INTERNAL MEDICINE GRAND ROUNDS

THE ROLE OF IMMUNOLOGY IN CLINICAL TRANSPLANTATION

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The Role of Immunology in Clinical Transplantation

A. Historical perspective

In the early 1940's Peter Medawar performed the experiments that established a scientific basis for transplantation studies and showed that allograft rejection is immunologic. Antibodies against infectious organisms were being studied intensely but the role of lymphocytes was not yet known.

Mitchison (1954) performed the experiments that demonstrated the importance of lymphocytes in the rejection of grafted tumors. Billingham, Brent and Medawar showed that adoptive transfer of accelerated rejection of allografts of skin was accomplished with lymphoid cells but not with serum.

Fruitful relationships developed between scientists who worked in transplantation immunology and the surgeons who brought the applications to the clinic. Identical twin kidney transplants were performed in Boston (1956).

Schwartz and Dameshek (1959) discovered they could inhibit antibody formation in rabbits with 6-mercaptopurine. Very shortly thereafter, Calne (1961) was performing kidney transplants in non-identical twins and using 6-mercaptopurine.

Table 1. Immunology in Clinical Transplantation

1943 - Medawar	graft rejection is immunologic
1954 - Mitchinson	adoptive transfer with lymphocytes, tumor grafts
1956 - Billingham, Brent	adoptive transfer with lymphocytes, skin grafts
- Merrill, Murray	identical twin kidney transplants
1959 - Gorer, Snell	H-2
- Schwartz, Dameshek	6-mercaptopurine
1961 - Calne	kidney transplants with 6-MP
1962 - Gowans	long-lived lymphocytes
1964 - Terasaki	microcytotoxicity test for HLA typing
- Bach	mixed lymphocyte cultures
- Woodruff	anti-lymphocyte serum
1965 - Dausset, Amos, Payne, van Rood, Terasaki	HLA antigens
1966 - Kissmeyer-Nielsen	hyperacute rejection
1969 - Patel, Terasaki	crossmatch
1976 - Borel	Cyclosporine A
1977 - Bodmer	HLA-DR typing

Interest in histocompatibility was stimulated by the prospect of these clinical applications. Dausset, Amos, Payne, van Rood,

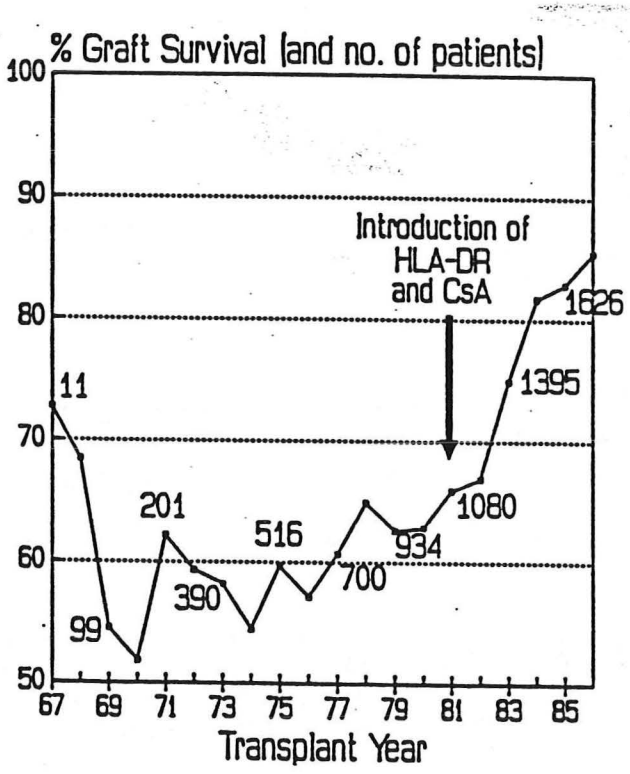
Teresaki and others became convinced that the leucocyte antigens discovered using sera from women immunized through pregnancy represented the antigens of the major histocompatibility system in man.

From this humble beginning developed the HLA system as we know it today. It is now known to be not only the main barrier to transplantation of organs, but also the basis of differentiation between self and non-self and therefore the basic key to all of the immune response.

Hyperacute rejection of the transplanted kidney was recognized first by Kissmeyer-Nielsen (1966) and then by many others. Patel and Terasaki (1969) showed that it could be prevented by careful crossmatching of recipient serum against donor lymphocytes. The pre-operative crossmatch is still one of the most important functions of the tissue typing laboratory. We are still learning about the crucial role antibodies play in kidney transplantation.

The report by Borel (1976) describing the new immunosuppressive drug cyclosporine A, appeared in 1976 and has had enormous impact.

The development of HLA-DR typing in the workshop organized by Walter Bodmer (1977) was certainly an important development. Before that time, the class II antigens could only be determined by tedious cellular techniques. It is now well established that



(From: Thorogood et al., Transplantation, 1988)

the class II HLA antigens have a powerful effect in clinical transplantation.

B. Evaluation of the results of kidney transplantation.

B.1. Improved graft survival in recent years.

Thorogood et al. examined graft survival at 1 year in Europe over the 20-year period from 1967 to 1986. They observed a progressive improvement which was quite dramatic from 1981 onwards. Cyclosporine was observed to have a powerful effect. Multiple factors affecting graft survival were examined. Transplant year had an effect demonstrable also in patients not treated with cyclosporine. HLA matching was observed to influence graft survival independently of year and immunosuppressive therapy.
A similar analysis performed by Terasaki and coworkers, included first cadaver kidney survival rates at 1 month and at 1, 5 and 10 years.

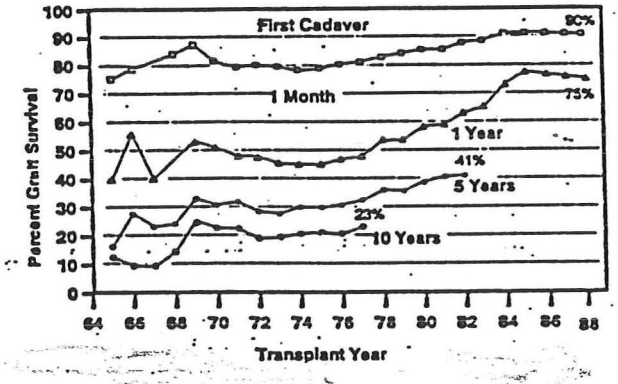


FIGURE 2. Cadaveric kidney survival rates (1 month and 1, 5 and 10 years) of transplants since 1964. (From: Terasaki et al., Immunology Letters, 1989.)

The period covered is from 1965 to 1988. In 1965 25% of the grafts failed in the first month. After 1984 this failure rate was reduced to about 10%. Much of this improvement is thought to be due to better management in the early post-operative period.

The Terasaki registry data has also been presented as shown in Figure 3.

Most of the difference appears to occur early. The lines depicting graft survival after the first year for the different time periods are essentially parallel. The half-life, which is a measure of the long-term loss rate, has been fairly constant at 7 to 8 years and

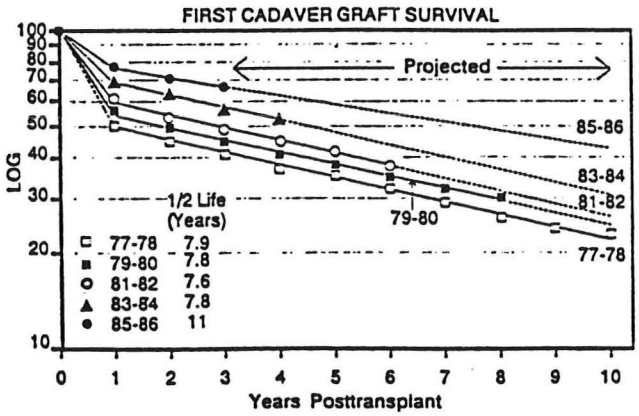


FIGURE 3. (From: Terasaki et al., Transplantation Proceedings, 1989.)

has not changed over a span of over 20 years of experience. Only the last period has an increased half-life of 11 years. But it was based on only 3 points and is therefore a projection. It was concluded that the 10-year graft survival at present is probably about 40%. Which means that 60% of the transplants performed today will probably be lost in 10 years.

B.2. The center effect

One of the puzzling observations in both U.S. and European registries of kidney transplants has been the so-called center effect. Certain groups consistently have better results than others. The differences have been observed for the early period,

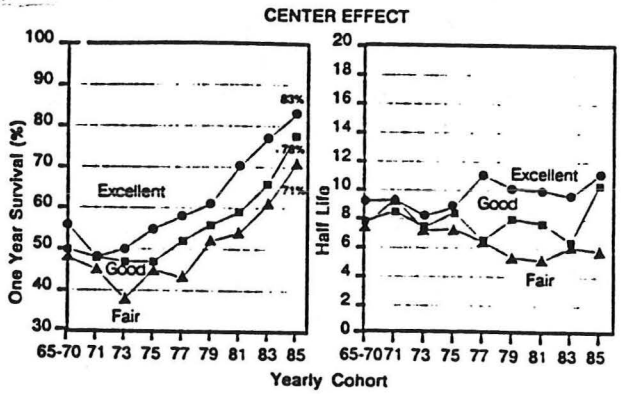


Fig 4.

(From: Terasaki et al., Transplantation Proceedings, 1989.)

long-term graft retention and even patient survival. In figure 4, it can be seen how this center effect has been maintained over a period of many years. Excellent centers have a cadaver donor half-life of about 10 years compared to a half-life of about 6 years in the fair centers.

The center effect appears to be due to several factors many of which are difficult to quantitate. Differences in the quality of management seem to be more important than overt immunosuppression protocols. Other factors are inclusion of high risk patients, the effect of race and patient compliance. The latter two will be discussed briefly next.

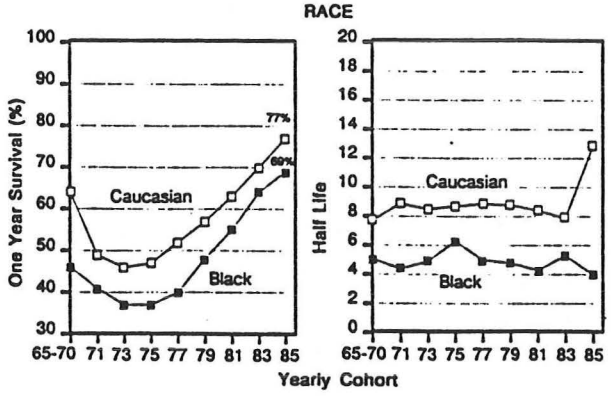


Fig 5.

(From: Terasaki et al., Transplantation Proceedings, 1989.)

B.3. Difference in kidney graft survival related to race

It has been known for a long time that in the U.S., Caucasian recipients have better results than Black recipients. The long-term trend shown in Figure 5, is quite clear. Caucasian recipients had an 8 year half-life, while in Black recipients the half-life was about 5 years. The reasons for the difference are not known but the following factors are considered.

Propensity to hypertension and vascular disease in Blacks more than in Caucasians. The possible role of histocompatibility differences since most patients receive kidneys from Caucasians and HLA typing is less developed for Blacks. Socioeconomic factors are also cited. Particularly factors that might have an effect on patient compliance.

B.4. Patient compliance

Discontinuation of immunosuppression has been recognized as a major cause of rejection and graft loss. Faithful compliance with life-

long intake of medications needs to be established by efforts in patient education and frequent reinforcement. A network of nurses, coordinators and assistants, which provide the interface between the transplant physicians, the local physicians and the patients, plays a crucial and all-important role.

It is of interest that a similar effect was also seen in kidney transplants from living-related donors (Fig. 6). When one looks at 1-haplotype and 2-haplotype matched recipients, the White and Asian groups showed similar graft results, while Black recipients did significantly worse.

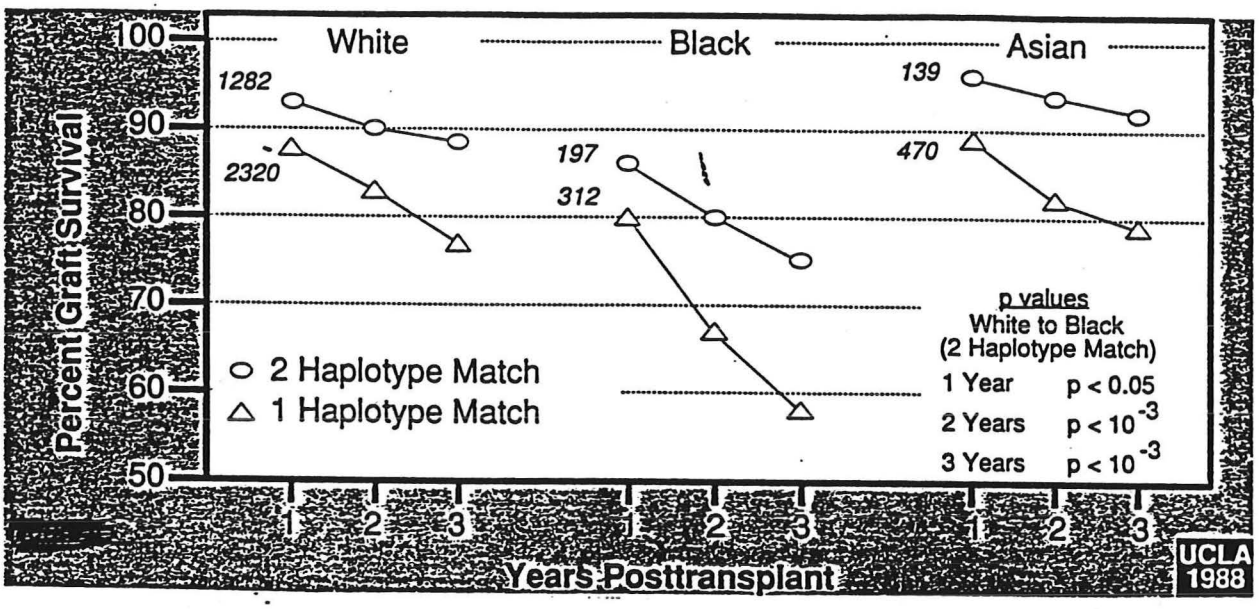


FIGURE 6. (From: Cicciarelli, Clinical Transplants, 1988.)

C. Role of transplantation antigens

C.1. Major histocompatibility antigens

The major histocompatibility complex, the HLA region on chromosome number 6 of man, is the most intensely studied segment of the genome. The structure of most of the alleles is known in detail.

At the protein level, the class I antigen HLA-A2 has been crystallized and its three-dimensional shape has been visualized. The HLA antigen has a little pocket which can hold peptide antigens to present them to T cells. From this work, the role of the MHC antigens in the immune response is much better understood.

In regard to transplantation, the important advance has been the definition of the polymorphic sequences. DNA sequencing of the genes of all the major haplotypes has been performed and it is now

possible to construct synthetic oligonucleotides and to use them for typing.

The picture that emerges is that of a very complex patchwork with extensive sharing and apparent interchanges of sequences between the genes of different alleles.

Table 2. Sharing of amino acid sequences between different HLA class II alleles.

	Amino Acid Position				
	70	71	72	73	74
DR4/Dw14,Dw15	Q	R	R	A	A
DR1	-	-	-	-	-
DRw14.2	-	-	-	-	-
DR2	D	-	-	-	-
DR3	-	K	-	G	R
DRw11.2	D	E	-	-	-
DR7	D	-	-	G	Q
DRw8	D	-	-	-	L

During the past year our laboratory has devoted a major effort to the development of DNA typing. The procedure we are using depends on specific amplification of the DNA we wish to examine by the polymerase chain reaction (PCR). Thus we would amplify DRB1 molecules of all DR4 haplotypes. To do this we take advantage of a sequence in the first hypervariable region of DRB1 which is unique to all DR4 alleles. The actual typing is then performed by dot blot hybridization.

Figure 7

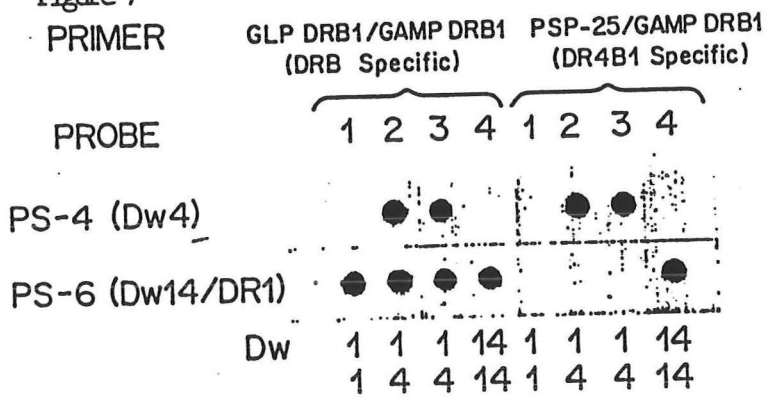


FIGURE 7. (From: Gao et al., Human Immunology, in press.)

In this figure the reason for allele-specific amplification is illustrated. On the left, amplification was performed with generic DRB primers. On the right, amplification was DR4DRB1 specific. Because of the sharing of the sequence between DR4, Dw14 and DR1 false-positive hybridization occurs when all the DRB genes are probed and the subject carries DR1 in addition to DR4.

These techniques will now be applied to matching for transplantation. Instead of the old concept of matching for antigens and also for some "public" and "private" specificities as they could be defined by serology, it will be possible now to match for epitopes. With the complete knowledge of the chemical structure, the magnitude of matching or mismatching can be more precisely quantitated. Park recently pointed out that one of the most difficult problems faced by tissue typing over the years has been the excellent survival of badly-matched transplants. A more quantitative approach may show which bad matches are functionally significant.

However, analysis of the results of the major registries does show an important effect of matching in kidney transplant survival even today.

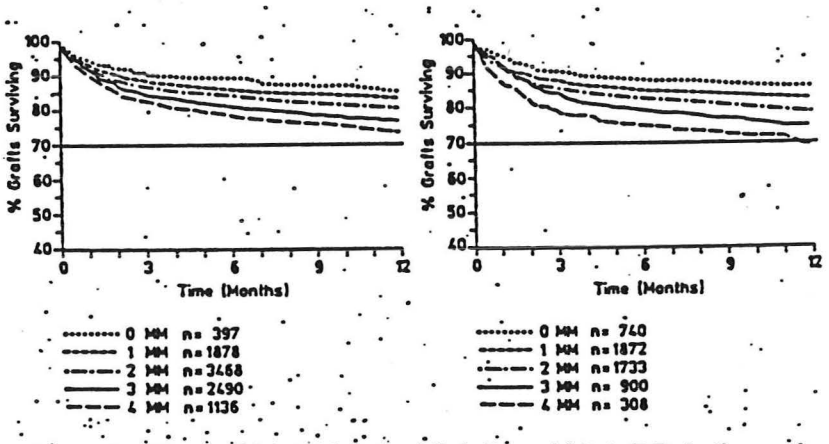


Figure 8 Effect of Matching for HLA-B and HLA-DR Antigens in First Cadaver-Donor Kidney Transplants.
(From: Opelz et al., New England Journal of Medicine).

Data from the Opelz registry, published last year, show the effect of HLA-B and HLA-DR antigen matching in two groups of patients receiving first cadaver kidney transplants. The chart on the left shows results with locally obtained kidneys, on the right, kidneys shipped between centers. In either case, graft survival correlated with the number of HLA antigens mismatched. Similarly, graft survival in second cadaver-donor transplants correlated with HLA match (Fig. 9). At 1 year, graft survival was 81 ± 2.8 percent in

0 mismatched, shipped kidneys, compared to 60.38 percent in 4 antigen mismatched organs, transplanted locally (p <0.001).

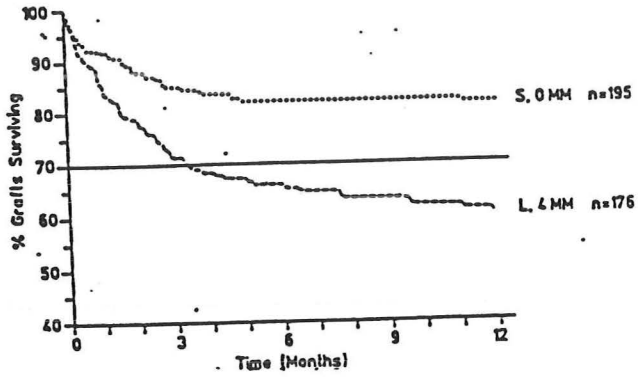


FIGURE 9. Graft survival in second cadaver-donor transplants. (From: Opelz et al., New England Journal of Medicine, 1988.)

The data of the UCLA registry is shown (Fig. 10) as percent 1-year survival, percent 3-year survival and half-life. Patients transplanted after 1980 are considered to have had adequate HLA-DR typing for the analysis to be valid. In each of the three yearly cohorts, the 0 A,B,DR mismatched transplants clearly had superior graft survival. The half life of such grafts performed in the 85-86 period was 11.3 years, compared to 6.3 years for kidneys transplanted in the same time period with no HLA antigens in common.

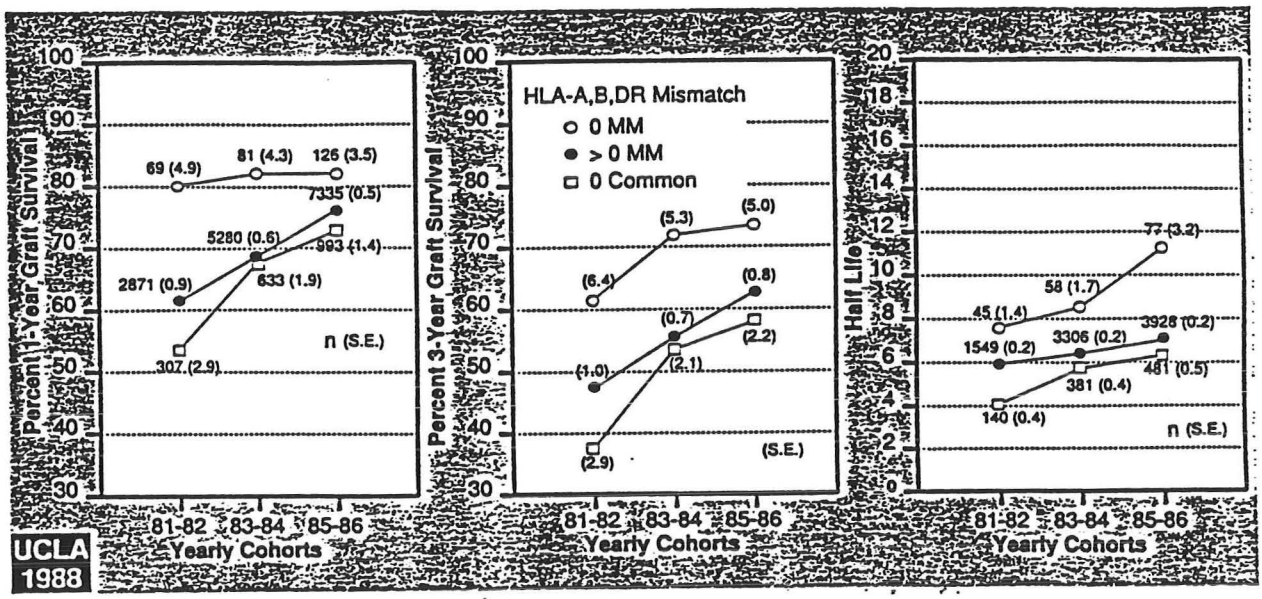


FIGURE 10. HLA-A,B,DR matching and long-term outcome. (From: Cho et al., Clinical Transplants, 1988.)

Similar results were reported by San Filippo et al., from the SEOPF registry (Fig. 11). Here shared kidneys with good matches are again compared with locally transplanted organs with only 1 or zero HLA antigens matched. The difference between the two groups showing the beneficial effect of HLA matching over a 2-year period is quite substantial.

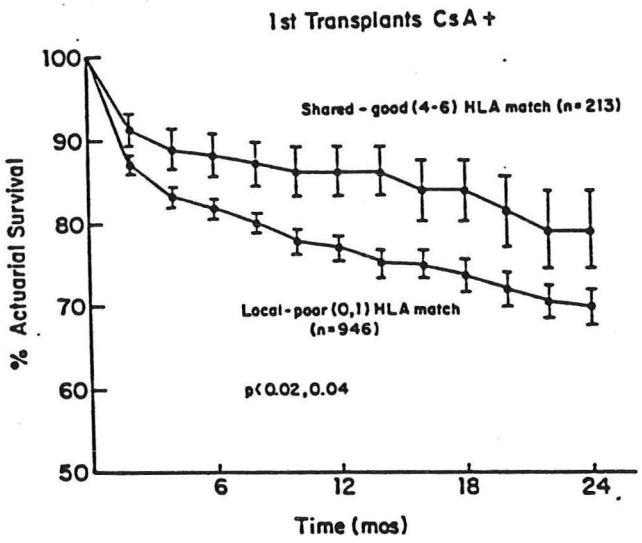


Fig 11 Comparison of Actuarial Survival

FIGURE 11. Comparison of actuarial survival. (From: Sanfilippo et al., Transplantation Proceedings, 1989.)

Fig. 12, shows data collected from the centers served by the New England Organ Bank. The results are based on antigens matched, considering only HLA-B and HLA-DR. The group with 4 matched antigens did very well and those with zero matches had a significantly lower graft survival.

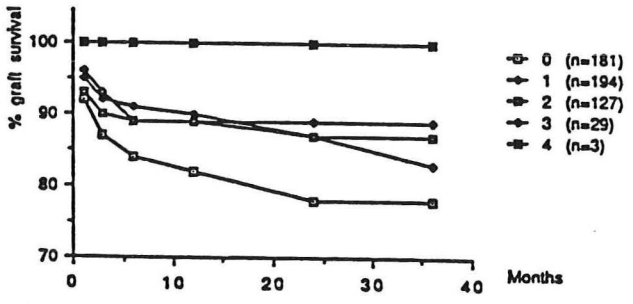


FIGURE 12. First cadaver grafts treated with cyclosporine. (From: Carpenter et al., Transplantation Proceedings, 1989.)

HLA matching has also been shown to influence the results of heart transplantation. However, the analysis is difficult because relatively few well-matched organs are transplanted. In this study there were only 73 cases with less than 2 HLA-B,DR mismatched antigens. The difference between these better matched and the rest of the recipients was of borderline significance (p = 0.05).

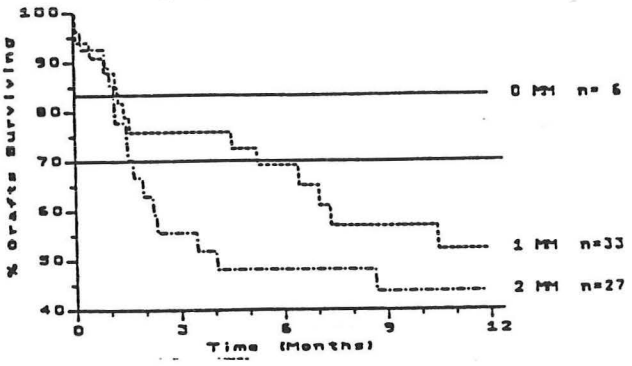


FIGURE 13. Actuarial survival rates of first heart transplants. (From: Opelz, Transplantation Proceedings, 1989.)

C.2. Minor histocompatibility antigens

Minor histocompatibility antigens are targets of host-versus-graft and graft-versus-host reactions that occur when organs or tissues are exchanged between members of the same species who, although genetically not identical, are matched for the major antigens (MHC). Genes encoding minor antigens map outside the MHC.

Clinically, the effect of mismatching for minor histocompatibility antigens is observed when kidneys transplanted from an HLA-identical

Table 3. MHC restricted cytotoxic T cell responses against minor histocompatibility antigens

CTLs Derived From	HLA Typing CTLs	Minor Histocompatibility Antigens
1. AML ♂ post bm grafting	HLA- (A2), A2, (B27), B(62)	HA-1
2. AA ♀ post bm grafting	HLA-A1, (A2), B7, B8	HA-2
3. AML ♂ post bm grafting	HLA-(A1), A11, (B8), Bw60	HA-3
4. CML ♀ post bm grafting	HLA-(A2), A3, B18, B44	HA-4
5. AML ♀ post bm grafting	HLA-(A2), A29, (B44), B49	HA-5

( ) MHC restricting antigens

(From: Goulmy, Transplantation Review, 1988.)

sibling donor are rejected or when a recipient of HLA-identical bone marrow develops graft-versus-host disease.

Relatively little is yet known about these antigens in man. Goulmy has succeeded in generating cytotoxic T cells against the human H-y antigen and also against several other specificities which have been designated HA-1 through HA-5. A review written by Goulmy last year summarizes these experiments in detail.
Recognition of the HA antigens is class I HLA restricted. HLA-A2, A1, B8, B44 are the antigens most often used. Elegant experiments with cultures of bone marrow cells have demonstrated that the antigens are expressed in myeloid precursor cells.

D. The antibody barrier

Development of antibodies against HLA antigens constitutes the most formidable barrier to kidney transplantation.

Anti-HLA antibodies are induced by various stimuli including blood transfusions, previous transplants and pregnancy. Once formed they may persist for years. On many lists of patients awaiting transplantation 25% or more may have such antibodies and since the antibodies act as an impediment to transplantation, the proportion of highly sensitized patients waiting to be transplanted tends to increase.

To detect anti-HLA antibodies before transplantation, serum from prospective recipients is screened regularly against panels of lymphocytes. Broad reactivity with such a panel usually means a long search for a compatible kidney.

Lymphocyte crossmatching is done immediately before transplantation. The recipient's serum is incubated with the potential donor's lymphocytes and lymphocytotoxicity is generally used to assess antibody activity.

The tissue typing laboratory must avoid two serious dangers:

1) a very sensitive crossmatch must be done to avoid hyperacute rejection;

2) inappropriate denial of a transplant (because of fear of rejection) must also be avoided.

Some of the known facts surrounding this dilemma will now be discussed.

Table 4. The antibody barrier in kidney transplantation

1. Preformed antibodies to donor HLA antigens cause hyperacute rejection
2. Early non-function correlates with presence of antibodies
3. Early graft failures can be predicted by more sensitive crossmatches
4. Rejection is associated with appearance of anti-donor antibodies in serum
5. Chronic vascular rejection is believed to be antibody mediated

While high dose steroids and anti-lymphocyte antibodies are quite effective in the treatment of cellular rejection, antibody-mediated rejection is generally refractory to such therapeutic pressures.

The panel reactive antibody (PRA) is usually expressed as the percentage of the panel donors whose lymphocytes are killed in the cytotoxicity test by a recipient's serum.

The group from Birmingham, Alabama looked at three groups of patients with high PRA (Table 4). In the first group the high

Table 5. Effect of transfusions on antibody formation and transplantation in sensitized recipients*

	High PRA Group		
	Sustained N = 46	1-peak N = 22	Several peaks N = 24
Patients transfused	83%	9%	29%
Transfusion number	15.3	1.9	4.0
Patients transplanted	4%	32%	17%

*modified from Deierhoi et al., Transpl. Proc. 1989.

reactivity of anti-HLA antibodies was sustained over a long period of time, in the other two the PRA fell by more than 30%. Two things were clear: patients with sustained elevated PRA were those that received a greater number of transfusions during the period of study; their chances of receiving a transplant were very small.

D.1. Antibodies against T cells

Because of the selective distribution of HLA molecules on lymphocytes, antibodies against class I antigens (HLA-A,B and C loci) bind to both T and B cells, while those against class II antigens (HLA-DR, DQ and DP loci) attach to B cells. When the patterns of reactivity against panel lymphocytes are analyzed it is sometimes possible to recognize the specificity of the antibodies present and these donor antigens can then be avoided. In most cases however, the patterns are too complex for easy recognition.

Not all antibodies that react with T cells are directed at the HLA antigens. Some autoantibodies (as may occur in patients with systemic lupus erythematosus and other conditions) as well as antibodies against other unidentified antigens of lymphocytes can also be the cause of panel reactivity with recipient serum. Many of these other antibodies are of low avidity, tend to react preferentially at low temperatures and frequently are IgM rather than IgG.

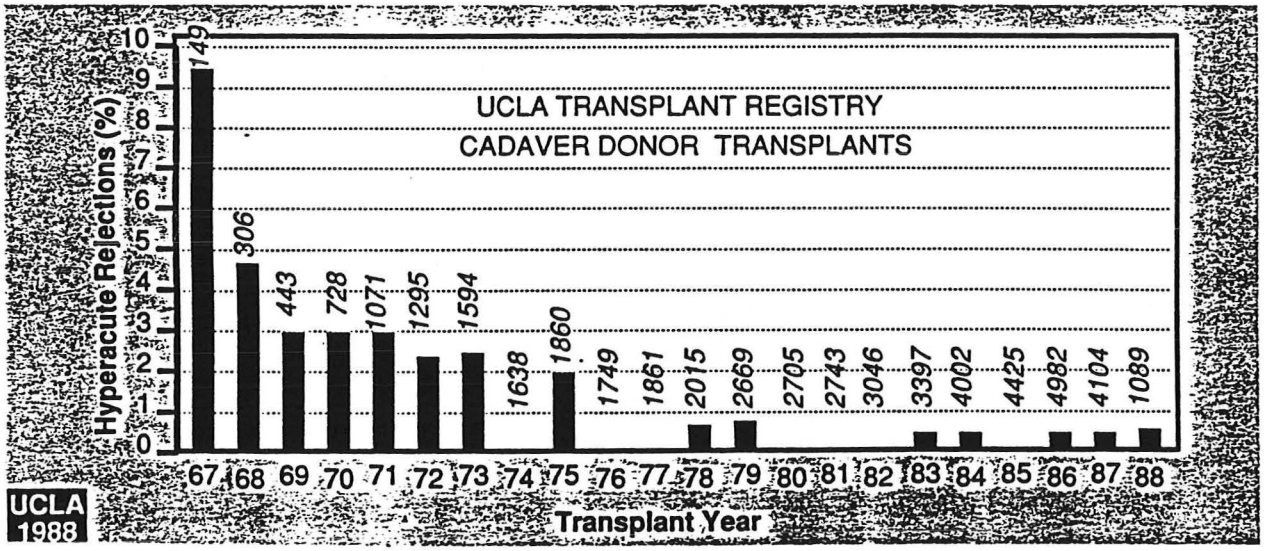


FIGURE 14. Yearly incidence of hyperacute rejections reported to the UCLA Transplant Registry. (From: Cecka et al., Clinical Transplants, 1988.)

The incidence of hyperacute rejection, as reported to the UCLA transplant Registry is shown in Figure 14, taken from a paper by Cecka and Cho. The importance of crossmatching with donor T cells was only recognized in the late 1960's. When its use became

widespread the incidence of hyperacute rejection was markedly reduced.

Sensitization as detected by cytotoxicity against panel T cells appears to have a significant effect on transplant outcome.

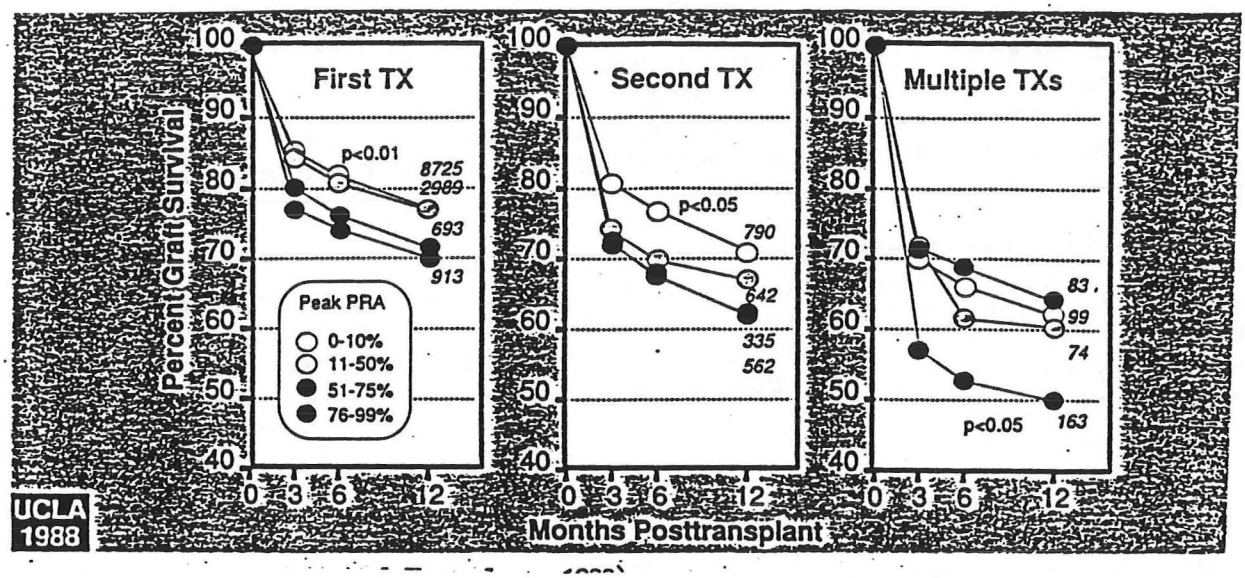


FIGURE 15. The effect of sensitization on transplant outcome. (From: Cecka et al., Clinical Transplants, 1988.)

Recipients of first transplants with PRA greater than 50% had worse graft survival. For second transplants even moderately sensitized (11-50% PRA) had worse outcome. For recipients with third or fourth grafts, graft survival was lower even when no antibodies were detected but those with high PRA had the worst outcome.

D.2. Antibodies against B cells

The question of whether B cell antibodies are also deleterious to kidney allografts has been debated extensively. Many transplants with a positive B cell crossmatch against the donor have been performed without evidence of harmful effects. However, some centers have observed early rejection when the recipient had high titer antibodies against the B cells of the donor.

Because of the differential expression of HLA antigens on lymphocytes, class II antigens are detectable on B cells but not on resting T cells, class I antigens are expressed on both. Thus antibodies positive on B cells and negative on T cells are usually anti-class II antigens. However, in some cases low level class I antibodies are also detectable on B cells and not on T cells,

because B cells have more antigen and may be more susceptible to lysis.

Table 6. Different types of antibodies cytotoxic for B cells

Antibody Type	Absorption with platelets	Susceptible to DTT
Class I	yes	no
Class II	no	no
"Auto-antibodies"	no	yes

In addition, a third type of B cell antibodies, usually of the IgM isotype, are auto-antibodies or antibodies directed at other non-HLA antigens such as B cell surface immunoglobulins.

Mohanakumar and coworkers recently examined the reactions of 17 sera from recipients that were transplanted with positive B cell crossmatches. Ten of them had class I specificity although they were not reactive with donor T cells. Four demonstrated class II specificity and the remaining three were IgM antibodies which were inactivated by DTT. Details of these experiments are given in Table 6.

Table 7. Definition of B cell antibody specificity by differential absorption

	Crossmatch Results (B-AHG-CDC) After Absorptions with				
Recipient	Unabsorbed/ Untreated	Platelets	B-LCLs	DTT Treated	Antibody Specificity
RS	Pos—1:4	Neg	nt	nt	I
MB	Pos—1:2	Neg	nt	Pos—1:2	I
KO	Pos—1:4	Pos—1:4	Neg	Pos—1:4	II
DF	Pos—1:4	Pos—1:4	Neg	Pos—1:4	II
TB	Pos—1:4	Pos—1:4	nt	Neg	Auto
RC	Pos—1:2	Pos—1:2	nt	Neg	Auto

(From: Phelan et al., Transplantation Proceedings, 1989.)

Irreversible graft rejection occurred only in the group with anti-HLA class I antibodies.

Noreen and coworkers have also reported a deleterious effect in kidney transplants performed with a positive B cell crossmatch against the donor. Part of their data is given in the following table.

Table 8. Graft survival of kidney allografts performed with a positive crossmatch against B cells of the donor*

B cell Crossmatch	Number recipients	Graft Survival 1 yr	2 yrs	p
Negative	665	88%	82%	
Positive	49	71%	66%	0.0006

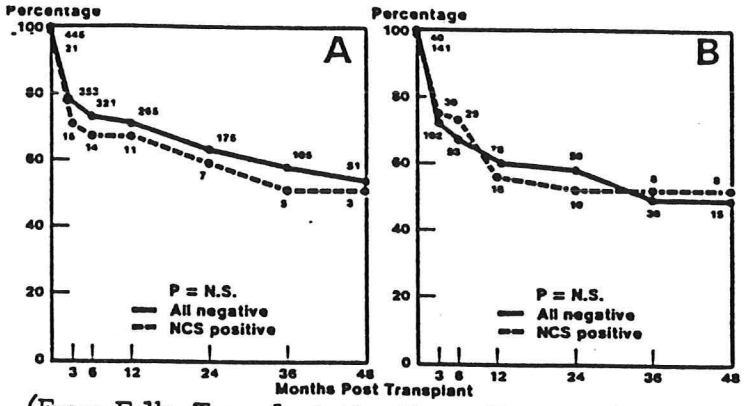
*Modified from Noreen et al., Transpl. Proc. 21:691, 1989.

D.3. Historical versus current serum crossmatches

The risk of hyperacute rejection in recipients who at the time of the transplant have antibodies against HLA antigens of the donor is well established. This knowledge led to a policy of not transplanting if a positive crossmatch against donor cells is obtained with any of the collected recipient sera. However, accumulation of sensitized recipients who could not be transplanted because of old sera with high PRA, led Cardella and coworkers in 1982, to initiate a study in which patients were accepted for transplantation if their current serum was crossmatch negative against donor T cells, even though there might exist past sera that were positive. The first series of 15 patients, and a follow-up study including 61 recipients, showed no statistically significant differences in graft survival between patients who had "historical" positive crossmatches and those recipients in whom all the sera were negative.

Subsequently, the American Society for Histocompatibility and Immunogenetics conducted a survey which was reported by Goeken in 1985, and essentially confirmed the reports from Toronto. However, this study lacked a concurrent control group and the wording of the conclusions had to be made with reservations. They ended saying that "while a remote positive crossmatch may remain a relative contraindication, it need not be considered an absolute contraindication to transplantation".

Depending on their particular bias, some groups accepted these new findings with enthusiasm and implemented very liberal policies in which only the serum of the day of the transplant was considered relevant for crossmatching against the prospective donor. Other centers were slow to accept such a drastic change in transplant practice. The period of time for calling a serum "historical" or "remote" varied greatly, with many centers requiring 3 or 6 months.



(From: Falk, Transplantation Proceedings 1985).

FIGURE 16. Positive crossmatch study: actuarial graft survival of first transplants (A) and multiple transplants (B).

Then reports appeared suggesting that anti-donor antibodies did have an adverse effect on graft survival, and that this was particularly true in recipients who had previously rejected one or more kidneys.

Table 9. Positive historical crossmatches decrease graft survival in primary and regrafts: actuarial graft survival (%)

Months	Pos X-M (Primary)	Neg X-M (Primary)	Pos X-M (Regraft)	Neg X-M (Regraft)
1	76.9 ± 6.7%	92.8 ± 1.2%	73.6 ± 8.6%	85.6 ± 3.1%
3	74.3 ± 7.0%	89.0 ± 1.4%	65.4 ± 9.4%	81.5 ± 3.5%
6	68.8 ± 7.5%	87.4 ± 1.5%	53.1 ± 9.9%	78.9 ± 3.7%
12	67.9 ± 7.4%	86.2 ± 1.6%[a]	50.7 ± 9.8%	78.9 ± 3.7%[b]

(From: Turka et al., Transplantation Proceedings, 1989.)

Turka et al. examined retrospective crossmatches in 70 recipients who had at least one positive crossmatch against donor T cells six or more months prior to transplantation (drawn from a series of 742 cadaver kidney transplants). The analysis showed that positive historical crossmatches against an allograft donor can be detrimental to graft survival. However, no hyperacute rejection was seen.

The authors concluded that "despite the adverse effect of prior donor-specific antibodies on graft survival, the majority of these patients can still be successfully transplanted. Given the rising proportion of sensitized patients... (centers) may purposefully

choose to sacrifice improved graft survival to increase the probability of transplanting these individuals".

D.4. Flow cytometry crossmatch

Garovoy et al., reported in 1983, that flow cytometry was a more sensitive method for detecting harmful antibodies against donor histocompatibility antigens. In more recent work these authors evaluated 117 cadaveric kidney transplant recipients. All were negative by T cell cytotoxicity with antiglobulin yet 38% were found to have anti-donor T cell antibodies by flow cytometry. Their results are summarized in the following table.

Several other groups have reported results of crossmatching which essentially confirmed these observations. Flow cytometry was found to be more sensitive, it detected certain non-complement-fixing antibodies that were missed by other techniques, and it appeared to be predictive of early graft rejection. The following results are taken from a report by Opelz and coworkers.

Table 10. Role of flow cytometry crossmatching (modified from Garovoy et al., 1985)

Patient Group	Number	1 yr Graft Survival
PRA <10%		
FACS T Negative	68	70%
FACS T Positive	35	48%
PRA >10%		
FACS T Negative	19	72%
FACS T Positive	26	36%

Terasaki and coworkers found that patients sensitized by a previous graft were much more likely to reject if they were crossmatch positive against the donor by flow cytometry. In fact, their results showed no difference in primary kidney allografts.

Table 11. Pretransplant flow cytometric crossmatches and graft outcome within two months posttransplant

	Irreversible/ Chronic Rejection	Reversible Rejection	No Rejection
Positive crossmatch (n = 11)	5	5	1
Negative crossmatch (n = 131)	15	51	65

Fisher's exact test, positive versus negative:

Irreversible/chronic versus reversible or no rejection: $p < 0.01$.

Irreversible/chronic or reversible versus no rejection: $p < 0.01$.

(From: Daniel et al., Transplantation Proceedings, 1989.)

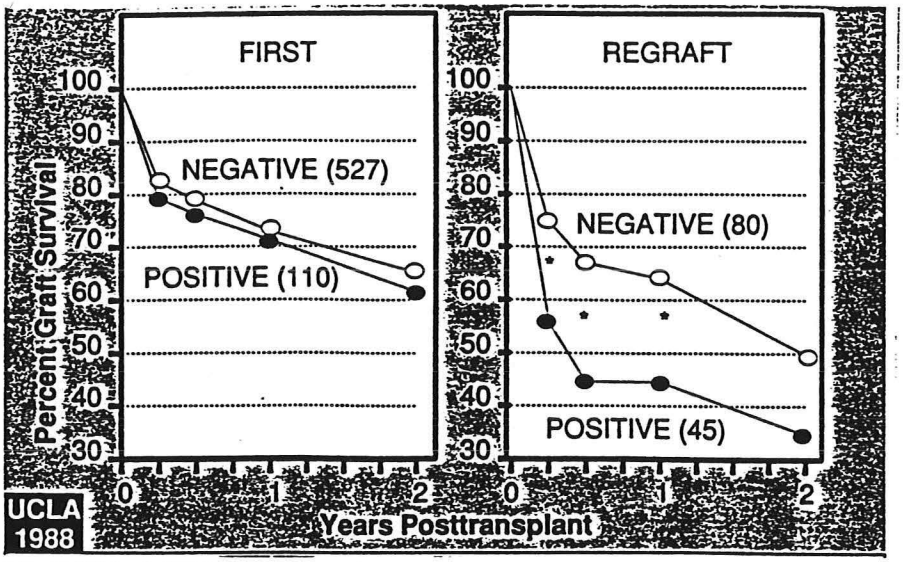


FIGURE 17. Graft survival and the FCXM. (From: Cook et al., Clinical Transplants, 1988.)

Also Kerman found that positive flow cytometry crossmatches in patients with negative crossmatches by the antiglobulin technique correlated with transplant outcome in regrafted patients and not in recipients receiving their first kidney allograft.

In a recent discussion, Terasaki suggested that antibodies present after rejection of a first graft are different from antibodies elicited by pregnancy and transfusion. Three differences were noted:

1) patients with antibodies receiving regrafts have a higher incidence of delayed graft function, while first graft recipients with antibodies do not show that effect;

2) historic positive antibodies appear to be more important in second graft recipients, than in first grafts;

3) flow cytometry crossmatching correlated with the outcome of regrafts, but not of first grafts.

D.5. Management of sensitized recipients

Here we will discuss some of the strategies that have been proposed for management sensitized patients who cannot be transplanted because of the presence of antibodies.

Three main approaches will be reviewed. The first is the identification of those recipients who have autoantibodies or other T cell reactive antibodies not reactive with HLA antigens. Many of these recipients have been transplanted safely in spite of a positive crossmatch. The other two approaches are new ways to deal with sensitized recipients who have a variety of antibodies against class I HLA antigens. The first of these approaches is the identification and selection of acceptable mismatches. The final approach to be discussed consists of the removal of anti-HLA antibodies by plasmapheresis or by extra-corporeal immunoabsorption.

Autoantibodies are not uncommon in transplant recipients. We observed and reported such a case several years ago. The patient had a positive crossmatch with an HLA-identical sibling donor. On further testing, the antibodies were found to react with the patient's own cells. The reason for the development of autoantibodies in this case was not clear. In other patients a cause can be found. Some have SLE, in others it is a virus infection, or a drug that appears to trigger autoantibody

Table 12. Differentiation between HLA antibodies and autoantibodies

Laboratory Procedure	Results	
	HLA Antibodies	Autoantibodies
Temperature of incubation	broad	$4^{0}C$ pos, $37^{0}C$ neg
Reducing agent (DTT)	no effect	inactivated
Reactivity with CLL cells	positive	negative
Reactivity with autologous T cells	negative	positive
Wash after incubation	positive	some become negative

production. Most of these antibodies react preferentially in the cold and are of the IgM type. Several laboratories have begun to utilize reducing reagents such as diethyltriethol (DTT). Brief exposure of the serum to such a chemical inactivates IgM, but does not change the activity of IgG antibodies.

Terasaki recently summarized the combined experience of several centers on the use of DTT to eliminate crossmatches due to IgM antibodies.

It should be pointed out that not all IgM antibodies are proven to be harmless. Several cases of recipients having high titer antibodies against class II HLA antigens were reported to have rejected and all of them apparently had IgM antibodies.

TABLE 13

Crossmatch Positive - DTT Negative Transplants

	n	1-Month Function	
Iwaki	29	28	
Barger	17	14	
Al Arif	15	15	
Senitzer	5	5	Function at 1 Month
Langley	3	3	
Total	69	65	94%

(From: Terasaki et al., Clinical Transplants, 1988.)

While an HLA-matched donor will be virtually always non-reactive with anti-HLA antibodies, perfect matching is in practice difficult to achieve. Several groups have therefore undertaken to try to determine whether "acceptable mismatches" could be identified.

The approach used is based on careful work up of the antibody profile of a given recipient in the hope of identifying which HLA antigens, although not present in the recipient, are nevertheless not recognized by the serum antibodies. Panel analysis and microabsorption have been used for this purpose. Application of the microabsorption method is illustrated in Table 13.

It has been suggested that many people develop tolerance to non-shared maternal HLA antigens and that acceptable mismatches tend to coincide with non-inherited maternal (but not paternal) haplotypes. Other groups have not been able to confirm these interesting observations.

TABLE 14. Application of the microabsorption method in determining acceptable mismatches

Patient S: HLA: A2, A11, B51								PRA*: 100%	
Platelets				Lymphocytes				Antigen tested	Crossmatch
A1	A24	B8	Bw61	A1	B8	B37		B37	–
A1	A2	B7	B8	A1	B8	Bw57		Bw57	+
A2	A11	B27	Bw62	A2	B7	Bw62		B7	+
A2		B35	Bw60	A2	A3	B51	B35	A3	–
A3		B7	Bw35	A3	B7	B44		B44	+

Acceptable mismatches include A3 and B37.

*PRA: panel reactive antibodies.

(From: van Eijck et al., Transplantation Proceedings, 1989.)

Removal of anti-HLA antibodies prior to transplantation is another strategy used to transplant highly sensitized recipients. Two methods have been employed: a) plasma exchange and b) extracorporeal immunoabsorption. The latter utilizes a special column that depletes IgG by letting it bind to Staph protein A. In addition, the recipients may be given immunosuppressive drugs to avoid resynthesis of the antibodies.

Preliminary results have been quite encouraging. These recipients were all highly sensitized with an average PRA of 92%. They were given cyclophosphamide and prednisone to prevent antibody resynthesis while waiting for their transplant.

TABLE 15

TRANSPLANTATION				
Patient no	Time (wk) between IA and transplantation	Time (mo) post transplantation	Current creatinine (μmol/l)	No of rejection episodes
1	4	23	220	1
2	30	14	170	1
3	49	8	150	1
4	6	Failed at 1 yr		2
6	4	Never functioned		1
7	36	3	124	0
8	10	3	144	0

(From: Palmer et al., Lancet, 1989.)

E. T cells in graft rejection

The role of lymphocytes in allograft rejection was established in the early days of transplantation immunology. It soon became clear from the work of Billingham, Brent and Medawar that adoptive transfer of accelerated rejection of skin grafts could be accomplished easily by the injection of sensitized lymphocytes while serum from immune mice was largely ineffective.

In the early 1960's, Gowans labelled lymphocytes and found that small lymphocytes were long-lived, recirculated through the blood and underwent blastic transformation on coming in contact with allogeneic tissue. The commonly used surface markers of lymphocytes had not yet been discovered and the functional aspects of these cells were largely unknown.

It now appears that many different types of lymphocytes can be involved in the rejection of organ allografts. Class II antigens appear to elicit the strongest response in CD4-positive T cells which include helper cells, cytotoxic cells against class II antigens, as well as cells involved in delayed-type hypersensitivity. Differences involving class I MHC antigens induce responses mainly in CD8-positive T cells. Especially cytotoxic T lymphocytes that can be involved in killing of cells in the transplanted tissue or organ. Thus a somewhat different complement of host T cells will invade and exert their functions in allografts depending on the antigens for which donor and recipient are mismatched.

TABLE 16. Kidney-infiltrating T-lymphocyte subset and sub-subset markers in rejecting and stable grafts. Reduced numbers of T-suppressor inducer ($CD45R^+CD4^+$) and T-suppressor effector ($CD11b^+CD8^+$) cells during rejection

Subset	Proportion of Cells (%)		p Value for Difference[1]
	Rejection	No Rejection	
$CD4^+$/Lymphs[2]	17.9 ± 1.9 (55)[3]	18.7 ± 1.7 (75)	0.8
$CD8^+$/Lymphs	31.8 ± 2.5 (55)	22.1 ± 2.1 (75)	0.004
$CD4^+/CD8^+$ Ratio	0.72 ± 0.1 (55)	0.98 ± 0.08 (74)	0.04
$CD45R^+/CD4^+$	4.4 ± 0.8 (25)	30.7 ± 2.3 (35)	0.0001
$CD11b^+/CD8^+$	3.7 ± 0.8 (42)	14.6 ± 1.3 (50)	0.0001

[1]Mann-Whitney U-test.
[2]For example, $CD45R^+/CD4^+$ = percent CD45R-positive cells within the $CD4^+$ subset.
[3]Mean ± S.E.M. (n).

(From: Totterman et al., Transplantation Proceedings, 1989)

E.1. Infiltrating T cells

The infiltrating inflammatory cells can be investigated by immunohistologic techniques using monoclonal antibodies for the various surface markers. Of particular interest are some of the newer markers that have been claimed to identify the functional subsets of CD4 and CD8 T cells. Thus CD45R-positive CD4 cells were shown to inhibit immunoglobulin production and to inhibit the proliferation of other T cells. These cells have been called "suppressor inducer" cells. The effects have been shown to be mediated by CD11b-positive CD8 cells which have been called "suppressor effector" cells.

In these experiments a decrease in "suppressor inducer" and "suppressor effector" T cells appeared to correlate with rejection.

Attempts have been made by several investigators to isolate the T cells from allograft biopsies to grow them in vitro in long-term tissue culture. Usually these experiments involve addition of IL2 and stimulation with donor accessory cells. Clones of T cells were then characterized on panels of stimulating target cells and by inhibition with monoclonal antibodies. These studies are very laborious and appear to have little practical application.

E.2. T cells in the blood

Monoclonal antibodies and flow cytometry have been used extensively to monitor T cell subsets in kidney transplant recipients.

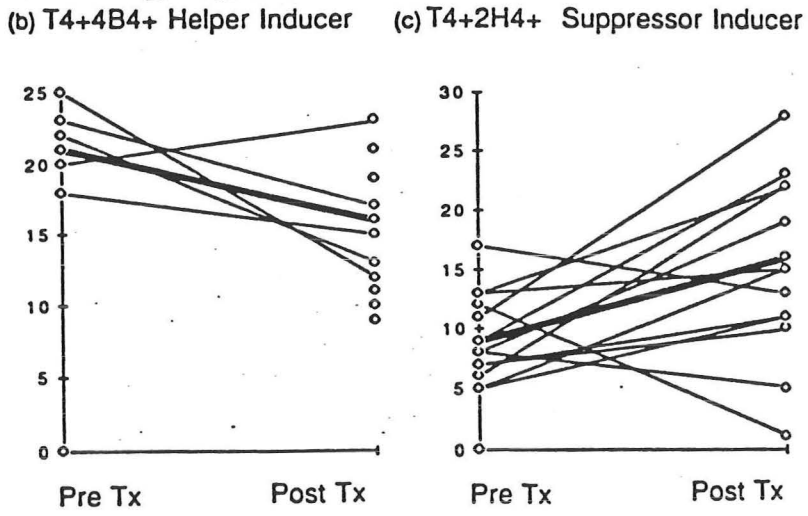


FIGURE 18. (From: Ramos et al., Transplantation, 1989.)

Leendert Paul and coworkers, observed that a decrease in the percentage of CD8-positive cells after transplantation appeared to correlate with either transplant rejection or CMV infection.

Ramos et al., in the laboratory of Carpenter, in Boston, studied the number of suppressor-inducer, helper-inducer and suppressor-effector T cells as defined by CD4, CD8, 2H4 and 4B4 monoclonal antibodies.

Patients with stable allograft function showed an increase in suppressor-inducer CD4-positive cells and an increase in CD8-positive suppressor-effector T cells.

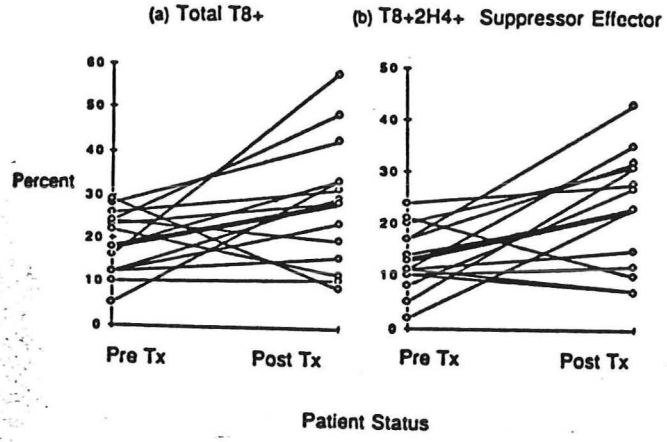


FIGURE 19. (From: Ramos et al., Transplantation, 1989.)

E.3. Which T cells reject grafts?

As discussed above, there is no simple answer. The phenotype of the infiltrating T cells that predominate in rejecting grafts and the T cell subset that can be used successfully to transfer allograft rejection depends in large part on the nature of the mismatched antigens. Thus, CD8-positive cytotoxic T cells destroy grafts mismatched for class I MHC and CD4-positive killers are important in class II MHC differences. In the usual situation, involving multiple MHC differences many different types of T cells are involved.

Minor histocompatibility antigens which also play a role in rejection of many grafts appear to be restricted by class I MHC antigens and are attacked primarily by CD8-positive cytotoxic cells.

F. State of the art and new approaches

The results of kidney transplantation have improved during the last few years. It is difficult to be sure how much of the better graft survival obtained is due to the use of cyclosporine A. This drug certainly has had a marked effect.

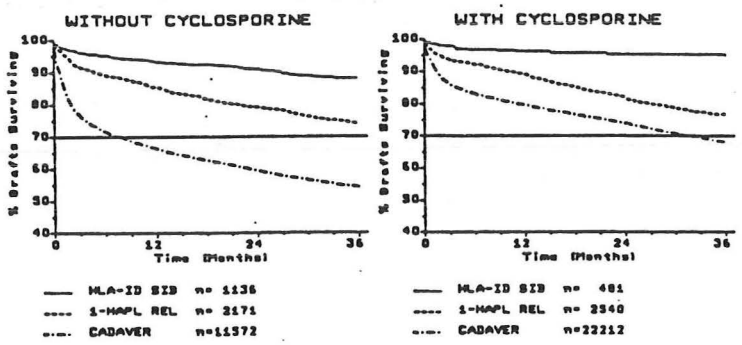


Figure 20. Actuarial graft survival with or without cyclosporine. (From: Opelz, Transplantation Proceedings, 1989.)

Other immunosuppressive drugs, which hopefully will be less toxic are becoming available soon.

There is an extensive list of monoclonal antibodies that could be used to prevent or to treat rejection. Most of the current experience is with OKT3. Preliminary results are being published with antibodies against other surface molecules of T cells such as the IL2 receptor. The clinical studies are laborious and lengthy. The advantages of monoclonal antibodies is that they offer reagents of uniform activity that can be better standardized that crude polivalent sera.

The evidence in favor of HLA matching to achieve long-term graft survival is overwhelming. Well-matched kidneys tend to survive many years more while the mismatched ones generally return to hemodialysis and possibly retransplantation.

The ultimate goal has always been to induce true specific tolerance to the alloantigens of the donor. Studies by Brent and coworkers in cynomolgus monkeys are of interest in this regard. Also of interest are the experiments of David Sachs on the induction of tolerance by grafting mixtures of autologous and allogeneic bone marrow cells into lightly irradiated recipients. These experiments have led to long-term chimerism and donor-specific tolerance without graft-versus-host disease and using non-lethal induction procedures in adult animals.

Liver transplants occupy a special position in organ transplantation. Hyperacute rejection is exceedingly rare and patients receiving combined liver and kidney transplants from the same donor enjoy a degree of protection of the kidney graft, even in the presence of preformed antibodies. Some of these effects may be due to dilution from massive transfusions given during the surgery. However, it is interesting that Pollard et al., have recently reported the release of large amounts of class I HLA antigens which could be detected shortly after transplantation. High levels of donor HLA were also found in the blood of longterm graft recipients with good graft function several years after transplantation.

Management of sensitized recipients is a major problem for which new solutions will have to be sought. With the availability of recombinant erythroproietin there will rarely be a need to submit renal transplant recipients to continued transfusions. Removal of antibodies using some technique of extracorporeal perfusion will be tested in those recipients who have already been sensitized.

Our group has been interested in the possibility of crossmatching donors and recipients for antigens that are not detectable in peripheral blood lymphocytes. We have therefore studied the reactivity of recipient sera with endothelial-specific antigens and more recently we have developed a crossmatch procedure using donor skin. Preliminary results suggest that crossmatching with donor skin may detect sensitization that is sometimes missed by the usual lymphocyte crossmatch and that the skin crossmatch may be able to predict early rejection in some recipients.

TABLE 17. Donor skin crossmatch and rejection during first week after transplantation.

Status of Recipient	Number Cases	Crossmatch Positive[a]	Early Rejection	Crossmatch Negative	Early Rejection
First graft	19	1	1	18	0
Re-graft	6	4	4	2	0
Total	25	5	5[b]	20	0[c]

[a]Staining of donor skin with recipient serum diluted 1:20 or more.
[b]Graft nephrectomy in 3; the other 2 had severe rejection with poor residual function.
[c]$p < 0.00001$.
(From: Moraes et al., Transplantation, in press)

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