *et al.*[118] have demonstrated the presence of alloantibodies, which had inhibited the 6th day primary mixed lymphocyte culture (MLC) response to donor and pooled third party stimulator cells regardless of the source of responder cells in the plasma of recipients after donor specific blood transfusion (DST). Horuzsko *et al.*[139] have reported the selective effect of noncytotoxic blocking alloantibodies produced after the transfusion of platelets on MLC and mitogen and soluble antigen-induced responses of human lymphocytes. These blocking sera mediated the suppression of the responder's cells in MLC test and associated the serum IgG fraction with either class I or class II specific antibodies. Such antibodies were not found in all recipients with successful outcome, which suggested that these antibodies may be developed after third party transfusion before transplantation. Fujiwara *et al.*[140] have reported a decreased relative response of MLR in renal transplant recipients surviving after 10 years when compared to the pretransplant MLR which had demonstrated the donor-specific immune unresponsiveness of cytotoxic T lymphocytes in living related donors.

Recently, Shoker *et al.*[135] have also demonstrated and characterized allotypic MLR-BF antibodies in serum from highly sensitized patients. MLR-BF antibody activity is present or lies in the fraction of immunoglobulin separated sera from known chronic rejection. These sera also contain lymphocytotoxic antibodies in the sera. These blocking antibodies were not detected in patients who did not demonstrate lymphocytotoxic activity in the sera. They further characterized this antibody and found that these blocking antibodies were predominantly associated with IgG3 subclass.

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Erratum

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In 'In this issue' writeup: in column 2, the last item of the bulleted list should read 'Validation of results against the real world' and not 'Validation analysis, visualization of results against the real world'.

We regret the error.

– *Editors*