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Allograft and Xenograft Acceptance under FK-506 and Other Immunosuppressant Treatment [a]

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We will focus on two issues, both involving, but not confined to FK-506: first, the meaning of the graft acceptance, which is, after all, the objective of immunosuppression for the transplant surgeon; and second, how to take the next great step of xenotransplantation.

GRAFT ACCEPTANCE

In June 1992 we published what is a new definition of graft acceptance.[1] Within a few minutes after the transplantation of any whole organ (as in the intestine) (FIG. 1), a brisk two-way cell traffic starts in which dendritic and lymphoid cells from the recipient move into and become an integral part of the graft. With effective immunosuppression, the result in the intestine is the formation of a graft chimera, in which the epithelium remains of donor HL-A phenotype, while the lymphoreticular stroma (including lamina propria, Peyer's patches, and mesenteric nodes) becomes principally that of the recipient.[2,3] This important discovery was reported only 14 months ago. Previously, only the liver, which is rich in these migratory cells, was known to be chimeric,[4,5] but in the past year, it has been realized that the same thing occurs with all organs. Furthermore, at the same time as the graft becomes a chimera, the replaced donor dendritic and lymphoid cells leave the graft and are seeded throughout the body of the recipient.[1]

In the human cases in which cell migration, repopulation, and chimerism were proved, the first evidence with immunocytochemical (monoclonal) techniques was confirmed in all cases with polymerase chain reaction (PCR) studies. For example, the PCR band of a donor-specific HL-A allele was demonstrated in biopsy specimens of

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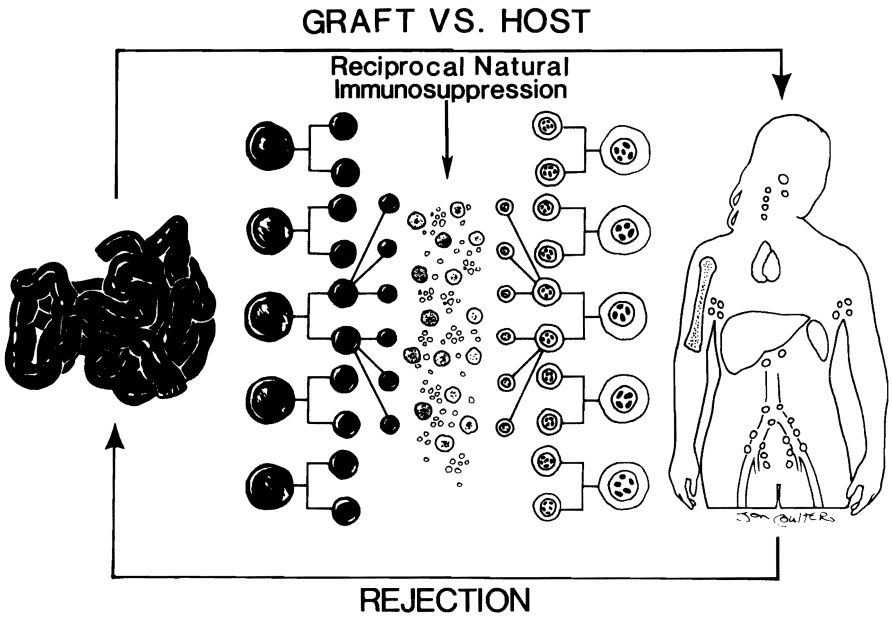

FIGURE 1. The cell migration and repopulation that occurs after the transplantation of any whole organ.[1] These phenomena and the consequent chimerism were first seen after intestinal[2,3] and liver[4,5] transplantation.

the liver, skin, and heart of the liver recipients years after transplantation.[1] In fact, systemic chimerism was easily detected in all 17 liver recipients studied from 10½ to 22 years after transplantation.

Organ grafts like the kidney and heart that are less well endowed with lymphoid tissue, but that contain dendritic cells go through the same process after transplantation, but on a smaller scale and with less easily detected chimerism. A few weeks ago, three of the longest-surviving kidney allograft recipients in the world underwent biopsy of their kidney allograft and their own skin and lymph nodes 28 to 29½ years after successful kidney transplantation had been carried out at the University of Colorado.[6] Because they had one or two haplotype HLA mismatches with their donors (and also in one case a different-sex donor), chimerism in the lymph nodes and skin could be detected with anti-HLA monoclonal antibodies and Y probes and confirmed with PCR. How redistributed donor dendritic cells survive this long, presumably involving clonal expansion initially and then chronic self-renewal, remains to be explained. The most conventional explanation is that dormant stem cells were in the grafts and were stimulated to respond by perioperative factors, of which Gm-CSF has been identified as a possibility in a recent paper from Inaba and Steinman *et al.*[7] A heretical possibility that cannot be dismissed out of hand is that the bone marrow-derived dendritic cell, once known as the passenger leukocyte, can *de*differentiate back to a pluripotent stem cell.

These important but unresolved matters aside, the bottom line is that cell migration is the secret of graft acceptance and a prime reason for drug efficacy. It is the explanation

for the two phenomena described in the title of our 1963 article that was the beginning of clinical kidney transplantation at a practical level—the reversal of rejection by steroids and the subsequent ability to reduce the intensity of immunosuppression (referred to as tolerance).[6] The consequences of the cell migration and repopulation were still demonstrable three decades later *in these same patients.* In retrospect, the extent of this cell movement was evident from the results of exhaustive skin test studies by two young immunologists, W. E. C. Wilson and Charlie Kirkpatrick, who were working with us, and by Dave Talmage in Denver. Nearly 80% (77% to be exact) of the skin reactions that were positive in the donor, but not the recipient crossed over to the previously negative recipient, along with the transplanted kidney.[8] When this did not occur (in the other 23%), it meant that the kidney transplant had failed. Kirkpatrick and Wilson speculated that the migration shown in the skin tests was "caused by adoptive transfer of donor cellular immunity by leukocytes in the renal vasculature and hilar lymphoid tissue." Unfortunately, these observations were made four years before Steinmuller showed the extent of intrarenal passenger leukocytes[9] and 10 years before these leukocytes were identified by Steinman and Cohn[10] as dendritic cells. Consequently, the skin test transfer could not be fully interpreted and eventually was attributed to "transfer factor,"[8] a subject on which the same Charlie Kirkpatrick has presented a paper in this volume.

However, even without a consensus explanation for rejection reversal and for what we had called tolerance in these early patients seen in Colorado, these observations, which soon were confirmed elsewhere, led to the empiric therapeutic dogma upon which our specialty of solid organ transplantation is based. The dogma calls for daily baseline treatment (in those days with azathioprine) plus intervention with the highly dose-maneuverable adrenal cortical steroids or antilymphoid agents to whatever level is required to maintain stable graft function. This creates a trial and potential 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 that was first emphasized in the classical studies of Pekka Hayry of Helsinki,[11] but preventing the immune destruction that is the natural purpose of the traffic.[12] In principle, 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 inactivated but living cells that normally cause graft immunogenicity and rejection become instead the missionaries subserving chimerism, graft acceptance, and ultimately tolerance.

THE XENOGRAFT BARRIER

The function of the lymphocyte can be disrupted at the level of antigen processing (claimed at one time for the drug deoxyspergualin), at an early stage in T cell activation as occurs with cyclosporine 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 antimetabolite drugs, of which azathioprine was the prototype, affect the immunocytes even more distally.

How these drugs work can be determined by isolating lymphocytes from biopsy specimens of rejecting organs and showing that these lymphocytes are suppressed with a given drug, and by measuring the cytokine content in the medium.[13,14] One of the first observations made with FK-506 was its ability to suppress clones of lymphocytes in cases of intractable rejection that had escaped under cyclosporine treatment. Subsequently, FK-506 clinical trials began in 1989 with "rescue" of recipients whose downhill course was arrested with this drug.[15] Slightly more than three years later, FK-506 has

TABLE 1. Graft Survival[a]

	Group	Treatment[b]	Without FK-506		With FK-506	
			n	Survial > 30 days	*n*	Survival > 30 days
Heart graft	1	None	6	0	—	—
	2	FK 506	6	0	—	—
	3	RS-61443	4	0	6	5
	4	BQR	6	1	5	5
	5	Cyclophosphamide	5	0	5	5
	6	Cyclophosphamide	5	0	5	5
Liver graft	7	None	8	0	—	—
	8	FK 506	10	5	—	—
	9	RS-61443	5	0	10	9
	10	BQR	7	1	7	6
	11	Cyclophosphamide	5	0	10	9
	12	Cyclophosphamide	5	0	15	12

NOTE: Animals alive at 30 days survived as long as FK-506 was continued out of 100 days no matter what the adjuvant induction drug.

[a] These experiments are a fraction of those performed. A full account of this work as well as the testing of numerous other compounds is provided in Ref. 16.

[b] Daily dose (mg/kg): for **FK-506** was 2.0 × 6, 1.0 × 25 (heart) or 1.0 × 30 (liver), and 0.5 on alternate day thereafter; for **RS-61443** was 20.0 × 15 (14) starting day before Tx (heart) or day of Tx (liver); for **BQR** was 4.0 × 3 and 3.0 × 12 starting day before Tx (heart) or 3.0 × 14 starting day of Tx (liver); and for cyclophosphamide was 7.5 × 15 (14) starting day before Tx (heart) or day of Tx (liver), except in Group 6 and 12, for which one dose of 80 mg was given 10 days preoperatively.

become the brooding giant of baseline immunosuppression that has allowed clinical intestinal transplantation to move onto center stage and may allow xenotransplantation to go forward in the way that I will describe now.

Ironically, the key drugs other than FK-506 that can unlock this door have been in our hands for more than 30 years.[16] They belong to the class of antiproliferative drugs that block enzymes required for the synthesis of ribonucleotides and thereby inhibit the DNA synthesis without which the final step of clonal lymphocyte expansion cannot proceed normally. All of these agents affect both T and B lymphocytes, but with some specificity. For example, azathioprine is more T cell-directed, as is discussed elsewhere in this volume, and cyclophosphamide has a greater anti-B cell effect.

Although the duality of humoral and cellular mechanisms of xenograft rejection has been common knowledge for years, the antibody component has been refractory to treatment. For example, a hamster heart is confronted in the rat by a moderate titer (1:16-1:32) of preformed heterospecific cytotoxic antibodies and subsequently by a rapidly gathering antibody storm that destroys abdominally placed cardiac grafts within three days in untreated recipients, before there is a trace histopathologically of immunocyte infiltration. By itself, FK-506, which prevents T cell activation and cytokine secretion, can prolong survival by only one day (TABLE 1). Monotherapy with either of two experimental "antiproliferative" drugs, RS-61443 and Brequinar, which suppress purine or pyrimidine nucleotide synthesis, respectively, tripled or quadrupled survival, but did not permit consistent chronic survival.[16]

However, when either of these two antimetabolite drugs or, more importantly, when the conventional anticancer drug cyclophosphamide was added to FK-506 for

the first two postoperative weeks, extended survival under continued FK-506 alone became routinely possible (TABLE 1). It was particularly noteworthy in the hamster-rat experiments that a single large dose of cyclophosphamide 10 days before transplantation permitted 100% success with daily postoperative FK-506. With either heart or liver, success was correlated with the ability to prevent the antibody response.

The conclusion from these studies is that prevention or mitigation of heterospecific antibody rejection by interdiction of the B cell proliferative response with antimetabolite drugs for a surprisingly short period after transplantation or even beforehand is the essential first step to successful xenotransplantation, and unmasks the potential of continuous therapy with T cell-directed immunosuppressants such as FK-506. Such combination therapy should be clinically applicable, providing the humoral antibodies do not act so rapidly that they cause hyperacute rejection in a matter of a few minutes or hours. This condition has been demonstrated empirically with baboon-to-human kidney[17] and heart[18] xenotransplantation. In 1963, six baboon-to-human renal xenografts functioned for 6 to 60 days. These organs (and two decades later, Baby Fae's baboon heart) eventually developed regional infarcts when the blood vessels developed these widespread occlusive lesions that were thought to be antibody-mediated.

After breaking through the antibody barrier, the process of xenograft acceptance involves the cell migration and consequent systemic chimerism that I described for allografts at the beginning of this paper. With a monoclonal antibody that recognizes LEW rat, but not hamster cells, we have shown that recipient dendritic and lymphoid cells are incorporated into heart or liver xenografts examined 100 days after transplantation. The displaced cells leaving these organs were ubiquitously distributed throughout the recipient where they could be detected easily with polyclonal anti-hamster antibodies and confirmed with polymerase chain reaction (PCR) technique.

This means that successful clinical xenotransplantation must be visualized along the same lines of the donor-recipient cellular intimacy that I described at the outset to be the fundamental means of allograft acceptance. To some this will be apocryphal, and to others it will define the magnificent unity of biology.

CONCLUSION

These and other viewpoints were considered at the University of Pittsburgh for two-thirds of a year before the decision was made to proceed with the baboon-to-human liver transplantation that took place there in 1992, just after this symposium ended. There was no more appropriate forum to have made these plans known in advance and to provide their justifications than at this remarkable meeting of scientists.

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