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Extrahepatic Manifestations in Liver Diseases

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State of the Art Lecture
Chimaerism after whole organ transplantation

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Throughout the modern history of transplantation, progress with kidney and liver grafting has been interchangeable – and then applicable with very little change to the thoracic organs and most recently the intestine. I will focus today first on why any kind of whole organ allograft and xenograft is accepted, because this not only defines the state of the art for the liver, but predicts the future of transplantation as a whole.

My personal interest in transplantation came via the back door of physiology, during metabolic investigations of the special (so-called hepatotrophic) qualities of portal venous blood. In the course of these inquiries I first developed a new experimental method of total hepatectomy[1] and then the operation of liver replacement[2]. By the end of 1959 we had clarified the surgical secrets of liver transplantation and had also completed a second project in dogs with a multivisceral transplant procedure[3]. Twenty-five years later this latter operation was performed successfully in humans and became the basis for several variations such as the cluster and liver–intestine procedures[4].

However, this research activity in 1958 through early 1960 was in a therapeutic vacuum because there was no such thing as practical immunosuppression. Pharmacological immunosuppression is dated to the classical paper on 6-mercaptopurine by Schwartz and Dameshek[5] in a non-transplant model. Within a few months this drug was shown to prolong survival of skin grafts in rodents[6,7] and kidney allografts in dogs[8,9]. Realizing by now that the road to my primary objective of liver transplantation would have to be through the simpler kidney transplant model, I moved from Northwestern University in Chicago to the University of Colorado in late 1961. There I began a clinical kidney programme.

KIDNEY TRANSPLANTATION AND CHIMAERISM

The programme was based on the simple laboratory discovery that canine kidney rejection under azathioprine could be reversed with prednisone in 88% of dogs[10], an incidence that proved to be the same in humans, as we reported in 1963[11]. The key points were summarized in the title of the 1963 article – the reversal of kidney rejection by steroids and the subsequent ability in successful cases to later reduce the intensity of immunosuppression (referred to as 'tolerance').

The explanation for these two observations was a mystery in 1963, but in retrospect a clue to the mystery was uncovered with exhaustive skin test studies (tuberculin, histoplasmin, coccidiodin, etc.) performed on these early Colorado kidney recipients and their donors. Skin reactions that were positive in the donor but not the recipient were found to cross over to the previously negative recipient along with the transplanted kidney 77% of the time. When this did not occur (the other 23%), it meant that the kidney transplant had failed. Wilson and Kirkpatrick, the immunology fellows who performed these tests, speculated (as it turned out correctly) that the migration of the skin tests was 'caused by adoptive transfer of donor cellular immunity by leukocytes in the renal graft vasculature and hilar lymphoid tissue'[12].

That this actually had occurred was proved 29 years later when some of these original kidney recipients were restudied, proving that there had been an exchange of lymphodendritic leucocytes between the transplanted kidneys and their recipients. These cells still survived nearly three decades later. The presence of the donor cells in the lymph nodes and skin of four recipients of kidneys from HLA mismatched donors was shown with immunocytochemical techniques that stained cells of donor phenotype. These appeared to be dendritic cells. The microchimaerism was confirmed with polymerase chain reaction techniques. In a fifth patient, a female who had received a kidney from her father, male donor cells with the Y chromosome were found in recipient tissues with fluorescent *in situ* hybridization, and confirmed with polymerase chain reaction (PCR) techniques. All of the studied recipients and their grafts were composite structures – no longer the same as at the outset[13].

LIVER TRANSPLANT RECIPIENTS

This was only the beginning of what quickly became a scientific detective story. Between April and July of 1992 evidence was obtained that an even more extensive exchange of tissue leucocytes occurred after liver transplantation, creating a composite graft as well as chimaeric composite host on an even larger scale than after kidney transplantation[14]. For the liver study we began by obtaining follow-ups on all 44 of our first 206 liver recipients who still were alive $10\frac{2}{3}$ to nearly 23 years after transplantation. Six of these patients had stopped their immunosuppressive medications 1–6 years after transplantation and had been drug-free for 5–13 years. The lymphocytes of treated as well as untreated patients reacted vigorously to the lymphocytes of third-party donors. The drug-free patients had achieved lasting immunological

tolerance. We also realized that many if not most of those still being treated probably no longer required immunosuppression.

Multiple biopsies were performed on six of the drug-free patients and on 16 more still under maintenance immunosuppression. Specimens were taken of the liver, skin, and a convenient lymph node. Using HLA markers, all 22 were demonstrated to be chimaeras with immunocytochemical and PCR techniques; by PCR, 75% were also blood chimaeras. This could also be documented with sex typing in a subgroup of nine women who had received livers from male donors. Sex chimaerism (the Y chromosome) was detected with fluorescent *in situ* hybridization or with PCR in every case. Using either the HLA alleles of chromosome 6 or the male Y chromosome, the hepatocytes, ducts, and endothelial cells of the allografts remained donor-specific while the Kupffer cells, dendritic cells and other stromal leucocytes were those of the recipients. The systemic chimaerism was usually in more than one site. In one female patient who lost her male graft after 12 years to recurrent viral hepatitis, tissue samples taken at retransplantation (which was successful) showed male cells in blood, skin, lymph nodes, jejunum and the aortic ellipse excised to accommodate a Carrel patch. At the time these samples were collected, this woman had been off medication for 7 years. In the autopsy specimens from another patient who died of B virus hepatitis after 18.4 years, chimaerism was found in essentially all tissues of the body[15].

Aside from their immunological implications the peripheralized chimaeric cells can profoundly alter metabolism[16]. In three additional patients who had undergone liver transplantation 26–91 months previously for metabolic storage diseases, enzyme transport by the seeded peripheral cells explained how amylopectin (in two patients with GSD IV) could be absorbed from the heart as had occurred. Donor cells (thought to be dendritic leucocytes) were detected with monoclonal anti-HLA antibodies and PCR in the myocardium, skin, and lymph nodes. In a patient with Gaucher's disease, donor cells or donor DNA were found in the recipient blood, bone marrow, skin, small bowel, and lymph nodes. In this patient the glucocerebroside deposits (Gaucher's cells) in the lymph nodes had diminished astonishingly over the 26 months post-transplantation.

Thus systemic chimaerism was detected in all 25 liver recipients who were studied from 2 to more than 20 years post-transplantation. The ability to find donor cells wherever they were looked for was striking. Because the same thing was found in the kidney recipients, although less prominently, we concluded that the same thing probably occurred with all kinds of grafts – but so much more extensively with the liver than with other organs, that this accounted for what has been called hepatic tolerogenicity.

WHY PRINCIPLES OF IMMUNOSUPPRESSION ARE NOT DRUG- OR ORGAN-SPECIFIC

These remarkable discoveries in kidney and liver recipients were made only a few months ago. Of course, none of this was known in 1963 when, without knowing why, the observations of rejection reversal and so-called tolerance

in kidney recipients led to the empirical therapeutic dogma upon which the transplantation of all whole organ transplantation is based[11,17]. The dogma calls for daily baseline treatment (in those early days with azathioprine) plus intervention with the highly dose-manoeuvrable adrenal cortical steroids (later augmented with antilymphoid agents) to whatever level is required to maintain stable graft function. This creates a trial-and-error situation for every patient as drugs are weaned.

Although the new drugs that have been added through the years have been increasingly potent, they can be viewed as traffic directors, allowing the cell movement to and from all kinds of grafts but preventing the immune destruction that is the natural purpose of the traffic. Apparently, it does not matter exactly how the immune reaction is disrupted, but only that this be achieved without killing all of the migratory cells. The emasculated but living cells that normally cause graft immunogenicity and rejection become instead the missionaries subserving chimaerism, graft acceptance, and ultimately tolerance. Disruption of the function of the lymphocyte can be at the level of antigen processing (claimed for the experimental drug, deoxyspergualin), at an early stage in T-cell activation as occurs with cyclosporin and FK 506, or distal to this with rapamycin which does not inhibit the secretion of cytokines including IL-2 but blocks their action. The so-called antiproliferative drugs (of which azathioprine was the prototype) work even more distally.

RE-EXAMINING TRANSPLANTATION IMMUNOLOGY

With the understanding that cell migration and repopulation is the basis of graft acceptance, no matter what the organ, we now can re-examine some controversies in transplantation immunology that have never been resolved – including why HLA tissue matching to govern the distribution of cadaveric organs has been so imperfect a tool. To understand these controversies we must turn the pages back 50 years, to when Peter Medawar planted the seed of our clinical specialty. If rejection was an immunological response, as Medawar claimed in 1944[18], what could be more logical in preventing it than to weaken the immune system. By 1951, Billingham, Krohn, and Medawar[19] and the American Morgan[20] had taken this crucial step, and had shown that skin graft survival was prolonged with cortisone acetate and ACTH – the first immunosuppressive drugs. The year before, Dempster and co-workers, of Hammersmith, showed mitigation of skin graft rejection with total-body irradiation[21].

Seemingly, these were small steps, but then in 1953 Billingham, Brent, and Medawar[22,23] raised expectations to a new level by showing the possibility of acquiring immunological tolerance, albeit only under the special circumstance of inoculation of immunocompetent adult spleen cells into fetal and perinatal mice. Main and Prehn[24] were able to mimic these developmental conditions in *adult* mice using supralethal total-body irradiation and bone marrow allo-reconstitution. When the reconstituted mice were shown to be tolerant to donor strain skin (the white patch), the clinical possibility of creating

radiation bone marrow chimaeras as a means to the end of solid organ transplantation seemed obvious.

These hopes were promptly dashed when the concept of GVHD and runt disease was delineated by Billingham and Brent[25]. However, what was *not* clearly recognized, then or later, was that these whole-animal models, and subsequently the experimental F_1 hybrid model, are almost artifacts in the sense that the interactions of the two-way cell migration and repopulation that I have been discussing were precluded in each case: by the immature state of one party (that was the Billingham, Brent, Medawar model), by the cytoablation used by Main and Prehn (and later bone marrow transplanters), or by genetic manipulation (the F_1 hybrid model). These were whole-animal analogues of the *in vitro* one-way mixed-lymphocyte reaction.

DIVISION OF TRANSPLANTATION INTO TWO FIELDS

Of course, this is hindsight 33 years later. Between 1959 and 1963 – and without really knowing why – the intellectual root that came from Medawar's seed divided into two branches. Although the issue from the roots looked like two separate trees when they surfaced the differences merely reflected different therapeutic dogmas. The bone marrow tree with its precondition of cytoablation mimicked the Billingham, Brent, Medawar model and was the *in vivo* version of a one-way MLR. HLA matching was crucial. Engraftment in a drug-free state (called tolerance) was a realizable objective only with perfect matching. This was not achieved clinically until 1968[26,27], but even with MHC compatibility, GVHD was a constant threat. The reason for the virulence of the GVHD with an HLA mismatch was the complete removal of a counterweight to the transplanted immunocytes.

The whole-organ transplanters who had broken ranks with their bone marrow colleagues, empirically developed the long-term immunosuppression which I discussed earlier with which success (called graft acceptance, not tolerance) did *not* depend on matching and could be accomplished without GVHD – even after the transplantation of lymphoid-rich organs such as the intestine and liver. The explanation for the GVHD resistance with the whole organs is envisioned as the interaction of cells coming out from the allograft with the immunocytes of the recipient (a two-way *in vivo* MLR). The term for the long-term coexistence of two populations of cells is mixed chimaerism.

Of course, the fact that mixed chimaerism interdicts GVHD is only half of the story. The other half is that the midfield cell interaction (which results in what we have called mutual natural immunosuppression) also mitigates rejection (the host versus graft reaction). The details of this donor-recipient rapproachment are not known, but it does seem clear that even organs like the kidney with a poor lymphoreticular constituency have enough dendritic cells (or whatever these leucocytes are) to sometimes induce for themselves donor-specific non-reactivity (tolerance). In the process the donor recipient interactions are envisioned as occurring on a sliding scale, in which each further level of histo*in*compatibility provokes variable countervailing increases

in the mutually cancelling donor versus recipient *and* recipient versus donor cell reactivity.

For renal allografts it becomes possible to understand why Terasaki, Opelz, and others have shown a large advantage only for six-antigen matched cadaver kidney but not for any matching that is less perfect. Most importantly, it becomes possible to understand why the vast majority of unmatched kidneys do well. For liver transplantation the reports from Cambridge and Pittsburgh become comprehensible that have shown an *inverse* relation between the quality of HLA match and survival of liver recipients, but again a difference that is measurable only within a few percentage points.

INDUCTION OF TOLERANCE

It seems obvious that the crucial variable distinguishing one organ from another is the lymphodendritic (not the parenchymal) component – and that these tissue leucocytes can be tolerogenic as well as immunogenic when effective immunosuppression is given. The liver, with its dense constituency of these cells, is high on the favourable list of tolerogenicity, with the lung and intestine following and the heart and kidney bringing up the rear. It is self-evident that the underprivileged kidney and heart could be brought to the same level of tolerogenicity advantage as the liver by the perioperative infusion of lymphoreticular cells obtained from bone marrow of the organ donor, or possibly from the spleen. Now the cycle is complete because this was the starting point for Billingham, Brent, and Medawar, and then Main and Prehn.

THE DRUG REVOLUTION

Of course, what I have said today is our current understanding of transplantation. Rather than limiting a search for better drugs, this insight should encourage their development, as can be illustrated by the different eras of liver transplantation. In July 1967 the first long-surviving liver recipients were produced under azathioprine, prednisone, after an effort which by then had consumed almost 10 years. However, acceptance of the procedure was slow over the next dozen years because of its high mortality. Roy Calne's introduction of cyclosporin[28] and the subsequent combination of this drug with prednisone[29] allowed a doubling or more of survival about a decade ago and brought liver transplantation to centre-stage[30].

Recently, the liver has been the lead organ in the next step of immunosuppression, made possible with the drug, FK 506, whose action is similar to cyclosporin[31]. The patient and graft survival with FK 506 has been improved a further 10–15% compared to the cyclosporin results in the Pittsburgh trials, and in the recent European multicentre randomized trials of FK 506 versus cyclosporin.

These trials suggest that we are at the dawn of another era in transplantation, signalled in addition by an emerging population of recipients of complete

cadaveric small bowel, either transplanted alone, with the liver, or as part of a multivisceral graft[32]. Of 23 such patients treated 4 months to more than 2 years ago, all but three are alive. Only one example of GVHD has been seen. The chimaerism I have been discussing has been obvious in every case. In the intestine with epithelial cells of the graft remain those of the donor while the lymphoreticular stromal substrate switches over to predominantly that of the recipient.

XENOTRANSPLANTATION

When organs are transplanted from a significantly disparate species, the first immunological hurdle is that of preformed xenospecific antibodies which quickly devascularize the graft and exclude it from recipient circulation by damaging its blood vessels[33]. If this barrier can be surmounted, the process of xenograft acceptance involves the same bidirectional cell migration and consequent systemic chimaerism as with allotransplantation. After hamster to rat xenotransplantation, the cells displaced from the xenografts can be detected in widespread rat recipient tissues with polyclonal rat absorbed anti-hamster leucocyte antibodies, and confirmed with PCR techniques[14]. As with allotransplantation, the chimaerism is more extensive after liver than after heart transplantation.

Chimaerism was observed recently in a patient who survived for 70 days after receipt of a baboon liver. Death was caused by infectious complications and by complications of biliary stasis rather than rejection or GVHD[34]. This means that successful clinical xenotransplantation must be visualized along the same lines of donor–recipient cellular intimacy which we believe is the fundamental means of xenograft as well as allograft acceptance.

SUMMARY

I have tried to present a unified view of transplantation to which the liver has contributed the central role[35,36]. Thank you for the honour of allowing me to present this to you.

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