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Liver Transplantation

Procedures and Management

PREFACE

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Cell Migration, Chimerism, and Graft Acceptance: a New View of Organ Transplantation

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Liver transplantation, as well as replacement of other whole organs, whether abdominal or thoracic, has now reached a high level of technical and management standardization.

Indeed, organ replacement has become an indispensable therapy for end-stage diseases of the liver, heart, kidneys and lungs.

However, though important progress has been made in the control and prevention of organ rejection (especially after Cyclosporin A and FK506 were introduced into clinical practice), immunological phenomena such as acute and chronic rejection are still responsible for a large percentage of post-transplant morbidity and mortality.

Further, we still know very little about the mechanisms involved in the acceptance of whole grafts under the usual immunosuppressive therapies available today.

Starzl et al. have recently shown how the exchange of migratory leukocytes between donor and recipient after whole organ transplantation leads to a state of long-term cellular chimerism. This chimerism could be crucial to the acceptance of all whole organ allografts and xenografts.

We believe this hypothesis radically changes the way we should look at whole organ transplants. Indeed, it is a speculation that on one hand enables us to deepen our understanding of transplantation immunology, and on the other hand it opens up the field for the development of new therapeutic strategies.

THE MICROCHIMERISM CONCEPT

Successful organ transplants were for a long time considered as compact alien grafts in a compact and

homogeneous recipient organism. The first doubts about this viewpoint were expressed in 1969 by K.A. Porter, who used karyotyping techniques to study female recipients of livers obtained from male cadaveric donors.

Porter showed that, following transplantation, the entire hepatic macrophage system, including the Kupffer cells, was replaced by female recipient cells within 100 days after the transplant [1, 2].

This was the first unequivocal evidence that whole-organ grafts in humans become genetic composites and that a state of chimerism is created after transplantation.

LOCAL MICROCHIMERISM

Even though from the 1970s onward a number of clinical reports have suggested the presence of local chimerism in long surviving human renal allografts [3-5] or in subhuman primates [6], the chimerical structure of the hepatic graft was for many years considered an exception within the context of whole-organ transplantation.

In 1991 it was shown, first on animal models [7] and then on humans, that also after small bowel transplantation the graft lymphoreticular system (lymphoid, dendritic and other leukocytes in the lamina propria, Peyer's patches and mesenteric nodes) is completely replaced by cells that are similar but belong to the recipient.

These findings led to the innovative hypothesis that graft chimerism might be a generic feature of all accepted grafts.

Further studies provided ample evidence of the existence of graft chimerism after transplantation of kidneys [8, 9] and thoracic organs as well [10-12].

SYSTEMIC MICROCHIMERISM

The discovery that local microchimerism occurred in all kinds of whole organ grafts brought up the old question of what happened to the donor cells that had disappeared from the graft. The importance of this question had been largely underestimated.

Since the discovery of chimerism in the transplanted liver, it had been assumed that the cells departing from the graft were quickly destroyed. This conviction was firmly rooted in spite of circumstantial evidence indicating that donor cells migrating from the engrafted organ will survive in the recipient organism.

In the case of kidney transplantation, for example, skin test studies (tuberculin, histoplasmin, blastomycin, coccidioidin, mumps, *Candida* and *Trichophyton*) on kidney donors and recipients in the early 60s (Starzl's early Colorado Kidney Transplant) [13] provided indirect evidence of the existence of systemic chimerism, though this interpretation was not considered plausible at the time.

Seventy-seven percent of the negative skin test patients who received kidneys from positive skin test donors became positive after transplantation. The remaining 23%, in whom the skin tests did not change to positive after transplantation, coincided with transplant failure. The authors of the study speculated that the secondary acquisition of the positive skin tests was "caused by adoptive transfer of donor cellular immunity by leukocytes in the renal graft vasculature and hilar lymphoid tissue" [13]. At the time, however, the possibility of cells migrating from the kidney graft to the recipient organism was considered untenable because the kidney was then thought to be a "leukocyte-poor" organ.

In the case of liver transplantation, the discovery that new donor-specific immunoglobulin (Gm) types appeared and were maintained in the blood of liver transplant recipients [2, 15] could have suggested, as far back as 1969, that donor cells departing from the graft remained vital in the recipient organism even for long periods after transplantation and were responsible for immunoglobulin production.

Similarly, the demonstration of the presence of soluble HLA antigens (Class I) in the circulation of liver graft recipients [16] was basically misinterpreted. It was considered, in fact, to be of hepatocytic origin, although we now know that these molecules are produced by histiocytes or dendritic cells, both of medullary origin.

Ramsey proposed in 1984 that cells migrating from the graft might be responsible for the production of anti-red blood cell antibodies encountered in recipients of ABO-unmatched livers [17].

This, as well as the other indirect evidence of systemic microchimerism, was unfortunately ignored at that time and correctly interpreted only later.

Direct evidence of systemic chimerism was only obtained in 1991, when Murase et al. used flow cytometry to demonstrate that, after intestinal transplant in rats treated with FK506, the stromal leukocytes leaving the graft then distributed themselves in large

numbers throughout the recipient's entire lymphoid tissue [18-20]. This cellular migration led to a state of systemic mixed allogenic chimerism lasting at least 45 days after the transplant. Interestingly, this condition of systemic chimerism did not lead, except in certain special strain combinations, to any signs of graft-versus-host disease [18-20]. Due to the time limit of the above mentioned experimental model (45 days), the long-term destiny of cells migrating to the recipient tissues was unclear throughout 1991. This experience served as the stimulus for definitive studies over the following months on long-term systemic chimerism in human recipients of kidneys, liver and other organs.

From April to July, 1992, direct evidence of systemic chimerism was sought both in kidney or liver transplant patients with a long term survival after successful transplant.

These studies were based on the distinctive features of two chromosomes, the sex chromosome Y and chromosome 6, in recipient and donor. On the one hand, cells with the Y chromosome were sought in the tissues and blood of females who had received the organ from a male donor, and on the other, cells with HLA alleles of chromosome 6 belonging to the donor were sought in the tissues of the recipient. Two techniques were used in the study on chromosomes Y and 6: cytostaining (which makes location and morphological characterization of phenotypically distinct donor and recipient cells possible) and polymerase chain reaction (PCR), which distinguishes the donor's DNA from that of the recipient [8, 21-25].

These techniques were applied to tissue biopsies obtained from a group of 5 living related kidney transplant recipients with graft survival from 27 to 29 years [26]. Some of these patients had taken part in skin test studies almost 30 years before. One of the five patients had suspended all immunosuppressive therapy and the other four were still taking azathioprine with or without steroids.

All five patients had received HLA-incompatible kidneys, so it was possible to distinguish between donor and recipient cells. In two cases, the donors were of the opposite sex.

Cytochemical analysis and PCR studies on graft biopsies demonstrated in all five cases that the interstitial leukocyte population was widely represented by the recipient's cells, whereas the nephrons remained genotypically the donor's. More importantly, in all five cases, skin and lymphonodal biopsies revealed the presence of the donor's cells (apparently dendritic leukocytes), demonstrating for the first time in a human being the presence of low-level systemic chimerism (microchimerism) in the host tissues almost 30 years after the transplant took place.

The fact that four of the five volunteer kidney donors were still alive also made it possible to demonstrate donor-specific non-reactivity by means of mixed lymphocyte reactivity (MLR) and/or cell mediated lymphocytotoxicity (CML) testing.

A similar study was also performed on a group of 25 liver recipients with grafts functioning well 2 to 22 after the transplant [25].

Analysis using cytochemical or PCR techniques on skin, lymph nodes, heart, lungs, spleen, intestine, kidneys, bone marrow and thymus demonstrated the presence of donor cells (systemic chimerism) in all 25 recipients. Interestingly, the number of chimeric cells found in the host organism after liver transplant was higher than that observed in long surviving kidney recipients.

Subsequent studies by A.J. Demetris et al. on liver transplant models in rats gave evidence that passenger leukocytes of the hepatic graft spread through the recipient's lymph nodes, spleen and thymus within a few hours after the transplant, without this migration being conditioned by the presence or absence of immunosuppressive therapy. However, these cells disappeared completely within the space of several days in non treated animals, whereas in rats given a brief course of FK506 (2 weeks), these passenger leukocytes survived, leading to a permanent state of low-level chimerism associated with indefinite liver graft survival [14, 27].

FUNCTIONAL CONSEQUENCES OF MICROCHIMERISM

The proof of the existence of a mixed chimeric state after whole organ transplants leads to the important question of what the functional significance of immunocompetent recipient and donor cells coexisting in the same organism might be. The relatively low number of cells from the donor in the recipient's tissues, even after transplant of a leukocyte-rich organ like the liver, could cause some doubt as to the actual functional importance of chimerism.

The virtually ubiquitous distribution of chimerism, however, suggests that the cumulative effect of donor cells, and consequently their functional importance, is considerable. There is also considerable evidence to indicate how important the role of these cells is, particularly after liver transplantation, where it is most easily demonstrated.

METABOLIC EFFECTS

The capacity of the chimeric cell population to have important metabolic effects was demonstrated in patients undergoing liver transplantation for type 4 glycogen storage disease (branching enzyme deficiency with amylopectin storage) and Gaucher's disease (B-glucocerebrosidase deficiency and storage of glucocerebrosides) [23]. Although it was thought that only a bone marrow transplant could be used to treat such disorders (since the enzymatic deficiency involves all cells), surprisingly, 2-8 years after liver transplant, complete resorption of the storage material in the recipient's tissues was witnessed.

Chimeric cells from the donor found in all the recipient's tissues had caused resorption of the storage

material, probably via a co-culture effect on the enzyme-deficient recipient cells (present in a much higher number).

These observations lead to the hypothesis that a similar cellular interaction may also occur in the more complex immunological processes.

IMMUNOLOGICAL INTERACTION

The question remains as to what mechanism allows systemic chimerism to persist for such a long time after transplantation. It is likely that a certain number of tissue leukocytes present in the transplanted organ do not reach complete differentiation, as was once believed, but retain the capacity to migrate and proliferate.

Under immunosuppressive therapy these pluripotent, progenitor cells present in the interstitium of the graft give rise to immunocompetent cell lineages (dendritic cells and other leukocytes), which replicate indefinitely in the recipient's tissues.

Inaba et al. recently demonstrated the possibility of generating a large number of dendritic cells from precursors grown from mouse bone marrow cultures, blood or whole organs supplemented with GM-CSF [28]. How and under what stimulus the continued proliferation of these cells occurs *in vivo* is not clear. Starzl et al. suggested that chronic mutual stimulation of the donor and recipient cell populations might occur after the transplant [24, 27]. In this context, as noted by Bandeira et al., the tolerogenicity arising from such an interaction shares many of the characteristics associated with immunity [29].

According to this hypothesis, the two coexisting immunocyte populations interact resulting in a state of immunologic balance. This balance is thus the result of a "mutual natural immunosuppression".

The very existence of this persistent cellular interaction depends on the permissive role played by the immunosuppressant drugs, which allow long-term microchimerism to establish itself once the mutual host-graft leukocyte migration has taken place.

The development of this non-responsiveness requires bidirectional alloactivation of a host-versus-graft reaction (HVG - i.e. rejection) as well as graft-versus-host activity (GVH), causing reciprocal and persistent stimulation of the two immune systems (Fig. 25.1). Alterations in this state of dynamic equilibrium can therefore result in either rejection or a GVHD. In successful cases of organ transplantation, however, this cellular interaction between the donor and the recipient under immunosuppressive therapy can drastically reduce the danger of a host-versus-graft reaction as well as rejection.

In particular, this mutual natural immunosuppression is effective even in the case of organs with poor lymphoreticular consistency, like the kidneys or heart.

Similarly, this mutual immunologic stimulation is responsible for the well-known resistance to GVHD of leukocyte-rich organs such as the liver, small bo-

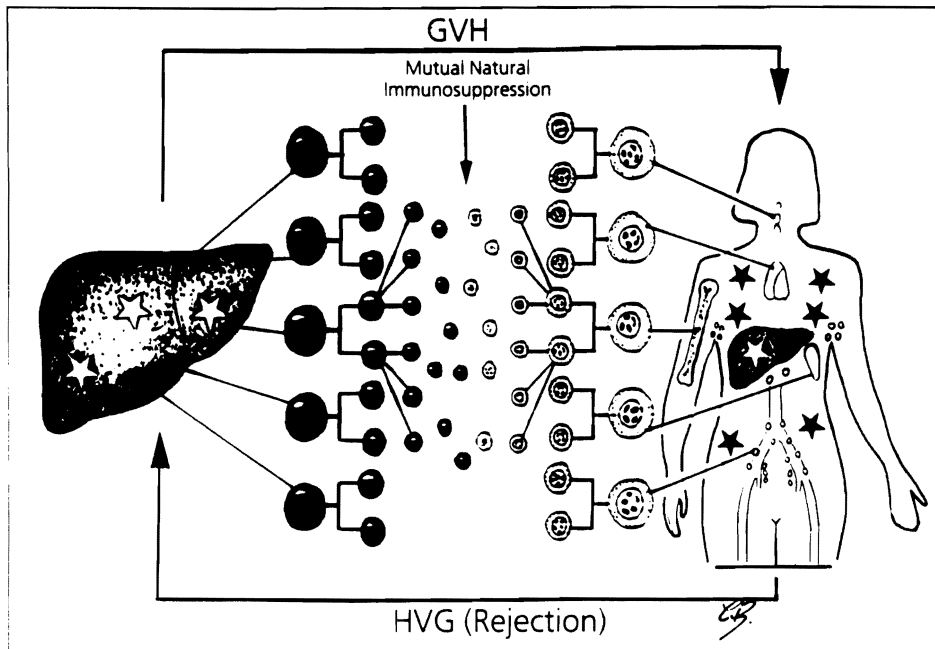


Fig. 25.1. The mutual engagement of migratory leukocytes from the graft and the recipient under potent pharmacologic immunosuppression. GVH: Graft-versus-host; HVG: Host-versus-graft. (Modified from [25]).

wel or combinations of intra-abdominal organs (multivisceral transplantation) [30].

It was known as far back as the 70s that a GVHD can be avoided in mice even after bone marrow transplantation if the recipient immune system is maintained intact [31]. Some years later Ildstad and Sachs validated these studies by producing various mixtures of donor and recipient bone marrow cells *ex vivo* and then creating mixed allogeneic chimeras by infusing the mixtures into cytoablated recipients who subsequently did not develop GVHD [32].

These studies experimentally recreated what occurs in clinical practice when treating recipients of whole organ transplantations with immunosuppressants. The key to the success obtained empirically in a clinical setting lies in permitting the interaction between the two cell populations and in the need to avoid altering this interaction by ablating or weakening one of the two populations in favour of the other. It therefore becomes essential to avoid compromising recipient reactivity with pretreatments or impoverishing the immunological component of the graft.

Since both cell groups receive the same protective immunosuppression, the more successfully the equilibrium between the two cell populations is maintained, the more reasonable it is to expect resistance to GVHD or to rejection.

MUTUAL CELL ENGAGEMENT AND TISSUE MATCHING

Also the limited correlation between HLA matching and outcome in whole organ cadaveric transplantation [33-35] finds a rational interpretation in the presence of a mixed long-term chimerism [8, 21, 25].

Interaction between donor and recipient coexisting leukocytes intensifies the higher the degree of histoincompatibility, reaching progressively higher grades of mutually cancelling donor-vs-recipient and recipient-vs-donor reactivity (Fig. 25.2).

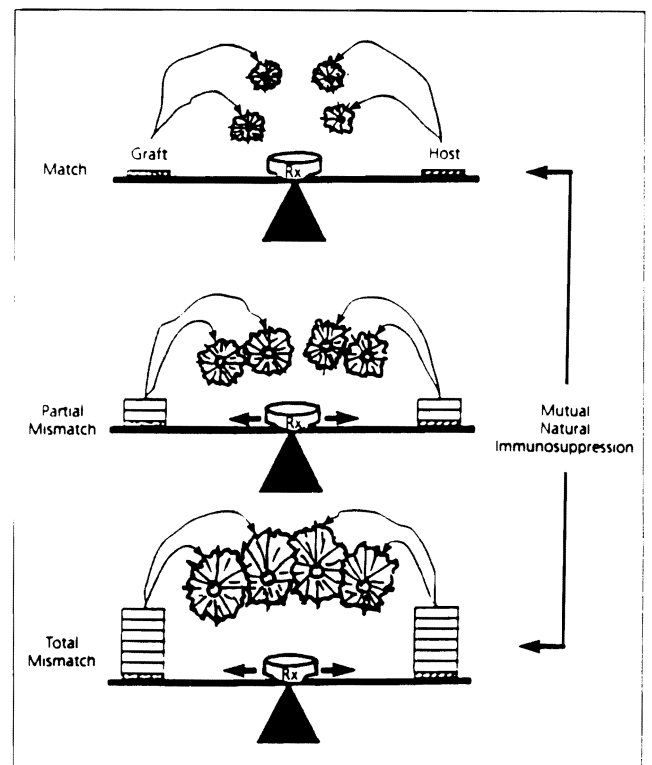


Fig. 25.2. The donor-recipient leukocyte interaction is a sort of buffer against GVHD on one hand and HVG reaction on the other. Veto and suppressor cells are suggested to be the result of the interaction. RX Immunosuppressive therapy. (Modified from [25]).

The permissive action of current immunosuppressants allows mutual cell engagement between the two systems to occur and a condition of rapprochement to be reached. In the case of liver transplant, this vindicates the two reports from Cambridge [36] and Pittsburgh [37] which produced evidence of an inverse relationship between tissue matching and survival of recipients. It also becomes easy to understand how even in the case of six-antigen-matched cadaver kidney allografts, the clinical advantage is minimal compared with kidney transplants with a lesser degree of matching.

THE DENDRITIC LINEAGE

The most prominent chimeric cell by morphological criteria is the dendritic cell [8, 21-25, 27]. It seems likely that this type of leukocyte plays a crucial role in determining the tolerogenic action of chimerism after whole organ transplantation.

In a normal immune response (whether rejection or GVHD), the efficient response of T cells to a given antigen requires professional antigen presenting cells (APCs) to correctly present the antigen to the T cell. To activate an effective immune response, it is then essential for the T cell to receive a co-stimulatory signal from the APCs themselves [38].

Among these APCs, dendritic cells [39-41] are probably the most important in the context of chimeric interaction, since they can modify the expression of cell interaction, MCH and adhesion molecules, thereby determining the way in which the antigen signals are heeded by the T cells [42].

Obviously, other lineages can also be decisive in these complex immunological interactions.

CELL MIGRATION AND TOLERANCE

Although a discussion of the concept of tolerance is not our purpose here, it is important to define the relationship between cell migration and tolerogenicity. Several authors recently stated that thymic clonal deletion is inadequate as an explanation of acquired transplantation tolerance [43].

The presence of a long-term microchimerism after whole organ transplantation, on the other hand, and the consequent, persistent interaction between the donor and the recipient immune system could well be consistent with and lend further weight to certain tolerance theories, such as peripheral (non-thymic) clonal deletion and anergy.

Coutinho and Cohen have defined the acquired tolerance as a high (not anergic) level of sustained immune activity in immunological networks [29, 44, 45]. This immunological interaction is probably more complex than that of the idiotypic system originally postulated by Jerne [46].

The cell migration-chimerism concept can therefore be seen as a mechanism which uniformly explains achievement of donor specific non-reactivity, independently of the characteristics of the immunosuppressant used or, in certain animal models, without any immunosuppression at all. The problem remains of identifying the finer mechanisms of microchimerism tolerogenicity.

It would be interesting, for example, to understand the relationship between drug-induced tolerance and the kind of acquired tolerance produced with intrauterine or neonatal splenocyte inoculation in the mice models of Billingham, Brent and Medawar [47].

On the basis of studies in drug-free models of tolerance induction, the theory has been put forward that T-cell receptor (TCR) occupancy can lead to the production of negative regulators of IL-2 production, the so-called anergy proteins [38, 48]. These negative regulators are inefficient during a normal immune response because they are diluted out by the vigorous cell replication driven by IL-2. However, if clonal expansion is inhibited by immunosuppressive drugs (IL-2 inhibitors or synthesis inhibitors) or by the absence of a co-stimulatory signal (in drug free models) [38], these negative regulators accumulate, leading to a state of anergy.

The use of non T cell depleting monoclonal antibodies or monoclonal antibodies against adhesion molecules (ICAM-1, LFA-1) can also be envisaged [49].

As suggested by Coutinho [44], it has been postulated that in fully successful cases, there has been complete integration of the donor's immunocompetent elements into the existing recipient immunological network [24, 27].

HEPATIC TOLEROGENICITY

According to Starzl et al., cell migration and repopulation represent the basis for all whole organ graft acceptance.

However, not all organs carry the same density of immunocompetent cells potentially capable of interacting with the host immune system (Fig. 25.3).

The abundance of leukocyte lineages, including Kupffer cells, in the liver is a particularly striking feature of this organ, with important implications for these cells in determining hepatic tolerogenicity.

Among the various aspects of the liver's "immunological advantages", the possibility of obtaining liver allograft or xenograft acceptance after a limited course of immunosuppression [26, 50-52] or even with no treatment at all (some rat strain combinations, swine) [53-57] is well known. The liver can also induce a state of unresponsiveness to donor tissues in the recipient. Its tolerogenic action does in fact assist acceptance of other organs and tissues transplanted either simultaneously or subsequently from the same donor.

The liver is also characteristically more resistant to the preformed antigraft antibodies, and therefore to

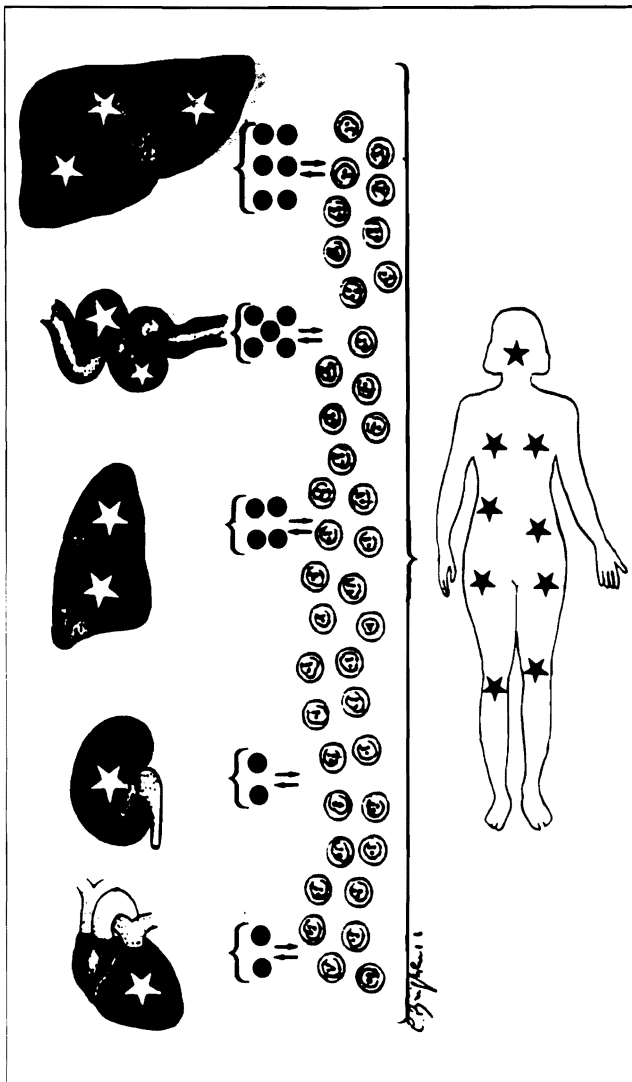


Fig. 25.3. The explanation for the variable ability to induce acceptance and ultimately tolerance of different organs. The tissue content of potentially migratory cells is liver > intestine > lung > kidney > heart. (Modified from [25]).

acute rejection, than organs such as the kidneys and heart. Its ability even to shield other organs against preformed allospecific [58] or xenospecific [59] anti-donor antibodies which cause hyperacute rejection is also documented.

According to the theory put forward by Starzl, these immunological characteristics of the liver graft are explained by its heavy endowment of migratory leukocyte lineages. In other words, the difference in terms of tolerogenicity between the various organ grafts depends on the extent of the leukocytic component of each type of organ and certainly not on its parenchymal characteristics.

In this context, the liver comes top of the list for "tolerogenic potential", followed by the intestine and the lungs, with the kidneys and heart bringing up the rear (Fig. 25.3).

Various experimental studies on the tolerogenicity of the spleen [60-62], intestine [7] and lungs [63-64] concur with this conclusion.

TOLEROGENICITY OF LEUKOCYTE "POOR" ORGANS

Although easier to demonstrate in leukocyte-rich organ grafts like the liver and intestine, potential tolerance processes also occur in organs with a decidedly lower leukocyte content, like the heart or kidneys, as demonstrated in 1992 [7, 18, 19, 65].

A wide range of studies has been performed on these organs in the past, but these concentrated on analysis of alloactivation and rejection rather than tolerization.

It was demonstrated, for example, that the allo-reaction in untreated animals starts peripherally in the graft and centrally in the recipient lymphoid tissues.

Extensive leukocyte migration after rat kidney transplantation in non-immunosuppressed animals had already been demonstrated in 1981 [66]. Larsen et al. had also demonstrated that, after a heterotopic heart transplant, the donor's dendritic cells are released into circulation and eventually home into the recipient spleen [68].

At a splenic level, certain authors [66-70] believe that the donor cells stimulate proliferation of the recipient's and vice versa, in what could appear to be an *in vivo* mixed lymphocyte response (MLR). This reaction seems to epitomize central allosensitization with tolerization.

It is likely that the processes of allosensitization (and tolerization) that occur centrally also occur at graft level.

Hayry and Willebrand showed that in human recipients of kidney grafts treated with Cyclosporine and steroids, needle aspiration biopsies appeared to indicate a bidirectional MLR [71, 72].

These and numerous other studies suggest that the differences with respect to leukocyte-rich organ transplantation seem to be quantitative rather than qualitative. Basically, the lower the number of migratory leukocytes involved, the higher is the tendency for allosensitization rather than tolerance. Despite this, it has been demonstrated that after mouse heart and kidney transplantation between weakly MHC compatible strains in untreated animals, a condition of tolerance can occur [73, 74].

UNSTABLE MIXED CHIMERISM

The chimerism concept, therefore, closely inter-relates tolerance, rejection and GVHD. This fact reunites the two disparate and far apart worlds of bone marrow transplantation and whole organ transplantation into a single and relatively homogeneous framework.

Until very recently, this clear-cut distinction between the two fields was in all probability the expression of two conflicting treatment dogmas (Fig. 25.4).

In the case of bone marrow transplantation, the conventional treatment requires optimal HLA matching. Recipient cytoablation, in fact, by preventing the establishment of cellular interaction between the donor and recipient immune systems tips the balance substantially in favour of the donor, which causes a GVHD in the event of an HLA mismatch.

The immunosuppressive treatment adopted after whole organ transplantation, on the other hand, permits mutual cell engagement and the establishment of a certain balance that drastically reduces the importance of HLA matching.

The aim in both whole organ transplantation and bone marrow transplantation is to avoid upsetting this interactive balance between the two immune systems, either in favour of the donor or recipient, thus preventing rejection or GVHD (Fig. 25.1).

Examples of failure to reach this immunological balance are well described in the classic studies of intestinal transplantation between certain rat strain combinations involving the Brown Norway (BN) strain [18, 20].

ACI, PUG or LEW rats that underwent intestinal transplantation from fully allogeneic donors and were given a daily course of FK506 for 14 days and then weekly did not develop either rejection or fatal GVHD. When, on the other hand, ACI intestine grafts were transplanted in BN rats, severe rejection occurred. Further, the combination of LEW or PUG donors to BN recipients proved vulnerable to GVHD after completion of the daily course.

In all the strain combinations, however, it was possible to detect bidirectional cell traffic, with leucocyte migration from donor to recipient and vice versa [18, 19]. The same tendency to rejection or GVHD under Cyclosporin was described in the case of WAG-BN strain combinations, with WAG as donor and BN as recipient [75].

It is still not clear to us why BN rats are "excellent donors" but "poor recipients".

The problem implicit in these observations is how to recognize and identify in a clinical context the more favourable donor-recipient combinations and thereby avoid the more difficult ones, such as, in the case of rat experiments, LEW, ACI or PUG to BN combinations.

In clinical experience, as in these experimental models, the signs of an unstable mixed chimerism are often very clear.

Although rejection is the major deterrent after liver transplantation in humans, the incidence of clinically significant GVHD accounts for around 5% of all cases in the early post-operative period. This disorder is characterized, symptomatologically, by dermatitis (often attributed in the past to drugs or allergy) [25], which can become serious and sometimes even fatal.

These patients can be treated effectively by increasing immunosuppression (especially the steroid dose) or more rarely, on the other hand, reducing immunosuppression. However, the presence of widespread skin disorder, gastrointestinal symptoms and a reduction in blood crasis are correlated with a high mortality rate [76].

Interestingly, it has been documented that in these patients chimerism is more extensive than in patients whose course presented no complications.

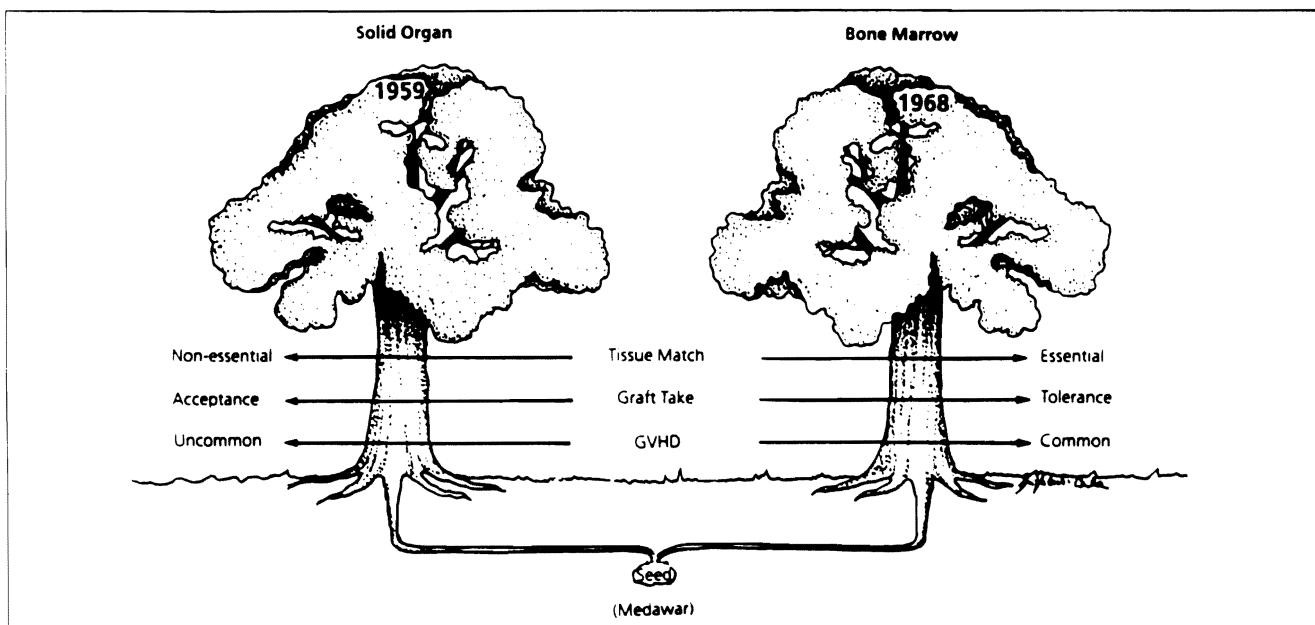


Fig. 25.4. The division of transplantation into two separate disciplines by divergent therapeutic dogmas. Therapeutic policies used in bone marrow transplantation precluded bidirectional cell migration. This phenomenon, on the contrary, was the fundamental basis for graft acceptance with the policies used in whole organ transplantation. (Modified from [25]).

A good example of the consequences of an unbalancing of the donor-recipient interface is provided by the case of a 56-year-old patient with gastric leiomyosarcoma and liver metastases, who was transplanted after an upper abdominal exenteration [77]. Just before the operation the patient had undergone total lymphoid irradiation (TLI) (single 550 rads dose thoraco-abdominal irradiation) and then infusion of 19×10^7 of non-purged bone marrow cells. After a few weeks the patient experienced a serious GVHD with a $> 80\%$ skin involvement. The situation was not mitigated by either increasing or reducing the immunosuppression treatment. After about a month and a half from the transplant he was given an intravenous infusion of 1.23×10^8 and 1.6×10^8 autologous unpurged bone marrow cells/kg (collected and stored prior to TLI). The GVHD dramatically resolved over the next two weeks and the skin rash cleared up completely. Interestingly, flow cytometry analysis of the donor and recipient circulating cells showed a 25 to 3% reduction of mixed lineage donor cells in the blood coincident with resolution of the GVHD.

Two important lessons were learned from this significant clinical experience. The first is the unfavourable effect of pre-operative TLI. Instead of "making space" for the new marrow cells, it risks upsetting the donor-recipient balance in favour of the donor, thereby causing a GVHD. The second is the potential value of native autologous bone marrow collected and stored before transplantation. This can be a useful treatment in the event of an unbalancing of the donor-recipient interface leading to GVHD.

The capacity of autologous marrow cells to block and resolve a GVHD sheds light, indirectly, on what happens during the mutual cellular interactions in mixed chimerism.

Although the number of bone marrow cells used to treat the GVHD may be considerably lower than those in the patient's blood, their therapeutic value is probably due to the fact that they are not previously exposed to the donor marrow. These "virgin recipient bone marrow cells" may be able to restore the immunological balance because they are not previously conditioned by the mutual cell engagement processes.

CLINICAL TRIALS

DRUG WEANING EXPERIENCE

On the basis of the hypothesis outlined in this chapter, the immunologic advantages of liver transplantation are related, as already mentioned, to the organ's abundance of migratory leukocytes. Similarly, the smaller tolerogenic potential of organs such as the kidney and heart may be explained by their smaller leukocyte component.

The existence of a bodywide mutual cell engagement and the consequent development of a donor

and recipient specific non-reactivity after whole organ transplantation is perfectly consistent with the fact that some patients can reach immunologic tolerance and a drug-free state.

The liver graft is obviously the organ most likely to be accepted in a drug-free condition on account of its considerable migratory cell constituency.

In a recently reported group of 44 human liver recipients who had survived 11-23 years after transplantation, 14% had suspended all immunosuppression 1 to 11 years post-operatively. These patients are clinically stable at the present time, with drug-free intervals from 5 to 13 years. Another 15 cases are showing no significant clinical result after interrupting immunosuppression, albeit with a shorter follow-up [25, 78].

The earliest suspension of therapy in this study is a liver recipient who came off immunosuppression at 6 months after transplant, with a follow-up of 3 years.

A trial of drug weaning has been started in Pittsburgh in patients with a rejection free course exceeding 5 years. The benefits of coming off drugs make the risk of rejection acceptable now that it can be treated so effectively with drugs like FK506 [79].

However, it is very difficult to decide when to suspend immunosuppression, since we have no way of knowing when a potential drug-free state has been reached.

This drug weaning experience in liver transplant recipients might be extremely useful for future clinical trials of drug weaning after transplantation of the heart or kidney, whose tolerogenicity can be approximated to that of liver by using bone marrow augmentation (see below).

BONE MARROW AUGMENTATION

The growing conviction that mixed cell chimerism is a crucial event in whole organ transplantation and in the subsequent induction of donor specific non-reactivity has posed the problem of how to augment this natural phenomenon.

Since the migratory interstitial cells are of hemato-lymphoid origin, the next logical step was to augment the immunocompetent component by perioperative exogenous administration of bone marrow. These considerations, and the clinical efficacy of autologous bone marrow cells in the treatment of GVHD in the case described above, have led to further attempts to use bone marrow augmentation to induce tolerance in whole organ recipients.

In the past, various advanced strategies featured intravenous infusion of donor marrow cells, splenocytes or donor specific transfusion at the same time as transplantation or shortly after. Such strategies are merely an augmentation of the normal post-transplant cell migration [31, 32, 80-82]. To reproduce the natural process as far as possible, however, these cells should be administered perioperatively, which is different from the so-called Monaco model.

Since December 1992, a clinical study has been running at the University of Pittsburgh on 16 recipients of various organs aimed at inducing a higher level of chimerism by infusing bone marrow cells from the donor at the time of whole organ transplantation (such a trial is still underway in Pittsburg and now includes nearly 30 patients) [83].

The clinical purpose of this study was to eliminate or at least reduce the need for chronic non-specific immunosuppression. Table 25.1 lists the relevant data for the study population. No irradiation or any other cytoablative conditioning was used.

The donor marrow was harvested from vertebral bodies and 3×10^6 of untreated bone marrow cells were infused intravenously immediately after the transplant. In all cases FK506 and steroids were used. The pancreatic islets were infused into the 3 diabetic patients intraportally at the same time as the bone marrow infusion.

All the patients and their grafts are doing well at the present time (follow-up of 3 to more than 13 months).

Some of these patients, however, showed (as expected) host-versus-graft (HVG) and graft-versus-host (GVH) reactions. In particular, 9 of the 16 patients developed mild to moderate acute cellular rejection over the first month and a half after transplant (Tab. 25.2). In all cases rejection was successfully treated simply by increasing the dose of routine immunosuppressant drugs, with one exception (heart transplant) which required an additional course of OKT3 therapy.

Two liver transplant patients, on the other hand, showed signs of GVH reaction characterized by skin rash without other organs being involved. These symptoms disappeared completely within 3 weeks. Steroids had to be increased in 1 case only.

Chimerism analysis in the recipients was performed by immunocytochemical staining of recipient peripheral blood lymphocytes (PBL) using donor and recipient-specific anti MHC class I monoclonal antibodies (mAb).

Chimerism was also assessed by flow cytometry and by PCR using donor and recipient specific DR probes. Fifteen of the 16 patients have demonstrable macrochimerism, the only exception being a kidney recipient with a perfect MHC match from a donor of the same sex who had no markers to be studied, the use of different technologies allowed cross-confirmation of results. The highest yield was with PCR, showing chimerism in 14 of the 16 cases. After male to female transplantation in 4 cases, all 4 recipients had Y chromosomes detectable, and in these cases there was an excellent correlation with the results obtained using HLA alleles. The yield with flow cytometry was 13/16, showing 0.6 to 5% circulating donor leukocytes 50 days to 1 year after transplantation. In the control group, on the other hand, the donor cells were almost undetectable 4 weeks after transplantation (Tab. 25.3). Furthermore, analysis by flow cytometry and immunofluorescent labelling of cytopins succeeded in showing multiple lineages of donor cells (T cells, B cell, NK cells, MØ and CD33+/

Table 25.1. Transplant groups.

Organ Tx	Controls (Allografts alone)	Study group (Bone marrow + solid organ)
Liver	4	5
Liver + Islets	1	1
Kidney	4	7
Kidney + Islets	0	2
Heart	1	1
Total (n)	10	16

Table 25.2. Incidence and severity of HVG reactions (rejection) in the bone marrow + whole organ transplantation group.

Organ	n	ACR	Severity of ACR
Liver	5	3	mild ACR
Liver + Islets	1	1	mild ACR
Kidney	7	2	mild ACR
Kidney + Islets	2	2	mild-moderate ACR
Heart	1	1	Grade 0-3A
Total	16	9	

Table 25.3. Percentage of donor cells in transplant recipients (detected by flow cytometry).

Organs	Control group (1) (X±SD)		Study group (2) (X±SD)	
	POD 3	POD 28	POD 3	POD 28
Liver	3.2±4.2	0.19±0.5	6.5±1.5	12.8±2.3
Kidney	0.2±0.3	0	4.2±3.8	5.6±0.4
Heart	0	0	7	3.6

(1) Liver (n = 5), Kidney (n = 4), Heart (n = 1)

(2) Liver (n = 2), Kidney (n = 2), Heart (n = 1)

POD = Postoperative day.

CD34+ Progenitor cells) in the recipient's blood up to 6 months after combined whole organ/bone marrow transplantation. Interestingly, flow cytometry and PCR on the aspirated bone marrow of all 16 cases showed the presence of donor progenitor cells 6 months after the combined transplant. Quantitation of the chimerism also was done with the technique of PCR co-amplification developed by Trucco and his associates. In all 15 testable recipients the density of blood chimerism was 1000 fold or greater than that occurring spontaneously [83, 84].

Post-operative immunologic monitoring was by mixed lymphocyte reaction (MLR) and cell mediated lympholysis (CML).

In most of the patients who underwent whole organ and bone marrow transplantation, the results of these tests showed a diminished donor specific reactivity and full proliferation capacity and cytotoxic response against third party lymphocytes for up to 5 months after transplantation.

Patients who had received allografts alone, on the other hand, remained responsive to both the donor and third party stimulators/ effectors for up to 13 months post-transplant.

These results indicate that donor bone marrow augmentation enables us to reach a higher level of chimerism (macrochimerism) than after whole organ transplantation alone (microchimerism). It is also clear that we can perform a donor bone marrow engraftment without recourse to cytoablative conditioning of the recipient. This concept, by the way, has already been suggested by recent experimental findings.

Thanks to its capacity to augment the degree of chimerism and reduce donor-specific reactivity, bone marrow augmentation can thus induce early immunomodulation in the host.

For these reasons we can propose, for these patients, a reduction and/or early withdrawal of non-specific immunosuppression.

This clinical experience, however, has also shown that loss of donor specific non-reactivity in humans occurs with considerable variability.

This variability had already been stressed in the series of Pittsburgh articles on chimerism. The result of mutual cell engagement may thus vary from clinical stability without immunosuppressant drugs, to

stability dependent on immunosuppression, or to instability (despite therapy), either in the form of rejection (most commonly) or GVHD (less common but most frequent with the liver, especially after bone marrow augmentation).

The use of bone marrow augmentation in an organ like the liver, which is in itself highly active in inducing cell chimerism, requires careful assessment of all the risks and benefits involved. In fact, we still don't know whether leukocyte augmentation will improve natural hepatic tolerogenicity and thereby facilitate the achievement of a drug-free state, or whether, on the contrary, it will increase the risk of GVHD.

What is clear though, is that in all cases of bone marrow augmentation it should be mandatory to store autologous bone marrow for rescue therapy if GVHD occurs [77].

Donor bone marrow will perhaps become an extremely flexible therapeutic resource in all organ or cell transplants for facilitating graft acceptance and inducing donor-specific non-reactivity.

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