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Organ Transplantation from the Laboratory to the Clinic

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Organ transplantation is an extraordinary success story, developing rapidly from slow and distressing beginnings to a standard life-saving treatment now given to more than 1 million recipients worldwide.

Experimental and clinical organ transplantation started nearly 50 years ago, with experiments on immunosuppression in 1959, performed at the Royal College of Surgeons of England research laboratories. After initial failure to prolong canine renal allografts using total body x-irradiation, drug-induced immunosuppression was investigated using the anti-leukaemia drug, 6-Mercaptopurine, which had been shown by Schwarz and Damashek to prevent antibody formation in rabbits.[1] 6-Mercaptopurine proved to be superior to x-irradiation. Following publication of this work,[2] 6-Mercaptopurine was used on a few cases at the Royal Free Hospital in London and in Paris by Professor Kuss.[3,4]

Since these early experiments were encouraging, a number of compounds were tested, one of which was Aziothioprine, which was slightly superior to 6-Mercaptopurine in prolonging canine renal allografts.[5] Following this work a clinical programme was started at the Peter Bent Brigham Hospital using Aziothioprine as the main immunosuppressant.[6] The early results were disappointing until corticosteroids were added to the regimen. This combination developed enthusiastically by Dr. Starzl in Denver was used in kidney transplantation by approximately ten centres worldwide.[7] In 1963 Dr. Starzl performed the first clinical liver transplant, but the patient and three others did not survive long enough to justify the procedure, so a moratorium was established on liver transplantation for four years in Denver.[8]

Some of the pigs which had liver transplants survived for many years without immunosuppression at any stage.[9] The technique worked out in the pig was then applied to man in 1968 in Cambridge[10] and at about the same time Dr. Starzl had resumed his clinical programme in Denver[8]. The search for better immunosuppression led to some effective polyclonal antilymphocyte globulin preparations developed by Woodruff in Edinburgh, Starzl in Denver and Najarian in Minneapolis. They found it difficult to obtain consistently effective non-toxic products, but gradually refinements in production yielded better results.

The next important improvement in immunosuppression was the development of Cyclosporin, from the Sandoz laboratory by Borel using *in vitro* studies of antibody formation and skin grafts in mice.[11] Cyclosporin was investigated and was found to be extremely effective in prolonging heart allografts in rats, kidney allografts in dogs, liver and cardiac allografts in pigs.[12-14] This led to the first use of Cyclosporin in clinical organ transplantation in Cambridge.[15] The dose derived from the animal experiments proved to be much too high, and a side effect of Cyclosporin in man, not observed in animals, was nephrotoxicity

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which, once recognised, resulted in drastic dosage adjustment.

Cyclosporin was a watershed in organ transplantation and enabled much better early results with liver, heart and eventually lung and pancreas transplants. Instead of approximately ten centres worldwide performing solid organ transplants, soon there were more than 1000. A standard regimen of immunosuppression was Aziothioprine, steroids and Cyclosporin attempting to obtain maximum immunosuppression and minimum side effects of each individual agent.

In Cambridge an active programme of kidney and liver transplantation was established and the first vascularised pancreas transplant[16] in the UK was performed in the Cambridge unit, as was the first intestinal transplant[17] and the first heart, lung and liver transplant in collaboration with the associated cardiovascular unit in Papworth.[18] The Cambridge department had a special interest in developing better immunosuppression. Prograf (Tacrolimus) had been shown to be an effective immunosuppressant by Ochiai in Japan.[19] This work was repeated and then moved to larger animals where considerable toxicity was observed.[20] Another macrolide, Rapamycin, was investigated and found to be slightly less toxic.[21] The mechanism of action was later found by others to be different from the calcinurin inhibitors. Rapamycin alone is not nephrotoxic; it was first used in the clinic in Cambridge and was found to be a useful additional immunosuppressant but not so powerful as the calcinurin inhibitors. In the meantime Pittsburgh group under Dr. Starzl had demonstrated the value of Prograf in clinical transplants, where the toxicity in man was shown to be less than in the large animal studies in Cambridge.[22]

Monoclonal antibodies were first discovered in Cambridge by Kohler and Milstein.[23] Waldmann and colleagues in the Dept. of Pathology found an extremely powerful lympholytic monoclonal antibody called Campath[24] (named after the Cambridge Dept. of Pathology). This is derived from rats and is effective against the human CD52 epitope present on all lymphocytes. This antibody was used to treat rejection in Cambridge and was found to be very effective but the patients had a high incidence of dangerous viral infections.[25] The humanised version of the antibody was used as a induction agent in kidney transplants after reports from Wisconsin which suggested that powerful induction with an "immunotoxin" could produce long lasting immunosuppression without any other drugs in monkeys with renal allografts.[26] The use of Campath combined with very low maintenance immunosuppression, half-dose of Cyclosporin instead of full-dose of three drugs, has been followed up for more than eight years now with encouraging results. 80% of the patients have not received any maintenance cortiocosteroids so this form of immunosuppression which has been called *prope* (almost) tolerance results in good graft survival, an excellent quality of life for most patients and considerable reduction in the cost of maintenance immunosuppression.[27-29]

Gene therapy for the treatment of diabetes and haemophilia using the human insulin and factor VIII genes and the Lentivirus as a vector has now been attempted. This work has reached a stage for study in large animals. A novel source of foetal stem cells taken from the lining of the human umbilical cord is used in these experiments. The cells, which are of two types, epithelial and mesenchymal, proliferate extremely well. One million cells after two weeks are expanded to one billion. Gene therapy is studied in two ways; one via transfection *in vitro* of amnion cells and then transplanting the amnion cells into diabetic or haemophilic animals. The amnion cells have been studied also from the point of their apparent lack of immunogenicity. They secrete HLA-G which has a powerful immunosuppressive effect and maybe part of the mechanism by which the mother and foetus can live in harmony.[30]

A similar vector to the one used in Singapore has been used by another group in Sydney and has made an important advance in transfection, by injecting the vector into temporarily ischaemic livers of diabetic rats and mice.[31] This work is at an early stage but if the results obtained in rodents in Sydney can be reproduced in diabetic dogs, this would be a significant advance with the prospect of clinical application. The technique depends on rendering the liver temporarily ischaemic. It requires a major surgical procedure and therefore investigation of simpler, less traumatic methods of obtaining a similar high level of transfection is now being made.

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