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The Influence of Allogeneic Cells on the Human T and B Cell Repertoire

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Clinical transplantation is often complicated by rejection episodes, in which the immune system of the recipient reacts to the foreign transplantation (HLA) antigens on the graft. This immune response includes humoral and cellular components. In the first, B lymphocytes form antibodies to the HLA alloantigens. In the second, $CD8^+$ T lymphocytes recognize and react to HLA class I antigens, and $CD4^+$ T cells react to HLA class II antigens. The frequency and severity of these rejection episodes can be diminished by immunosuppressive drugs, HLA matching between donor and recipient, and immune modulation by blood transfusion. Effective HLA matching between donor and recipient is not always possible and often not necessary. Insight into the factors that influence the T and B cell repertoire after blood transfusion might lead to new approaches to improve graft survival.

EVER SINCE BILLINGHAM, BRENT, AND MEDAWAR SHOWED that the injection of allogeneic (a different individual's) cells into a newborn mouse induces lifelong immunological tolerance for the donor's tissues and organs in a proportion of recipient animals (*1*), transplantation immunobiologists have attempted to achieve a similar effect in the adult animal and in humans. However, in adult mice and rats tolerance can only be induced with physical, pharmacological, and biological immune modulators such as azathioprine, prednisone, cyclosporine A (CsA), total body irradiation, total lymphoid irradiation, antilymphocyte globulins, monoclonal antibodies, or combinations thereof. The multiplicity of protocols used underlines that in contrast to the immune system of the newborn, heroic suppressive measures are needed before the immune system of the adult will accept allogeneic cells as "self." The mechanisms leading to tolerance of allogeneic cells and tissues are only partially understood. It is clear that it is not due solely to deletion of the alloreactive T and B lymphocytes. In many instances, the function of these alloreactive cells is actively suppressed by regulatory mechanisms involving both T cells and humoral factors. Recently, the work done in this field has been lucidly summarized (*2, 3*).

The present overview is confined primarily to studies on the induction of tolerance in humans (and other primates), which although clinically relevant have less detailed immunologic mechanisms than do studies in rodents, because well-defined congenic inbred strains are not available, and because, often, in vivo experiments cannot be performed. The effect of pretransplant blood transfusions (PTBTs) on humoral and cellular immunity and on the outcome of the organ transplant will be emphasized. In humans as in all other species, individuals vary widely, both qualitatively and quantitatively, with respect to the specificities recognized by the T and B cell allorepertoire. In extreme cases, certain individuals may lack cytotoxic T lymphocyte precursors to specific alloantigens of the major histocompatibility complex (MHC; in humans, HLA). We shall refer to this situation as a "hole" in the T cell repertoire. The influence of individual variability and especially of the natural holes in the repertoire should be taken into account in attempts to induce transplantation tolerance in humans.

We shall first briefly review the state of the art in organ transplantation and its current challenges, then describe the events that led to the identification of the holes in the T and B cell repertoire, speculate on the possible mechanism, and finally suggest how these findings could lead to new approaches to the biological management of clinical organ transplantation.

Historical Perspective

Fully 20 years after the report of Billingham *et al.* (*1*), it was realized that the infusion of allogeneic cells could down-regulate the homograft reaction although it did not induce tolerance in humans. Clinical renal transplantation, which started in 1955, played a central role in this achievement. It was first successfully done between monozygotic twins. Simultaneously it was shown that

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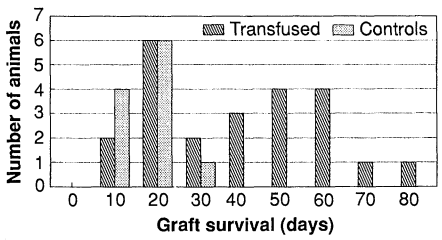

Fig. 1. Bimodal distribution of renal allograft survival in immunosuppressed monkeys given three pretransplant blood transfusions (PTBTs) from an unrelated donor. All recipients received daily intramuscular injections of azathioprine (2 mg kg^{-1}) and prednisolone (1 mg kg^{-1}), which were administered for a maximum period of 45 days, starting on the day of transplantation. [Reprinted from (*10*) with permission, ©1982 *Transplantation*]

blood transfusions can induce the production of antibodies to leukocytes (that is, HLA) (*4*). To overcome the rejection of allografts, in 1960 Schwartz and Dameshek introduced 6-mercaptopurine, an effective immunosuppressant that made clinical renal allografting a therapeutic reality, although less than 50% of renal grafts from related donors initially survived longer than 1 year (*5*).

The HLA system of alloantigens was recognized in the early 1960s and methods were developed to determine the tissue type of individuals. Prospective studies in nonhuman primates and human volunteers showed that choosing HLA-compatible donors prolonged skin graft survival in both related and unrelated donor-recipient pairs (*6*), indicating that HLA was functionally homologous to the murine MHC, H-2. It was then shown retrospectively that the prognosis of renal allografts was also significantly influenced by the degree of HLA compatibility, both with living related and unrelated donors (*6*). The importance of HLA typing was further documented by several reports that preexisting leukocyte antibodies could induce hyperacute graft rejection (*7*). This resulted in a reluctance to transfuse patients waiting for a renal transplant and forced the introduction of cross-matching before transplantation.

At the end of the 1960s organ exchange organizations were established to improve graft survival by donor-recipient matching (*6*). These activities generated registries that documented the outcome of the grafts in many patients. It was found, however, that the expected improvement did not occur; instead, graft survival rates worsened. Opelz identified the absence of PTBTs as a major cause of the poorer results. His findings were soon confirmed by many, though not all, centers (*8*).

Nowadays, intensive and effective immunosuppression combined with improved HLA matching, especially for the class II antigens, and (perhaps) PTBTs have led to a 1-year graft survival approaching 90%. There are, nonetheless, quite a few unsolved problems:

1) The difference between 90 and 100% survival is very significant, especially for the 10% of the patients who lose their graft and have a reduced life expectancy (*9*).

2) Immunosuppression results in complications such as CsA-induced nephrotoxicity and higher incidence of malignancies and infections.

3) PTBTs carry the risks of transmission of infections and alloimmunization.

4) Although HLA matching between donor and recipient improves graft and patient survival, many grafts that function well for years are completely or partially mismatched for HLA (*9*). Despite the overall worse prognosis for mismatched rather than well-matched grafts, the message is clear: HLA matching is an effective approach to improve overall graft survival, but it is not necessary for all individual patient-graft combinations.

The biological and clinical challenge is to determine for whom HLA matching is necessary, and who can do without it. We shall approach this challenge from the angle of the PTBT. Clearly the main weakness of the clinical evidence for the benefits of PTBTs in humans is the almost complete absence of well-designed prospective randomized trials. We shall review studies indicating that only some of the PTBTs improve graft survival and others do not. This will in part negate the above-mentioned criticism because, although all patients received a PTBT, the patients were divided into two groups: one in which PTBTs down-regulated homograft reactivity and another in which it did not. In this context, we shall also discuss an in vitro approach to the study of the mechanisms by which PTBTs have this down-regulatory effect.

The Importance of HLA Matching

Pretransplant blood transfusions appear to be effective in some individuals, but not in others. For instance in rhesus monkeys that received a suboptimal immunosuppression regime (azathioprine and prednisone) that did not prolong renal allograft survival by itself, PTBTs induced a significant increase in graft survival in 14 of 25 animals, whereas no effect was seen in the others (Fig. 1) (*10*). A similar heterogeneity exists in humans. The challenge is to identify the variables influencing the effectiveness of PTBTs.

One of these might be the time interval between the PTBT and the actual transplantation. In rodents, intervals between 50 and 3 days before transplantation have been described to be optimal in inducing a PTBT effect. The situation in humans seems to be different; PTBTs given up to 2 years before transplantation or longer may still be effective, whereas blood transfusions immediately before or during the operation were less effective (*11*).

The second variable, the number of PTBTs required for an optimal graft-enhancing effect, is also controversial. In several studies a single transfusion produced a clear PTBT effect, but in others the effects improved with increasing numbers of PTBTs. The number of transfusions needed for optimal effects might also depend on the specificity of the recipient's HLA class II antigens (*12*).

The third variable relates PTBT effectiveness to the cellular composition of the transfused blood: virtually every study indicates that the transfusion must contain leukocytes to induce the graft-protecting effect. Although "pure" platelet transfusion appeared to be promising in monkeys, the limited experience in humans has been disappointing (*13*).

Finally, what is the influence of HLA compatibility? In assessing the effect of HLA matching on the outcome of allografts to PTBT-treated recipients, one must consider the four immunogenetic combinations: (i) recipient and organ donor; (ii) PTBT, recipient, and organ donor; (iii) PTBT and recipient; and (iv) PTBT and organ donor. We have already discussed the effect of matching between recipient and organ donor and will abstain from discussing the effect of matching between PTBT and organ donor, although sharing of one RhLA DR antigen could lead to improved graft survival in the rhesus monkey (*14*), consistent with results obtained in rodents (*15*). In addition, donor-specific transfusions (DSTs), that is, the same individual donates both blood and organ and shares one *HLA* haplotype with the recipient (such as a parent or sibling), have excellent effects on graft survival (*16*). Given that one full *HLA* haplotype is mismatched between recipient and blood transfusion–organ donor, the results (almost identical to those for transplants between *HLA*-identical siblings) are remarkable. This raises the question of whether the sharing of one genotypically identical *HLA* haplotype between the recipient and the DST might have something to do with these excellent results.

Little is known about the effect of HLA class I matching between unrelated blood transfusion donor and recipient. Nubé *et al.* (*17*) observed a beneficial effect of such a matched PTBT on graft survival, whereas Albert *et al.* (*18*) were unable to confirm these

Table 1. Findings in two studies, showing that in hyperimmunized patients the leukocyte antibodies are less likely to be directed against the NIMA than the NIPA. For the combined studies, $\chi^2 = 14.1$; $P < 10^{-4}$. [Reprinted from (*25*) with permission, © 1989 *Immunology Letters*]

Antibody	First study		Second study	
	NIMA	NIPA	NIMA	NIPA
Negative	21	2	17	6
Positive	24	23	14	21

Table 2. HLA-DR mismatches are needed to induce renal allograft enhancement by DSTs. Patients were followed for at least 1 year and all clinical rejection episodes were confirmed histologically. No significant influence was observed for HLA-A, HLA-B, or HLA-DQ incompatibilities on the DST. Incompatibility and compatibility for HLA-DR between transfusion donor and patient are indicated. Incompatibility leads to a significantly better graft survival. $P = 0.012$ [From (*28*) with permission of the authors.]

Response	DST	
	HLA-DR incompatible	HLA-DR compatible
Rejection	6	9
No rejection	24	7

findings.

The influence of the HLA-DR match between blood transfusion donor and recipient was recently studied by Lagaaij *et al.* (*19*) in a cohort of patients who received a single blood transfusion from an unrelated donor. When the patients were divided retrospectively into two groups, those who shared one DR antigen with the blood transfusion donor and those who were completely DR-mismatched, a 20% difference in graft survival was observed, the latter having a graft survival rate similar to that of patients given no blood transfusions. These data suggest that sharing of a single DR antigen between blood transfusion donor and patient is essential to obtain a positive blood transfusion effect. The phenomenon is independent of the degree of compatibility between the patient and the kidney donor; within each match group, graft survival is superior when the blood transfusion donor shares an HLA-DR antigen with the recipient. This is in agreement with a report by Burrows *et al.*, who found similarly good graft survival in a small group of patients given transfusions of matched blood (*20*).

In a group of heart transplant patients treated with CsA, the necessity of HLA-DR matching between recipient and blood transfusion donor for a PTBT effect was again shown (*19*). In these patients rejection was diagnosed on the basis of the Billingham criteria (by histological biopsy) and not indirectly on the basis of clinical criteria only, as is often done in renal transplantation. Seven of eight patients who received one blood transfusion sharing a single DR antigen with the recipient had no rejection, whereas eight out of nine patients given a blood transfusion completely mismatched for HLA-DR had multiple histologically proven rejection episodes.

In patients who received a blood transfusion that matched at a single *DR* locus, fewer antibodies to the HLA class I alloantigens were formed than in a group of patients who received DR-mismatched blood transfusions (*19*). Further studies showed that if the blood transfusion donor was completely mismatched for HLA-DR, donor-specific cytotoxic reactivity increased after the transfusion, whereas after a transfusion with a single HLA-DR antigen match the donor-specific cytotoxic reactivity before and after blood transfusion did not change. Thus, in the almost 200 patients studied, graft survival was better, alloantibody formation less, and mixed lymphocyte culture (MLC) and cytotoxic reactivity remained unchanged when blood transfusion donor and recipient shared a single HLA-DR antigen (*19*). Preliminary data of van Twuyver, de Waal, and others show a significant decrease in cytotoxic T cell precursor (CTLp) frequency in five recipients after transfusion of blood from an unrelated donor who shared one *HLA* haplotype with the respective recipient (*21*). Against a third party, the CTLp frequency remained the same. Such a down-regulating effect was not observed after a completely DR-mismatched blood transfusion when either no change or an increase in CTLps was found. Heeg and Wagner also found a down-regulation of CTLps, which was dependent on the presence of $CD4^+$ cells, with a similar protocol in the mouse (*22*). These findings might provide a rational explanation for this effective approach to reducing homograft sensitivity in the clinic and might also be relevant outside the field of organ transplantation. Patients suffering from colon carcinoma who have received a blood transfusion have a significantly worse prognosis than those who have not been transfused (even if all other variables with an influence on outcome, such as hemoglobin level, had been stratified) (*23*). The potential management of this situation to prevent immunological down-regulation by using leukocyte-depleted transfusion is under study.

Noninherited Maternal HLA Antigens

Whereas transplantation happens to only a few of us and blood transfusion to many, all of us have been exposed to allogeneic cells from our mothers before, during, or after birth. What are the implications of this exposure for our B and T cell repertoire? We were confronted with this question when we studied patients who had formed broadly reactive antibodies against HLA in response to previous blood transfusion, pregnancy, or graft rejection. Such sensitized patients are difficult to transplant, because the cross-match with potential organ donors is almost always positive. A protocol was formulated to help these patients by identifying those HLA antigens (termed acceptable mismatches) to which they had not formed antibodies. A systematic study showed that these acceptable mismatches were often identical to or included in the noninherited maternal HLA antigens (NIMA) (*24*). About half of the patients with high panel reactivity do not form antibodies against NIMA, whereas this was not the case for the noninherited paternal antigens (NIPA) (Table 1).

Li Zhang and colleagues (*25, 26*) looked for a similar phenomenon at the level of the specific T cell precursors. The CTLp frequency to HLA of the mother was significantly lower than to that of the father in 32 children from nine families. In seven children, CTLps to the mother were not demonstrable at all, whereas they were present to the father in all cases (*25*). If we assume that having few CTLp cells leads to nonreactivity in vivo as well, these children may be tolerant for the noninherited antigens of the mother, a situation comparable to the one described as neonatal tolerance in mice. As in the latter case, the time and quantity of the exposure may be important, which may explain the apparent contradiction between these findings and the observation of leukocyte antibodies to the mother in the umbilical cord blood (*27*). It is conceivable that when maternal cells enter the fetal bloodstream later in pregnancy (when the immune system of the child is more mature), immunity rather than tolerance is induced.

A similar mechanism may be involved in the DR-matched blood transfusion effect and the low CTLp frequency against NIMA, in both cases one HLA-DR antigen is shared between donor and recipient and the other is mismatched. According to Lazda *et al.* (Table 2), the latter is an important prerequisite to obtain down-

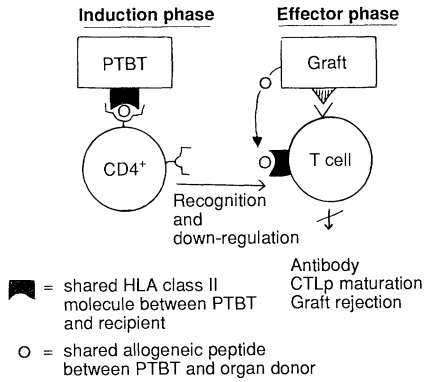

Fig. 2. Proposed mechanism. Induction phase: A one DR antigen–shared blood transfusion will, among other effects, activate $CD4^+$ T cells that recognize allogeneic peptides in the context of the shared HLA class II antigen. Effector phase: Recipient T cells that recognize donor antigen are activated and start to express HLA class II molecules. Allogeneic peptides shared between PTBT and organ donor will move to and be presented by the donor-specific activated T cells. These T cells serve as targets for the $CD4^+$ T cells induced by the blood transfusion, which will result in down-regulation of antibody formation, CTLp maturation, and graft rejection.

regulation of the homograft reaction (*28*). This might also be the reason why patients that receive a haploidentical bone marrow graft that is mismatched for HLA-DR have less graft-versus-host disease and slightly better survival than those mismatched for either HLA-A or HLA-B (*29*).

Toward the Mechanisms

Many different hypothetical mechanisms could lead to the PTBT effect, which we define as a down-regulation of the homograft reaction at the B and the T cell level. In this section we will focus on a T cell subset that might play a key role in the PTBT effect without claiming to know the precise mechanism of the PTBT effect.

The DR-matched blood transfusion effect, as opposed to NIMA-induced nonresponsiveness, was chosen for the study of the mechanism, because it has a control, the DR-mismatched transfusion. From the foregoing it is clear that to obtain a PTBT effect the following prerequisite sites are necessary: PTBT donor and recipient must share one DR antigen or a haplotype (*19*) and must be mismatched for the other one (*28*). Because HLA class II antigens play a central role and because both the B cell repertoire (HLA antibodies) and the T cell repertoire (CTL precursors) are down-regulated, it seems likely that a $CD4^+$ regulatory cell is involved.

Only a working hypothesis of the mechanism regulating the $CD4^+$ T cell can be suggested. Central to this hypothesis is the observation that PTBT works only if one DR antigen is matched and one is mismatched. The T cells of the recipient who is given a partial DR-matched transfusion will thus be confronted with not only alloantigens but also self DR antigens on the donor cells. These self DR antigens will presumably be loaded with donor peptides, including peptides derived from the mismatched class I and class II antigens (*30*). In contrast, the T cell repertoire of the recipient of a DR-mismatched transfusion will only be confronted with alloantigens and will not recognize them in a self DR-restricted fashion.

Our working hypothesis is that $CD4^+$ T cells restricted for self DR antigens recognize peptides of the mismatched antigens of the donor. They not only attack (or provide help for an attack) the allogeneic haploidentical DR^+ cells in the transfusate (down-regulating allogeneic stimulation), but also attack autologous activated (and thus class II–positive) $CD4^+$ helper cells that have taken up peptides from the DR-mismatched donor lymphocytes (down-regulating help) (Fig. 2). Such cells have been described (*31*). Whether this attack is made by the CD4 cells themselves or occurs indirectly via other cells is not yet established. In the rat, $CD4^+$ T cells are the mediators of PTBT-induced suppression of renal allograft rejection, consistent with the above speculations. For example, anti-CD4 treatment abrogates the induction of tolerance when peripheral blood lymphocytes are administered. In other words, $CD4^+$ cells are necessary for tolerance induction (*32*).

Such $CD4^+$ DR-restricted cells can only be part of the story. It remains to be explained, for instance, how donor specificity is achieved. We speculate that activated (class II) T cells with donor specificity are attracted to the graft and (because of their antidonor specificity) are more likely to take up donor peptides (Fig. 2). Only these CD4 cells are eliminated in the hypothesis proposed. It is possible that not only the recipient cells but also the blood transfusion donors might react against the alloantigens to which they are exposed, which leads to another consideration. As mentioned, the PTBT effect in humans is long-lasting. If the mismatched donor DR antigen is so critical, then PTBT mononuclear cells must remain viable for a long time and make the patient chimeric; hematopoietic stem cells in the buffy coat could qualify. This might also be the functional basis of an intriguing protocol introduced by Sachs in which T cell–depleted autologous or syngeneic mouse or swine bone marrow is mixed with non–T cell–depleted donor bone marrow that is MHC mismatched and reinfused into the irradiated recipient. The animals show a mixed chimerism and are tolerant of donor tissues (*3*).

Perhaps the main virtue of this hypothesis is that it is testable. We would predict that after a DR-matched blood transfusion. $CD4^+$ clones restricted by the matched DR antigen and that recognize peptides from the mismatched donor antigen can be isolated. Such clones might down-regulate CTLp maturation. Such clones will be absent after a fully DR-mismatched blood transfusion. To prove the presence of chimerism might be more difficult if the situation in the human is similar to that of the mouse, in which primarily the spleen and lymph nodes are chimeric.

The assumption that a DR-matched PTBT can down-regulate the number of CTLp that could otherwise be activated does not explain the improvement of graft survival when recipient and organ donor are fully DR-mismatched, although common epitopes on different alleles (for example, HV1 and HV2 in the case of HLA-DR3 and DR6) might provide a sufficient context for effective MHC restriction. Also the class I peptides shared between blood transfusion and organ donor may contribute to the specificity.

The above is in many ways reminiscent of the action of veto cells, which have been compared to antigen-presenting cells that do not up-regulate but down-regulate the CTLps, possibly by inducing anergy (*33*). The veto phenomenon has mainly been studied in the down-regulation of an allogeneic immune response across a class I difference only, whereas the almost immediate appearance of the phenomenon and its relatively short duration are not in agreement with the PTBT effect. Our working hypothesis is certainly not the first and most probably not the last to be formulated in trying to explain the PTBT effect in humans. For instance, Batchelor has hypothesized that suppression may be triggered not by class II peptides, but by T cell receptor–derived peptides (*34*). Other suggested mechanisms, mainly based on experiments in rodents, include aspecific and specific T suppressor factors, clonal deletion, down-regulation of IL-2 production (*35*), anti-idiotypic antibodies (*36*), enhancement (*37*), and combinations of these. As these are extensively reviewed in this issue, we will not describe them in detail here.

Clinical Relevance

To improve graft survival, it seems worth the effort to select blood transfusion donors that are matched at a single DR antigen (*19*). This matching might be of importance beyond the field of organ

transplantation. For example, patients suffering from colon carcinoma could be given completely DR-mismatched blood or leukocyte-depleted blood to prevent the induction of tolerance (*23*).

How does this hypothesis relate to our findings on NIMA? One would expect, on the basis of a diminished T and B cell response to NIMA, that maternal grafts should survive better than paternal ones, but they do not. The overall survival of grafts from unrelated females is, however, significantly worse than that of male grafts; the approximately equal survival of maternal and paternal grafts may be an expression of diminished homograft sensitivity specific for the maternal grafts (*38*). A prospective study on the relation between the CTLp frequency and graft survival outcome will have to give the answer. However, when rejection of a renal allograft is diagnosed on the basis of the histology, significantly less rejection is seen for maternal than for paternal grafts (*39*). At the serological level, Pohanka *et al.* (*40*) found no difference in antibody formation between maternal and paternal DST. However, studies by Bean *et al.* (*41*) and our own show a lower DST sensitization rate in cases of NIMA compared to NIPA.

Several studies have indicated that good graft survival in renal transplantation is associated with donor-specific cytotoxic nonresponsiveness (*42*). This nonresponsiveness does not involve the response to unrelated HLA alloantigens, which suggests that it is not merely an effect of the immune suppressive drugs, but rather a reflection of a specific immunoregulatory mechanism. One of the mechanisms involved might be specific clonal deletion. However, polyclonal activation of such a patient's peripheral blood mononuclear cells, which will bypass all specific immunoregulatory interactions (perhaps including the effect of the allopeptide-specific class II–restricted CD4 cells), resulted in an antidonor CTLp frequency of about 1:2000 (*43*), which is similar to the frequency of alloreactive cytotoxic T cells in normal individuals. Donor-reactive clones isolated after such a polyclonal activation are specific for all mismatched HLA class I and class II antigens of the kidney donor (*43*) and have a repertoire similar to that of graft-infiltrating cells from patients with an irreversible rejection (*44*). Thus, the potential donor reactive repertoire in cytotoxic-nonresponder patients is neither deleted nor changed with respect to the specificity. In agreement with this, the graft survival of cytotoxic nonresponsive patients is only marginally better than that of cytotoxic-responsive patients (*42*). The donor-specific cytotoxic T cell responses in such patients are probably suppressed by an active mechanism that might be induced or maintained, at least in part, by the suppressive drugs the patients receive. The interpretation of these findings is further complicated because cytotoxic nonresponsiveness can be reversed sometimes by the addition of interleukin-2 (IL-2), which could be consistent with observations showing that PTBTs down-regulate IL-2 production, IL-2 receptor expression, or both (*35*).

Studies of CTLp frequency may be useful for the prediction of graft prognosis. The CTLp frequency to HLA-A is significantly lower than that to HLA-B. This could be in agreement with the consensus that mismatches for HLA-A are of less importance for the prognosis of a graft than mismatches for HLA-B (*45*). Kaminski *et al.* (*46*) showed that a high frequency of host-specific CTLps in the donor is associated with the occurrence of severe graft-versus-host disease (GVHD) in bone marrow transplantation patients, whereas a low frequency is associated with an absence or mild GVHD. Similarly, in renal transplantation good posttransplant function coincides with low CTLp frequency against the donor (*47*).

We are therefore hopeful that we may finally have found the "Holy Grail" for which we have been searching: an in vitro test to predict graft outcome. The finding that PTBTs can down-regulate CTLp frequency might enable us not only to make predictions on graft outcome but also to study the responsible mechanism in vitro.

Concluding Remarks

HLA matching improves graft and patient survival but the polymorphism of the HLA system is so enormous that it is impossible to find a well-matched [defined as HLA-B and HLA-DR compatible or at most mismatched for one HLA-B antigen] donor for the majority of the patients. That leaves us with three options: (i) even more effective, but aspecific, immunosuppression; (ii) not only matching for expressed HLA specificities, but also for the specific holes in the recipient's T cell repertoire for mismatched HLA antigens; or (iii) one DR antigen–matched PTBT to induce specific immunosuppression.

A perfect HLA match is certainly an important and effective method for avoiding graft rejection, but it is achievable for only a minority of the patients and may not be essential for all patients. To overcome these logistic problems, we should attempt to manipulate the recipient's T cell repertoire by creating, modifying, or simply exploiting existing holes in the repertoire. That this is not a completely unrealistic option is borne out by the introduction of immune tolerance into humans and nonhuman primates. Strober *et al.*, by using total lymphoid irradiation, obtained operational specific tolerance in three transplantation recipients for 1 to 6 years (*48*). Borleffs *et al.* transplanted an allogeneic kidney into monkeys treated with three PTBTs from unrelated animals and CsA. Six months after transplantation, CsA was stopped and two of the four animals survived with good renal function for 4 and 6 months. These findings are compatible with the existence of (partial?) tolerance that eventually disappeared (*49*).

Before these futuristic protocols can be adopted, we need to obtain confirmation that a low CTLp frequency is a good prognostic sign and that such low CTLp frequency can be induced by exposure to NIMA or by a single DR antigen–matched blood transfusions. After this confirmation, the CTLp frequency determination, a costly and time-consuming procedure, needs to be streamlined and made applicable as a routine procedure in the clinic.

General Comments

Transplantation has come of age and, as we have shown, its future looks bright. However, transplantation is an elite, prestigious, and costly form of medicine. The surgeons predict that in the year 2000, 80% of surgery will be replacement surgery. Pittsburgh has led the way by transplanting the lung, heart, liver, and a kidney of a single donor into a recipient.

Obviously, such medicine raises almost as many problems as it solves. Apart from the cost implications there is the donor shortage with all its ugly commercial sequellae such as the selling and buying of organs. Here immunology has a major task and responsibility. Concerted effort should be put not only into prevention of the diseases that make transplantation a necessity, but also into studies leading to a better understanding of the homograft reaction. These ends cannot be obtained solely by the use of more powerful immunosuppressive drugs. What we need is an interactive protocol based on our insight into the homograft reaction, which might ultimately lead to the clinical application of xenografts. This may lead us out of our present predicament.

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