Int. J. Dev. Biol. 33: 189-195 (1989)

Organ transplantations: from basic research to clinical applications. A personal odyssey

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ABSTRACT. This article is a summary of the impact on contemporary medicine of organ and tissue transplantation. The article describes how, via trial and error, and beginning from basic research, the results of organ transplantation have steadily increased as has the number of organs that can be transplanted. Currently, the short-term results of most organ transplants, with the notable exception of the pancreas and the lung, are close to perfect; very few organs are lost any longer due to acute rejection. There is, however, little information on long-term results using the current modalities of immunosuppression, particularly on the effect of chronic rejection on late graft survival.

KEY WORDS: *Allograft rejection, immunopathology, organ transplantation*

The idea of transplanting an organ is millenia old: it existed in Chinese history, in Middle Eastern and Greek mythology, and materialized as Indian nose «transplants» more than 2000 years ago. The first allograft in man was probably performed by Tagliagozzi around 1590, who most likely was also the first surgeon to demonstrate skin allograft rejection (Tagliagozzi, 1597). However, it was not until the beginning of this century that Alexis Carrel and his associates developed methods for vascular anastomosis (Carrel, 1902), while it was during the Second World War that Medawar conclusively demonstrated the immunological nature of allograft rejection (Medawar, 1944). Both of these contributions received the Nobel Prize, awarded to Carrel in 1912 and to Medawar in 1960.

During the past 25 years clinical organ transplantation has drastically altered the picture of contemporary medicine: in 1970 in Finland we performed 16 renal transplant; 5 of which involved organs from deceased donors. No bone marrow, liver or heart transplants were done. In 1986 we performed 142 renal transplants, 127 using organs from cadaver donors, more than two dozen bone marrow transplants and approximately half a dozen liver and heart transplants. The heart-lung transplant programme is also now under way. In 1970, 30% of the cadaver renal allografts functioned after the first year; today nearly 90% of the grafts survive at least for one year. The one-year graft survival in liver and heart transplants averages 80% (Häyry, 1988).

Nature's miracle in transplantation: the embryo

Haploidentical grafts are rejected nearly as vigorously as an entirely unidentical allograft. Thus if skin or a kidney for example is transplanted from the offspring to the parent, the graft is rejected unless immunosuppressive medication is employed. The only exception to this rule is the embryo. For nine months, a human embryo matures in the womb of the mother and «rejection» occurs when the child is capable of life. Unfortunately, even now, we do not know precisley what the mechanism of graft acceptance is in this natural situation.

Graft rejection, a cellular and molecular concept

How has the remarkable success in clinical transplantation been achieved? To my undrestanding there is only one common denominator: biomedical research. This is what has brought the impossible within our reach.

In the field of transplantation, most research is basic research: its final applications may not necessarily benefit transplantation but may influence cancer biology, cellular immunology or molecular genetics. Approximately 20% is applied research, where the basic findings are applied from living animal transplants to human subjects. The remaining 10% is clinical research, mostly clinical trials in transplant recipients.

Transplantation in vitro

The *in vitro* research of relevance to transplantation carried out mostly during the 70's and early 80's was targeted to two major areas: investigators wished 1) to clarify the cellular and molecular mechanisms whereby lymphocytes, particulalry T lymphocytes, are triggered to proliferate as well as to elucidate their genetics and distribution; and 2) to attempt to determine the molecular nature of antigens responsible for this, i.e. the transplantation antigens.

Many significant contributions were published. One of the most important, which was awarded the Nobel Prize in 1980, was the discovery of the «special» transplantation antigens and the clarification of their genetics, particularly the structure of the «major histocompatibility gene complex» (MHC). Many of the functions of these molecules in natural immunological defense and in graft rejection were thereby explained (Dausset, 1958; Eliman *et al.*, 1970; Snell *et al.*, 1971; Thorsby, 1971). Finally, the structure of these molecules was also worked out.

Another equally important finding was the discovery that the two major components of the immune response, «cellular» and «humoral» immunity, are generated by different subsets of lymphoid cells, the T cells and B cells (Miller and Mitchell, 1969). This was followed by the development of reagents to identify and isolate further subpopulations of these two major populations, leading to the testing of their properties *in vitro*, and the elucidation of their functions, particularly in transplantation immunity.

A final observation of importance during this period was the discovery of interleukins, the secretory molecules regulating the stimulation (and cytotoxic events) of T cells, as well as the proliferation of, and antibody synthesis by, B cells, activation of mononuclear phagocytes, etc. (Aarden *et al.*, 1979). In the early 80's, genes for many of these molecules were cloned, and these molecules were synthesized by recombinant gene technology, first in bacteria and then in mammalian cells.

Our own contribution during this particular period included the demonstration that mixed lymphocyte culture-induced lymphoid T cells mediate specific cytotoxicity to lymphoid target cells (the MLC-CML-reaction), the first model whereby one component of the transplantation response could be analyzed entirely *in vitro* (Häyry and Defendi, 1970). Furthermore, we found that at least two T cell components participate in the generation of cytotoxic T cells (Häyry and Andersson, 1974). Unfortunately we were unable to define these components at that time given the lack of suitable reagents. It is now known that these components are T «helper» (CD4) and «cytotoxic precursor» (CD8) cells. Finally, we demonstrated that after priming in the MLC, T cells can respond to allogeneic cells alone (Andersson *et al.*, 1973) and that they develop into memory T-cells capable of responding rapidly when re-stimulated for a second time with the original antigen (Andersson and Häyry, 1973). After implanting the *in vitro*-primed T cells in nude mice *in vivo*, we found that this property is maintained for at least several months. A practical finding related to this observation is the primed lymphocyte typing test, PLT, which was the first test whereby class II transplantation antigens, the so-called antigens D, were defined.

Transplanation in vivo*: intra-graft events in allograft rejection*

The idea of conducting the whole transplant rejection in a test tube was a fascinating one, but impossible in practice. It was soon realized, primarily on the basis of histopathological and immunofluorescence studies, that the inflammatory response in the graft was far more complex than in a test tube, and that there was no way of investigating the various interactions between the graft and the different inflammatory populations by keeping them in *in vitro* culture. Therefore, the opposite approach was selected: isolation of the graft-infiltrating inflammatory cells *in vivo* and analysis of their functions *in vitro*.

To define the inflammatory components of graft rejection morphologically and functionally, we first employed the so-called sponge matrix allografts (Roberts and Häyry, 1976): a tiny piece of a viscous cellulose sponge was implanted into the peritoneal cavity of mouse strain A. After the sponge was filled with fibroblasts and macrophages, it was transplanted subcutaneously to mouse strain B. Rejection was generated, and inflammatory cells were recovered by squeezing the sponge. We found that the sponge allograft was infiltrated by specific donor-directed lymphoid cells, which exhibited a fast second-set response upon re-exposure to the relevant antigen and cytotoxicity to relevant target cells *in vitro*, and that in addition to cytotoxic T cells, other cytotoxic cells (of as yet undefined origin) were also concentrated in the sponge (Roberts and Häyry, 1977). Unfortunately, it was not possible to further investigate the relationship of the inflammatory components to the graft parenchyma with this relatively artificial model.

A second *in vivo* model was therefore set up: renal transplantation in the inbred rat. Methods were developed to isolate the inflammatory component from the renal transplant and, later, to grow a culture of the various parenchymal components of the graft, including the components of the nephron (glomerular mesangial and epithelial cells, proximal and distal tubular cells) and graft microvascular endothelium. We found that in addition to specific T cells, the graft was also infiltrated by B cells and —towards the end of rejection— by increasing numbers of mononuclear phagocytes (v. Willebrand and Häyry, 1978; Häyry *et al.*, 1979). Also large granular lymphocytes, the so-called NK cells, were present in the inflammatory event (Nemlander *et al.*, 1983). The activation of mononuclear phagocytes takes place *in situ* via activated lymphoid cells (Alfoldy *et al.*, 1984). At a final stage of rejection thrombocytes are also precipitated in the graft (v. Willebrand *et al.*, 1985). In addition to lymphoid target cells of the donor, the inflammatory cells were cytotoxic to the majority of the renal components *in vitro* (unpublished observation).

It was, therefore, pausible that the cytotoxic donor-directed components were responsible for the allograft damage. Although this view was generally accepted a few years ago, and appears in the textbooks even now, it may not be the case. Other groups (Doveren *et al.*, 1986; Oroz *et al.*, 1986) as well as ours (Manca *et al.*, 1987) have defined the frequency of donor-directed lymphoid cells in the graft. Limited dilution assay has demonstrated that after isolation of the inflammatory cells *in vitro*, with regard to the T cells, the frequency of donor-specific T helper cells is at the most 1:450 while that of the donor-specific cytotoxic T cells is at the most 1:250. We are currently estimating the frequency of donor-directed B cells in the inflammatory infiltrate, and preliminary results indicate that their frequency is not any higher; in fact it is lower. Taken together, at any stage of rejection, the total frequency of specific donor-directed lymphoid cells in the graft is less than 1 %. Thus it seems plausible that they are not the major pathway of inflammatory damage, but rather that most of the damage must be attributed to the remaining inflammatory elements. The question remains as to how these inflammatory components are regulated and what their actual mode of action is.

Regulation of inflammation

An early finding of interest and significance was that the expression of transplantation antigens in human renal allografts was not stable, but was influenced by extraneous factors. In the resting stage (i.e., in the donor) human kidney expresses transplantation antigens, particularly class II histocompatibility antigens, only in graft microvascular endothelium and, to a lesser extent, in the remainder of the vascular endothelium (Häyry *et al.* , 1980). This is in clear contrast to rat tissue, where class II MHC antigens are distributed primarily inside the proximal tubular cells. Very little expression is seen on the endothelial component (v. Willebrand *et al.*, 1980). After transplantation, in well-functioning human kidneys, we were unable to trace the MHC antigens of the graft endothelial cells. However, upon rejection these antigens were widely distributed not only in the endothelial cells but also in the tubular cells (Häyry *et al.*, 1981a, b). Later studies have shown these alterations to be regulated by inflammatory lymphokines, secreted in large quantities *in situ,* particularly by gamma-interferon (Pober *et al.*, 1986). Thus, graft immunogenicity is not stable, but may be altered by regulation of antigen presentation (Ferry *et al.*, 1987).

A second important observation made both in Helsinki and elsewhere was that similar or closely-related lymphokines regulate both MCH antigen expression and endothelial cell permeability to inflammatory cells. During rejection, the white cell traffic to (Nemlander *et al.*, 1982) and from (Pedersen and Morris, 1970) the graft increases by more than two log orders in magnitude. Equally important was the observation that the alterations in vascular permeability are regulated by lymphokines secreted by inflammatory cells. A whole range of lymphokines including IL-1, IL-2, gamma-IFN and possibly others, have been found to

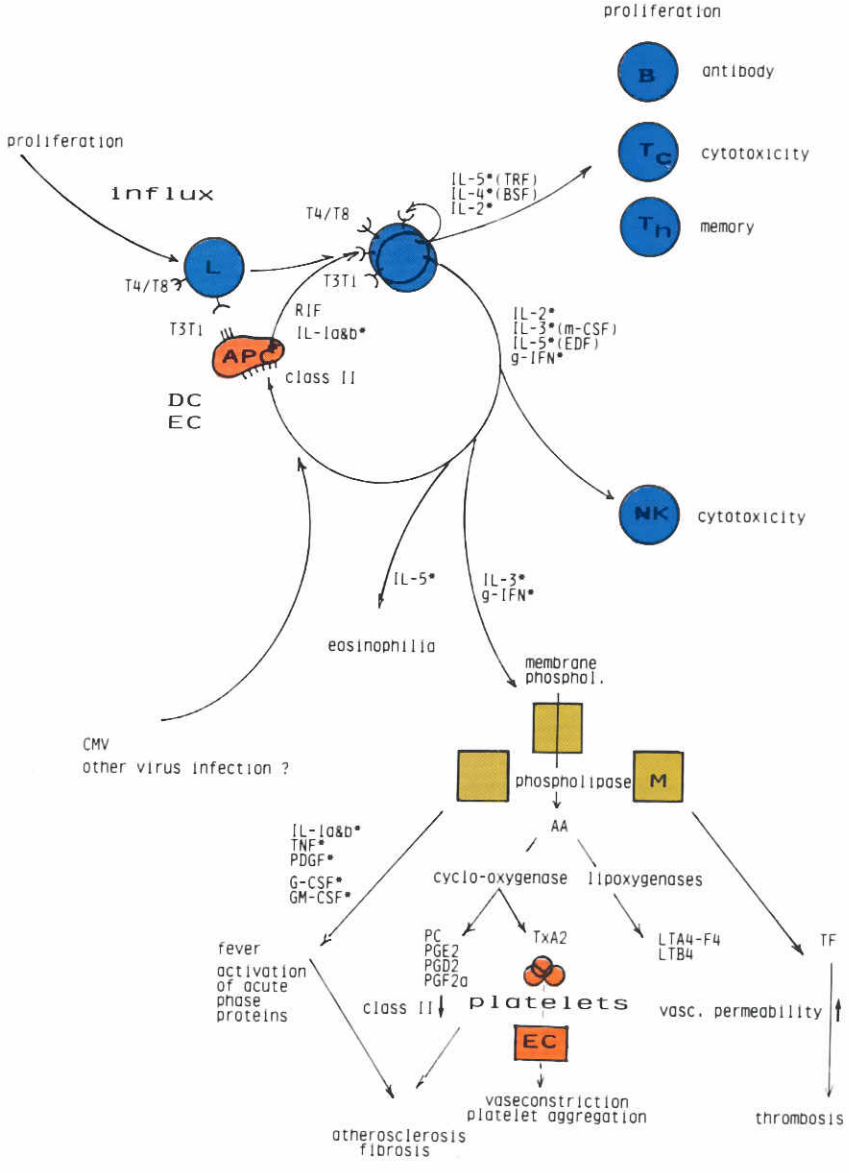

Fig. 1. *Major cellular and molecular pathways in allograft rejection. The antigen presenting cells (APC) display transplantation antigens on their surface and provide a «second signal», receptor-inducing factor (RIF), and/or interleukin-1 (IL-1 alpha and beta) to resting T cells possessing receptors (T3Ti) to this antigen. Both dendritic cells (DC) and endothelial cells (EC) can function as antigen-presenting cells. Obviously at least two types of T cells, designed as T4 and T8, collaborate in the induction of transplantation immunity. As a result of antigen presentation, the antigen-reactive T cells display interleukin-2 receptor (IL-2r) and secrete a variety of lymphokines including interleukins-2, -3, -4, -5 (IL-2, IL-3, IL-4, IL-5), and gamma-interferon (g-IFN). The net effect of these lymphokines induces T and B cell proliferation, including the generation of antibody-secreting (B) cells, cytotoxic (Tc) lymphocytes and memory (Th) lymphocytes, as well as making the natural killer (NK) cells cytotoxic. Several of these lymphokines, most notably IL-1, IL-2 and particularly gamma-interferon also effect the antigen-presenting cells, upregulating class II antigens on these cells and making them secrete more of the «second signal». This generates a vicious cycle, which is the driving force that keeps rejection going. Induction of MCH antigens on the APC is also a likely mechanism whereby CMV and possibly other virus infections can induce graft rejection. More «peripheral» consequences of the lymphokine response are blood eosinophilia, most likely due to IL-5, and activation of the mononuclear phagocytes. A central role in mononuclear phagocyte activation is displayed in eicosanoid synthesis. The various eicosanoid cascades, including the cyclo-oxygenase and lipoxygenase cascades,are generated by the interleukins. The activation of mononuclear phagocytes (M) leads to the further secretion of lymphokines, or rather «monokines», including alpha and beta IL-1, tumor necrosis factor (TNF), platelet-derived growth factor (PDGF), granulocyte colony-stimulating factor (GC-SF) and monocyte colony-stimulating factor (GM-CSF). In acute rejection, prostacyclin (PC), PGE-2, PGD-2 and PGF-2 alpha seem to operate as «good» prostacyclins, downregulating class II expression. The «bad» prostacyclins, particularly thromboxane A2 (TxA-2) and lipoxygenase products, LTA4-F4, are «pro-inflammatory» prostacyclins inducing vascular permeability and platelet deposition in the graft. Some of the clinical manifestations of rejection, particularly fever and the activation of acute phase proteins, are due to alpha and beta IL-1. These lymphokines, together with the «bad» prostacyclins, particularly thromboxane-A2 (TxA2), also collaborate in the generation of graft atherosclerosis and fibrosis, the basic manifestations of chronic rejection.*

increase leukocyte adherence to endothelial cells, the penetration of leukocytes through the endothelium and their appearance in tissues (Leszczynski and Häyry, 1988a, b).

The effect of interleukins on class II expression seems to be a direct one, though it can be modified by prostacyclin synthesized by most endothelia. Prostacyclin downregulates the level of class II expression. Instead, the effect of interleukins on endothelial permeability seems to be regulated by the 5-lipoxygenase pathway of eicosanoids, possibly by LTB4 and related products (Leszczynski and Häyry, 1989). In addition, many of the clinical consequences of acute rejection, including fever (interleukin-1 effect), and blood eosinophilia (interleukin-5 effect), can be explained by the inflammatory interleukins.

Taken together, these intra-graft changes generate a vicious cycle inside the allograft: increased antigen expression induces increased immune response, and increased immune response induces in turn an increased amount of inflammation. Some theoretical considerations of this vicious cycle are given in Fig. 1.

Applications to man

Clinically, three modes of rejection have been distinguished. Hyperacute rejection results from preformed antibodies which can be excluded by a negative cross-match before transplantation. In acute rejection, cell-mediated, lymphoid and mononuclear phagocyte-mediated mechanisms predominate. Such rejections are usually recorded during the first weeks or months after transplantation. In acute rejection, the specific donor-directed lymphoid component is clearly demonstrable. Chronic rejection, which takes place later is dominated by graft atherosclerosis: specific components are difficult to demonstrate or are non-existent.

Transplant aspiration cytology

An important result of these basic studies was the development of aspiration cytology methods for the monitoring of *in situ* events in renal allografts (Häyry and v. Willebrand, 1981; v. Willebrand and Häyry, 1983). During the immediate postoperative follow-up, aspiration cytology has largely replaced other invasive methods, particularly needle core biopsy. Currently, aspiration cytology has been extended to liver allografts, to the monitoring of lung allografts (by bronchoalveolar lavage) and to the monitoring of pancreatic allografts. Five international workshops have been organized on this technique.

Immunosuppressive chemotherapy

Unlike in rodents, which may be rendered «tolerant» to the alien graft, tranplantation is not possible in human subjects without immunosuppressive chemotherapy.

The development of human transplantation parallels the development of chemical immunosuppression. Early attempts at immunosuppression using total body irradiation met with very poor results. Approximately 30 years ago Schwartz *et al.* (1959) demonstrated that 6-mercaptopurine, which is an anti-cancer drug, entirely abolishes antibody response to human serum albumin in rats and hamsters. This observation of «induced immunological tolerance» was applied successfully to dog renal transplants by Roy Calne during the following year (Calne, 1960). However, it was quite toxic. Within months, Hitchings and Elion of Burroughs-Wellcome had developed a new derivative from 6-mercaptopurine, originally called BW-322 and later azathioprine (Hitchings and Elion, 1963). This derivative proved far less toxic. It induced graft acceptance for variable periods of time not only in the dog but also in many human subjects. In 1988 Hitchins and Elion were awarded the Nobel Prize for this and related studies.

Azathioprine was first combined with low-dose whole-body irradiation, irradiation of the grafted kidney (either before or after performing the transplant) and with the use of adenocorticotropic hormone (ACTH) and cortisone. Rejection was still a problem: only the routine application of steroids together with azathioprine finally made long-term graft acceptance possible (Starzl *et al.*, 1963).

The results were still far from remarkable: when in 1976 I reviewed the worldwide results of human renal transplantation (Häyry, 1976), the average one-year cadaver renal allograft survival was still well below 50%, and one-year mortality was nearly 35%.

Clinical trials

When a new mode of treatment is developed, its efficacy is compared to the pre-existing one(s) by using randomized clinical trials. In transplantation, this is particularly applicable to the prophylactic treatment of rejection, where new immunosuppressive drugs and methods have become available since the advent of immunosuppression.

After the basic features of transplant response were elucidated, it was possible —on the basis of available literature— to define the sites of action of the various drugs on the inflammatory cascade. The main sites of action of the major immunosuppressive drugs, azathioprine (AZA), cyclosporine-A (CSA) and steroids (MP) in the inflammatory cascade are defined in Fig. 2.

Our first clinical trial derives from 1979-1980 (Table 1). In this trial we investigated the impact of the steroid dose on graft outcome (Häyry *et al.* , 1984). In an open, controlled, non-randomized experiment we found that with AZA and a low steroid dose, a 44% rate of cadaver renal transplant function was obtained at the end of the first year. This was very close to the international average. When the steroid dose was tripled for the immediate postoperative period, one-year graft survival increased to 68%. The new treatment had no impact on patient survival, which remained at about 80%.

TABLE 1

UNIVERSITY OF HELSINKI, DEPARTMENT IV OF SURGERY CADAVER RENAL TRANSPLANTS[a]

Years of trial	Drug combination		Patients[b]	Graft survival	Patient survival
1979	I	AZA + low MP	136	44%	80%
1980	vs. II	AZA + high MP	68	68%	84%

[a] Häyry, Ahonen, Kock *et al.* (1984), *Scand. J. Immunol. 19:* 211.
[b] Open, controlled, non-randomized. First and subsequent grafts.

In the second clinical experiment, performed in 1981-1982, we investigated the effect of a new drug, cyclosporine (Table 2). Ninety-six patients were randomized in three treatment groups, AZA plus high initial steroids (our best group so far),

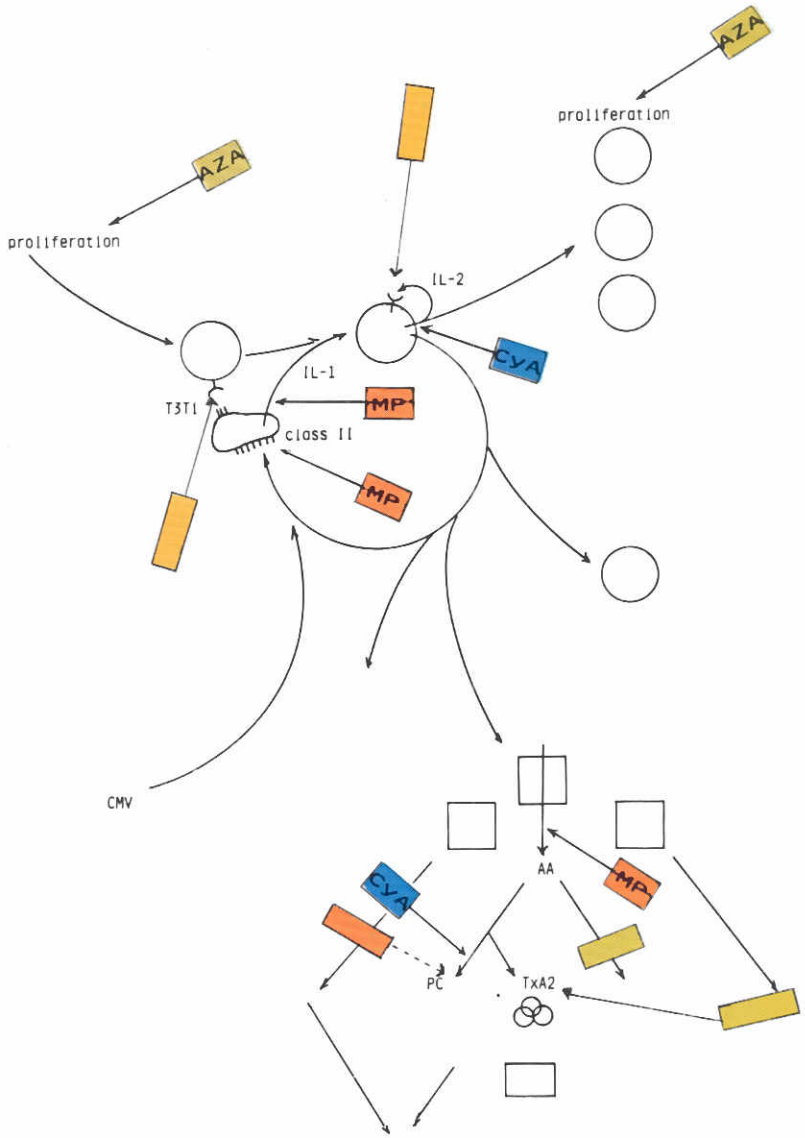

Fig. 2. *Sites of action of drugs commonly used in rejection prophylaxis and treatment. Azathioprine (AZA) is an anti-metabolite affecting cell proliferation. It is moderately specific to lymphoid cells, particularly to T cells, and inhibits the proliferative response both before and after antigen confrontation. Steroids (methyl-prednisolone, MP) reduce IL-1 synthesis and class II expression on the antigen-presenting cells (APC), as well as reducing the phospolipase A2 enzyme of the arachidonic acid cascade. Finally, cyclosporine, the most specific of these, inhibits transcription of the IL-2 molecule. Cyclosporine might also have a second site of action on the eicosanoid pathways. This is most likely an inhibitory action on prostacyclin endoperoxidase or possibly some other endoperoxidase. This second site of action is most likely responsible for the negative (toxic) effects of cyclosporine on the kidney.*

CSA alone or CSA plus high initial steroids, the drug dosages being comparable in the three treatment groups (Häyry *et al,*. 1982; Häyry *et al.*, 1988). We found that with regard to graft survival, CSA monotherapy was clearly inferior to the remaining two regimens. Graft survival was 69% with AZA and steroids, 66% with CSA and steroids but only 62% with CSA alone. Patient survival in all three groups was around 90% at the end of the first year.

During 1982-1985, the possibility of employing three-drug immunosuppression with AZA, CSA and steroids was investigated in small series in crder to define an optimal dose for these three drugs when used together. Compared to recorded controls, the triple-drug treatment appeared superior to any previous two-drug combinations. Long-term consequences of triple-drug treatment were, however, not determined.

Therefore, in 1986-1987, a trial was performed to investigate whether it is possible to remove one of the three drugs in a randomized manner 10 weeks postoperatively (Häyry *et al.*, 1988). The results are given in Table 3. We found that it is possible to remove any one of the three drugs with no negative consequences to the graft or to the graft recipient. In fact, graft survival in all four groups, i.e. in patients who continued on

TABLE 2
UNIVERSITY OF HELSINKI, DEPARTMENT IV OF SURGERY CADAVER RENAL TRANSPLANTATION[a]

Years of study	Drug combination		Patients[b]	Graft survival	Patient survival
1981-1982	I	AZA + high MP	32	69%	88%
	vs. II	CyA	32	62%	91%
	vs. III	CyA + high MP	32	60%	81%

[a] Häyry, Ahonen, von Willebrand, *et al.* (1983), *Transplant. Proc. 15:* 2842, and unpublished data.

AZA, CSA plus MP, AZA plus CSA, AZA plus MP, or CSA plus MP, was nearly 90%, approximately equal to patient survival. In this study only one graft was lost to acute rejection; the rest of the losses were due to death of the recipient with a functioning graft.

Taken together, these studies demonstrate an increase in one-year graft survival from 48% to nearly 90%. Today, acute irreversible rejection is no longer a problem. Future studies will be conducted to investigate the impact of immunosuppression on long-term transplant success. To obtain a comprehensive picture of immunosuppression in the recipient, not only the graft but also the patient and his various organ systems are being investigated. An experimental model is urgently needed, preferably in the rat, and further clinical experiments must be performed.

TABLE 3
UNIVERSITY OF HELSINKI, DEPARTMENT IV OF SURGERY CADAVER RENAL TRANSPLANTATION[a]

Years of trial	Drug combination				Patients[b]	Graft survival[b]	Patient survival[b]
	Initial		Subsequent				
1986-1987	AZA CyA low MP	I	AZA CyA low MP		32	88%	88%
		vs. II	AZA CyA		32	91%	91%
		vs. III	AZA low MP		32	88%	88%
		vs. IV	CyA low MP		32	88%	93%
		Altogether			128	89%	91%

[a] Häyry, Ahonen, von Willebrand *et al.* (1987), *Transplant. Proc. in press,* and unpublished data.
[b] Open, controlled, randomized. First grafts only.

From the Department of Zoology to the Transplantation Laboratory

The tiny embryo that was implanted to grow in Sulo Toivonen's Division of Developmental Biology laboratory in the Department of Zoology in the 1960's has grown into a mature adult. This development has taken place via several different «stadia»: the implantation was performed by Sulo Toivonen, Lauri Saxén and Tapani Vainio at the Department of Zoology more than 20 years ago; the embryonal maturation was completed at the Wistar Institute with Vittorio Defendi and Hilary Koprowski, and the delivery took place at the Third Department of Pathology with Erkki Saxén and Kari Penttinen, about 15 years ago. Since then, the newborn has grown, and more people have joined the transplantation group, e.g. Leif Andersson, Peter Roberts, Eeva von Willebrand, Juhani Ahonen, Irmeli Lautenschlager, Risto Renkonen, Arto Nemlander and Anu Soots, to mention just a few. All of them have contributed to the growth and guidance of the maturing youngster. Now, 20 years later, what was once an embryo has grown into a full-fledged laboratory that is serving all components of transplantation activity in this country.

It is a pleasure for the staff of the Transplantation Laboratory to congratulate Professor Emeritus Sulo Toivonen on his birthday and to offer our very best wishes to the *Primus Motor* of so many areas of experimental research in Finland!

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