AN ASSESSMENT
OF A NEW IMMUNOSUPPRESSIVE AGENT
15-DEOXYSPERGUALIN (15-DS) FOLLOWING CARDIAC AND
RENAL ALLOTRANSPLANTATION AND CARDIAC
XENOTRANSPLANTATION IN PRIMATES - DOES
15-DEOXYSPERGUALIN INDUCE GRAFT NONREACTIVITY?

Ph.D.-THESIS

by

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Dedicated to

Professor Bruno Reichart M.D.

formerly

Head of Department of Cardiothoracic Surgery
University of Cape Town, RSA

now

Head of Department of Cardiac Surgery
University of Munich, FRG

for his active support throughout my carreer

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ACKNOWLEDGEMENT

I would like to thank my supervisors, Professor Bruno Reichart and Professor John Odell for their major support throughout the study period.

In addition I, would like to express my gratitude to Dr. Paul Human, Dr. Andreas Hildebrandt and Dr. Dieter Boehm for their assistance and F. Barends, J. Kloppers, J. Place, P. Madlingozi and F. Snyders for their skilled technical contribution.

I am further grateful to Professor Alan Rose and the Dept. of Pathology for elaborating the histological specimens, the Dept. of Chemical pathology for analysing the blood tests, Dr. Robin May and the Dept. of Tissue Immunology for their help with regard to the cyto-immunological monitoring and the Dept. of Pharmacology for Cyclosporine level determination.

15-Deoxyspergualin was generously supplied by Behring Werke, Marburg, FRG and Cyclosporine A by Sandoz Pharmaceuticals Corp., Johannesburg, South Africa.

The study was supported by grants of the "Chris Barnard Fund" and by supplies from the Cape Provincial Administration.

Finally, I would like to express my deep appreciation to the University of Cape Town for all the support and for its strong attitude rejecting racial segregation in constitution of the student body.

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ABSTRACT

15-Deoxyspergualin (15-DS) is a new immunosuppressive agent which showed promising results after experimental allogeneic heart, liver, kidney and pancreas islet cell transplantation in rodents. After renal and hepatic transplantation in rats, long-term graft tolerance has been achieved by using 15-DS; however, very little is so far known about the efficacy of 15-DS in larger animals.

In order to assess the immunosuppressive potentials of 15-DS in a preclinical experiment, heterotopic cardiac (n=27, group I) and classical renal (n=25, group II) allotransplantations were performed in Chacma baboons. The following immunosuppressive regimens were used:

Groups IA and IIA served as control groups and received no medication.
Groups IB and IIB were treated with 15-DS alone (4 mg/kg/ day) for postoperative days 0-9.
Groups IC and IIC were treated with Cyclosporine A (CyA, 10-40 mg/kg/day) for postoperative days 0-30.
Groups ID and IID received the combination of 15-DS (for postoperative days 0-9) and CyA (postoperative days 0-30).

Acute rejection episodes were diagnosed using cyto-immunological monitoring and weekly myocardial or renal core biopsies.

After cardiac transplantation, the mean graft survival was 11.0 days for group IA, 28.2 days for

group IB ($p < 0.05$; IB vs. IA), 32.4 days for group IC and 43.1 days for group ID ($p < 0.025$; ID vs. IA).

After renal transplantation, the corresponding figures were 12.3 days for group IIA, 8.5 days for group IIB, 30.4 days for group IIC and 148.9 days for group ID ($p < 0.025$; IID vs. IIA).

After cardiac and renal transplantation, acute rejection was the main cause of graft failure. Treatment related side effects, mainly gastrointestinal complications, were observed only in primates which were treated with 15-DS alone, but not when 15-DS was combined with CyA. No major infections occurred.

After cardiac transplantation, permanent graft nonreactivity was not achieved, but a delayed rejection occurred within a mean of 21.8. days after immunosuppression had been stopped.

Following renal transplantation, graft nonreactivity was observed in group IID after initial treatment with 15-DS and CyA. In this group, 4 out of 8 animals (50%) were graft tolerant up to 340, 256, 244 and 164 days after treatment discontinuation.

Thus, the combination of 15-DS and CyA led to a significant prolongation of graft survival in both groups. Using the same drug combination, long-term graft nonreactivity was achieved in a primate model after renal transplantation.

In a second study protocol, the efficacy of 15-DS in combination with CyA was tested after heterotopic

cardiac xenotransplantation and compared with other immunosuppressive protocols.

Heterotopic heart transplantations were performed in the neck using Vervet monkeys as donors and Chacma baboons as recipients. The following groups were investigated:

Group I (n=8): Control, no immunosuppressive medication.

Group II (n=5): CyA in combination with azathioprine and methylprednisolone.

Group III (n=6): CyA, azathioprine and methylprednisolone in combination with antithymocyte globulin (RATG) for postoperative days 0-9.

Group IV (n=7): CyA, azathioprine and methylprednisolone were combined with 15-DS for postoperative days 0-9.

Due to severe treatment related side effects which were observed in group IV, further immunosuppression was modified as follows:

Group V (n=5): 15-DS was combined with CyA and methylprednisolone only.

Acute rejections were treated with 500 mg methylprednisolone i.v. as a bolus dose for 3 to 5 consecutive days.

The graft survival after xenogeneic heart transplantation was best in group III with 43.3 days on average compared to 10.3 days in the control group. However, 2.3 acute rejections per animal still occurred, which in most cases led to graft failure in these animals.

In the 15-DS-treated group IV, the graft survival was prolonged to 20.1 days on average. Only 0.14 acute rejections per animal and biopsy occurred, but severe gastro-intestinal complications and infections were observed, which made further experiments necessary to minimize these treatment related complications. However, by omitting azathioprine, the mean survival rate in the modified treatment group V was 35.6 days.

In both 15-DS treated groups, a significantly reduced number of acute rejection and a decreased incidence of hyperacute rejections was noted when compared to the other treatment protocols.

In conclusion, 15-DS is a promising immunosuppressive agent, but when given alone, graft survival results are not satisfactory. In combination with CyA, however, significant improvement of graft survival was achieved after cardiac and renal allotransplantation and after cardiac xenotransplantation in primates.

After cardiac allo- and xenotransplantation, the number and severity of acute rejection episodes were significantly reduced by the addition of 15-DS.

After renal allotransplantation, long-term graft nonreactivity was observed in 50% of the animals, when initially treated with 15-DS and CyA.

These findings definitely justify further studies with 15-DS, as it may be a useful adjunct in clinical immunosuppression.

PART I: INTRODUCTION

I.1. Immunosuppression after heart transplantation.

Allogeneic heart transplantation (HTx) has become an accepted clinical therapy for end stage cardiac diseases (1). Sufficient immunosuppressive therapy is, however, mandatory to avoid lethal rejection crises. "Conventional" immunosuppressive therapy, which was used for the first 10 years of cardiac transplantation, consisted of azathioprine, prednisone and antilymphocyte globulin. Severe rejection and infection were the major cause of death, leading to a 1-year survival rate of only 60% after heart transplantation. The results have improved dramatically since the introduction of cyclosporine A-therapy (2,3). This drug is, however, not the panacea hoped for: Severe acute rejection episodes can still occur and represent,besides severe infections, the major cause of death following transplantation. In addition, Cyclosporine A (CyA) has major side effects, such as nephro- and hepatotoxicity. A correct dosage schedule is therefore necessary and blood levels have to be measured regularly.

To improve immunosuppression and to reduce side effects of CyA, this drug is currently combined with azathioprine and low dose prednisone. This drug regimen has led to an actuarial 1-year survival rate of 81% after HTx for adult patients (3). This current immunosuppressive therapy has not yet reached the final state of the art, since acute rejection still occurs and severe side effects are still observed.

For these reasons, transplantation research should continue to concentrate on improving immunosuppressive therapy. One of the ideals in every field of organ transplantation would be the induction of tolerance against the graft; long term immunosuppressive thera-

py would then not be necessary. As a consequence of this, not only allogeneic transplantation would be easily managed, but also xenogeneic transplantation using foreign species as organ donors would become possible. The need for xenogenic transplantation is becoming more prominent due to the shortage of suitable donor organs for adult recipients as well as for small infants who are waiting for heart replacement. There exist several congenital heart defects which can be best treated with heart transplantation (e.g. hypolastic left heart syndrome) or even using combined heart and lung transplantation. Using current immunosuppressive therapy, experimental results after xenogeneic heart transplantations are not satisfactory, predominantly because of hyperacute and humoral rejection episodes which cannot be treated successfully at present (4). In addition, further problems may be caused by infectious diseases (especially virus infections), which may be transmitted by the xenogenic donor.

In order to improve experimental and clinical results after allogeneic and xenogeneic transplantation, a new immunosuppressive drug, 15-Deoxyspergualin (15-DS) was tested for potency and side effects in this study.

I.2. 15-Deoxyspergualin - a new immunosuppressive drug and its mode of action.

15-DS is a derivative of spergualin, a guanidine-like structure. Its isolation from bacillus laterosporus and its anti-tumour activity was first described by IWASAWA et al. (5). While the exact mode of its immunosuppressive action is not yet fully understood, it has been documented that 15-DS plays an important role in antigen presentation and/or recognition of target antigens by effector cells: Within the first few days after transplantation, 15-DS reduces the

expression of target antigens on the transplanted cells. This phenomenon has been demonstrated after skin and renal transplantation in rodents (6). After the 3rd postoperative day, the expression of major histocompatibility complex class I antigens (MHC class I) on transplanted kidney cells completely disappeared. Since MHC class I structures are the most important target antigens for the response of cytotoxic T-lymphocytes, it is possibly imaginable that the non-expression of these structures limits the antigen recognition by T-effector cells (7). This action may be the first step with regard to graft nonreactivity. There was no influence of 15-DS on MHC class II expression (8). This is in contrast to the action of CyA which affects the expression of MHC class II (9). When 15-DS was started on the 4th day after transplantation, the rejection process had already started, since the expression of target antigens was not inhibited (6). Analysis with the Fluorescence-Activated-Cell-Sorter revealed that 15-DS also reduces the expression of MHC class I on splenic macrophages. In the peritoneum, a new macrophage population was created, demonstrating a weaker MHC class I expression when compared to the original population (6); this second macrophage population was not induced by CyA therapy. The MHC class I and II expression on lymphocytes was not affected by 15-DS. These results confirm the different mode of action of 15-DS and CyA. Further studies would be necessary to show whether perfusion of the donor organ with 15-DS results in acceptance of the graft and in reduction of postoperative immunosuppressive therapy. This procedure was suggested by LITTLE II et. al.who used CyA for inhibition of alloantigen presentation in the donor organ (10).

Already in 1987 it was reported that 15-DS suppresses the macrophage function, but the exact mode of action was not understood (11). These authors used the technique of chemiluminiscence to demonstrate the suppression of macrophage function; the production of oxygen derived free radicals in monocytes was inhibited by 15-DS (12,13). Recently it was shown that 15-DS inhibits the

production of Interleukin I (IL-I) by in vitro cultivated and PHA-stimulated peritoneal macrophages (6). As a result of this process, the proliferation of cytotoxic lymphocytes is affected (14). This specific action of 15-DS is neutralized by the addition of comercially available IL-I. TAKASU et. al. confirmed this effect of 15-DS on IL-I secretion by in vitro cultivated hepatic epithelial and Kupffer cells after liver transplantation (15). This process might protect the transplanted liver from being rejected. The ability of Kupffer cells to secrete IL-I, has already been described (16). In addition 15-DS might block the IL-I receptor on T-lymphocytes resulting in inhibition of activation (17). Already in 1979 there was evidence that treatment of animals with macrophage suppressing agents induces the prolongation of survival of transplanted pancreatic islet cells (18).

Further reports describe the efficacy of 15-DS in treating acute rejection episodes after skin transplantation (19,20). An explanation for this may be found in its suppressive effect on macrophages (12): First, the presentation of antigen during the rejection process is mediated by phagocytosing cells (21). Second, CD4-positive T-lymphocytes are able to recognize their target antigen specifically and release cytokines which activate macrophages. These cells then destroy the graft, a process that can be prevented by suppression of their function (6,15). This "late" effect of 15-DS does, however, not affect the graft destruction mediated by CD8-positive lymphocytes which act independently of macrophages.

A further important advantage of 15-DS lies in its specific mode of action which is different to that of CyA. Because of their different working points, a combination of both drugs seems to be obvious. The function of lymphocytes (by CyA) and macrophages (by 15-DS) would thereby be affected resulting in a suppression of two immunologically essential cell populations.

Subsequently, 15-DS was shown to prolong graft survival after skin, heart, kidney and pancreas islet cell transplantation in rats (22,23,24). After renal and hepatic transplantation in particular, specific graft nonreactivity was achieved in long-term surviving rats (25,26). In islet cell transplantation in rats, the survival time was increased from a mean of 5 days to 38.3 days; in heart transplantation, from a mean of 8.5 days to 32.5 days and in kidney transplantation from a mean of 7 days to more than 170 days. Especially with kidney transplantation in rats, the long lasting graft acceptance may be explained by the induction of graft tolerance.

I.3. Design of the experimental study.

1. Very little is known about the efficacy of 15-DS in larger animals. In order to assess its immunosuppressive potential and side effects in a preclinical experiment, cardiac and renal allotransplantations were performed in Chacma baboons. A special point of interest in this study group was the potential induction of graft specific nonreactivity using 15 DS alone or in combination with CyA.

2. The efficacy of this drug was also assessed in a xenogeneic model. Concordant cardiac xenotransplantation was performed using Vervet monkeys as donors and Chacma baboons as recipients. In particular, the incidence of severe and hyperacute rejection episodes and graft survival rates were noted and compared with other immunosuppressive protocols consisting either of CyA alone or of CyA in combination with other immunosuppressive agents.

3. In all experimental groups, parameters like frequency and severity of acute rejection were noted, as well as the occurrence of treatment related side effects. The animals were followed using graft biopsies, routine biochemistry of the peripheral blood and immunological monitoring of the peripheral blood. Graft survival was also compared between the different treatment groups.

Part II EXPERIMENTS AND RESULTS

1. 15-Deoxyspergualin after cardiac and renal allotransplantation.

A major aim of the study was to assess the efficacy of 15-Deoxyspergualin (15-DS) after cardiac allotransplantation in a pre-clinical experimental model. Since graft tolerance has been reported after renal transplantation in rodents, a comparative study using 15-DS after renal transplantation was performed in primates as well.

1.1. Experimental animals, material and methods

1.1.1. Donor and recipient animals

Chacma baboons (Papio ursinus, 10 - 15 kg body weight) served as donors and recipients in all experimental groups. All animals received care according to the "Ethical Consideration in Medical Research, revised edition: 1987" set out by the South African Medical Research Council, Parow, 1989.

Donor and recipient animals were matched and compatible within the AB-blood group system. It was a precondition that donor and recipient animals were derived from different regions within South Africa in order to avoid transplantation within family members.

1.1.2. Anaesthesia and surgical procedure

After premedication with Ketamine (5 mg/kg b.w.), Pancuronium bromide (0,2 mg/kg b.w.) and Atropine (0,5 mg), anaesthesia was maintained by a combination of Halothane (1%) with oxygen 4 l/min and N_2O 6 l/min as inhalation.

1.1.2.1. Heterotopic cardiac transplantation

In 27 animals, heterotopic heart transplantation in the neck was performed according to the technique of MANN et al. (27).

Donor operation:
After routine midline sternotomy, the pericardium was opened longitudinally. The inferior (IVC) and superior (SVC) vena cava, aorta and pulmonary artery were dissected free. Thereafter the pulmonary veins were prepared. Intravenous heparin was administered (380 U/kg). A cardioplegic cannula was inserted into the ascending aorta. The SVC was ligated and the IVC clamped. The descending aorta was clamped and cardioplegic solution (St.Thomas solution, 15 ml/kg b.w.) was flushed through the ascending aorta into the coronary arteries. The IVC and right upper pulmonary vein were incised in order to avoid overdistension of the heart. In addition, the heart was cooled with topical cold saline solution. After perfusion with

cardioplegic solution, the IVC and all pulmonary veins were ligated. The heart was excised distally to the ligated vessels. Finally the aorta and pulmonary artery were dissected as distally as possible. The heart was placed in cold saline solution.

Recipient operation:
In the recipient animal, a skin incision was made anterior to the right sternocleidomastoid muscle. The muscle was retracted laterally and the carotid artery and the internal jugular vein were prepared. Particular attention was taken in order not to injure the vagus nerve. The carotid artery was clamped first and the aorta of the donor heart was anastomosed end-to-side to the carotid artery of the recipient by using runing 5.0 Polypropylene sutures. The internal jugular vein was next clamped and the pulmonary artery of the donor heart was anastomosed end-to-side to the internal jugular vein using running 5.0 Polypropylene suture (Fig. 1). After removal of the clamps, two cannulae were inserted into the left and right ventricles in order to remove the air from both ventricles. The heart usually defibrillated spontaneously. In case of ongoing ventricular fibrillation, the hearts were defibrillated by using internal defibrillation paddles. After careful haemostasis, the wound was closed. As antibiotic prophylaxis, the animals received 500 mg Ampicillin intraoperatively and twelve hourly for the first postoperative day.

1.1.2.2. Classical renal transplantation

Altogether 25 kidneys were transplanted in the usual clinical manner.

Donor operation:
After midline laparotomy, the abdominal aorta and IVC were prepared at the origin of the renal vessels. All side-branches of the renal

arteries and veins were ligated and dissected. After preparation, the IVC was clamped proximally and distally to the renal veins and incised. The abdominal aorta was similarly clamped proximal and distal to the renal arteries. A perfusion cannula was inserted in between the clamps. The kidneys were perfused using Euro-Collins solution (40 ml/kg b.w.) and the kidneys were cooled with topical cold saline. After perfusion, both kidneys were explanted en bloc with the abdominal aorta and the IVC still intact. The kidneys were placed in cold saline solution. The renal arteries and veins were prepared and dissected ex vivo by leaving big vessel cuffs around the distal ends of the vessels to facilitate the anastomoses.

Recipient operation:

After midline laparotomy, the right iliac region was prepared for implantation. The right common and external iliac arteries were prepared as well as the right common iliac vein. Any side braches were ligated and dissected. The external and internal iliac arteries were clamped distally and the common iliac artery was clamped

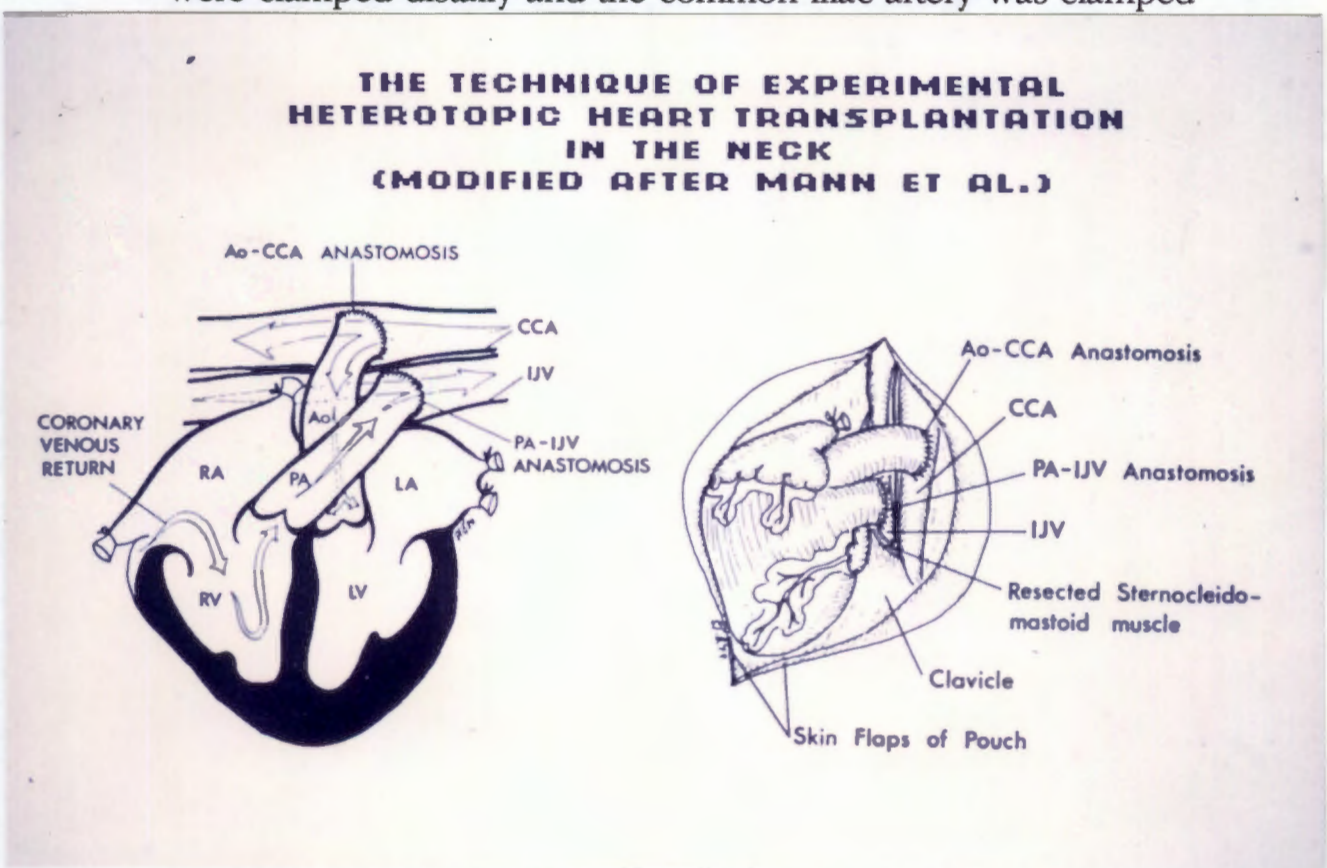


Fig. 1: Operative techique of heterotopic heart transplantation in the neck according to the technique of MANN (CCA = common carotid artery, IJV = internal jugular vein, Ao = ascending aorta, PA = pulmonary artery).

proximally. The common iliac artery was incised and the renal artery of the donor was anastomosed end-to-side using a running 6/0 Polypropylene suture. Thereafter, the iliac vein was clamped proximally and distally and the anastomosis was performed end-to-side also by using 6/0 Polypropylene suture (Fig. 2). After release of the clamps, the kidney was reperfused. The ureter was than anatomosed end-to-side to the bladder using the special technique according to Gregoir to avoid urinary backflow from the bladder into the transplanted ureter (28). The anastomosis was performed using 7/0 running Polypropylene suture. The distal portion of the ureter was tunnelled under the muscular layer of the bladder by using single 4/0 Polypropylene stitches. Thereafter, the recipient's own kidneys were removed after the vessels and ureters were separately ligated. After careful haemostasis, the peritoneum and muscular layers were closed using running sutures. The skin was closed using interrupted single skin sutures.

For antibiotic prophylaxis the baboons received 500 mg of Ampicillin intraoperatively and twelve hourly for the first postoperative day.

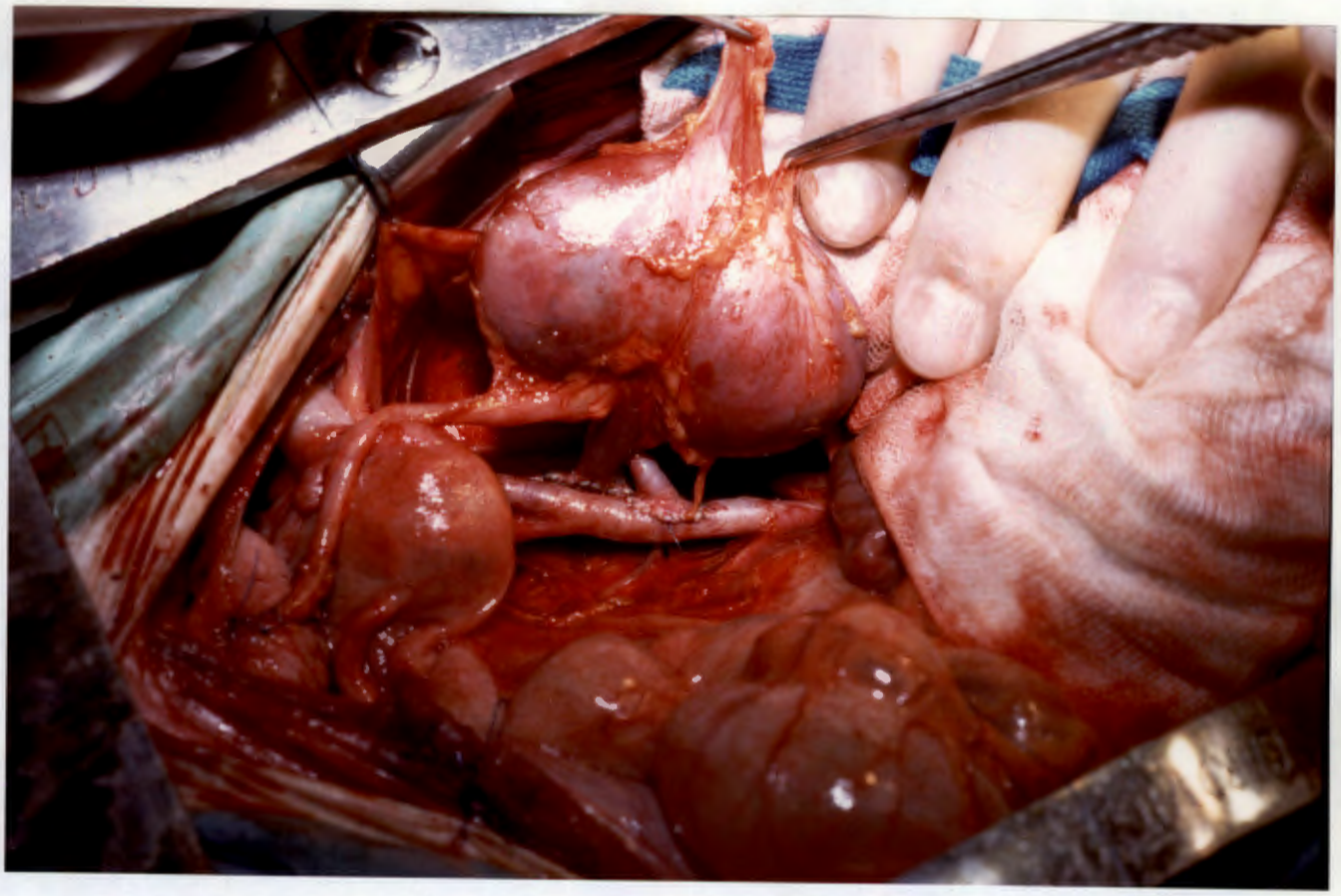


Fig. 2: Operative situs after classical renal transplantation. The renal artery is anastomosed to the common iliac artery and the renal vein to the common iliac vein. The ureter is anastomosed to the bladder tunnelled under the bladder muscle.

1.1.3. Immunosuppressive protocol

In this protocol, group I represents those animals following heterotopic cardiac transplantation, and group II the animals which underwent classical renal transplantation.

The immunosuppressive protocol is listed in Table 1. According to this protocol,

Groups IA (n=10) and IIA (n=6) served as control groups and received no immunosuppressive medication. For group IA, a histo-

ric control group was used which had been used for previous studies in our laboratory.

Groups IB (n=5) and IIB (n=6) were treated with 15-DS in a dosage of 4 mg/kg/day, administered intravenously over a period of 3 hours, from postoperative day 0-9, after which immunosuppression was stopped.

Groups IC (n=5) and IIC (n=5) were immunosuppressed only with CyA in a dosage of 10-40 mg/kg/day administered intramuscularly according to a whole blood trough level of 400-600 ng/ml from postoperative day 0-30.

Groups ID (n=7) and IID (n=8): In this group, 15-DS was given in a dosage of 4mg/kg/day, adminstered intravenously over a period of 3 hours from postoperative day 0-9. In addition, the animals received CyA in a dosage of 10-40 mg/kg/day administered intramuscularly, according to a whole blood trough level of 400-600 ng/ml from postoperative day 0-30. Thereafter no further immunosuppression was given.

IMMUNOSUPPRESSIVE PROTOCOL	
Groups IA and IIA	Control, no immunosuppression
Groups IB und IIB	15-DS, 4mg/kg b.w./d i.v. from p.o.day 0-9
Groups IC and IIC	CyA, 10-40mg/kg b.w./d i.m. from p.o.day 0-30
Groups ID and IID	15-DS, 4mg/kg b.w./d i.v. from p.o.day 0-9 *and* CyA, 10-40mg/kg b.w./d i.m. from p.o.day 0-30

Table 1: Immunosuppressive protocol after cardiac (group I) and renal (group II) transplantation.

1.1.4. Postoperative Monitoring

1.1.4.1. Clinical examination and graft palpation

Daily after transplantation, recipients were examined clinically. In the cardiac allograft group, the graft was palpated daily by the same person to monitor graft function. In group II, attention was paid as to whether the recipient animals were passing urine. It was not possible to exactly measure the urine output in the baboon cages.

1.1.4.2. Cyto-immunological monitoring

This method for early diagnosis of acute rejection using qualitative analysis of peripheral lymphocytes and their subsets has been described earlier (29,30).
Cyto-immunological monitoring was performed 3 times weekly during the whole postoperative course. In this test, the white blood cells per mm^3 are counted and a peripheral blood smear is prepared.
Mononuclear cells (lymphocytes and monocytes) are obtained from the remaining blood by centrifugation over a Ficoll Isopaque gradient (Ficoll Isopaque solution, density = 1.077 g/ml). Some of the cells obtained are spread on microscopic slides using a cytocentrifuge (Cytospin 1, Shandon Lab., Frankfurt, West Germany) and then stained with a panoptic leucocyte stain according to Pappenheim (May Grünwald solution, Merck Inc., Darmstadt, Giemsa solution, Merck Inc., Darmstadt, West Germany).

Under a light microscope (Hellfeld microscope, Zeiss Inc., Oberkochen, West Germany), the cells are separated according to the degree of activation into normal or activated lymphocytes and

lymphoblasts (Fig.3). The percentage of activated lymphocytes or lymphoblasts per 100 lymphocytes is calculated. If more than 3% activated lymphocytes per 100 lymphocytes are present, the mononuclear cell pattern of the recipient is described as being "activated" in terms of an acute inflammation process.

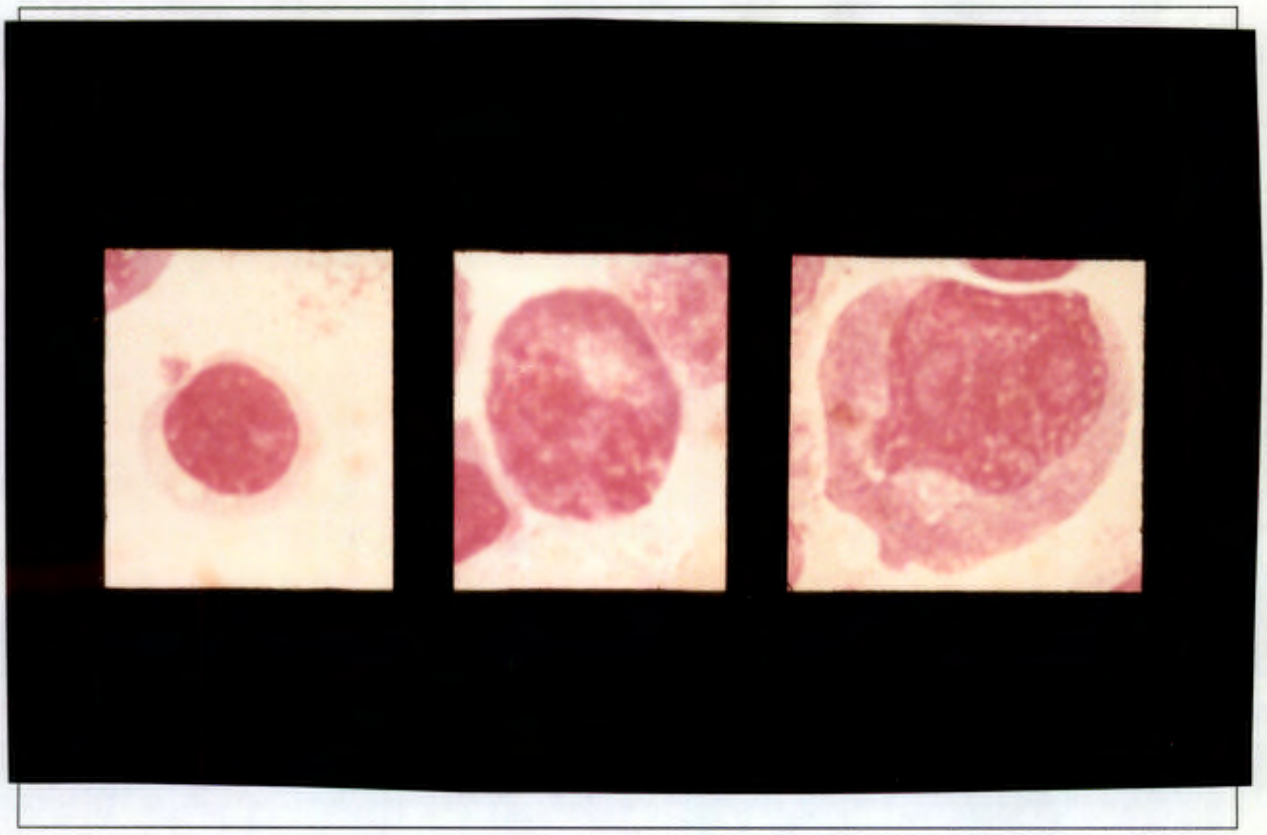


Fig. 3: A typical mononuclear concentrate of the peripheral blood during an acute inflammation process: a) normal lymphocyte, b) activated lymphocyte, identifiable by the noticeable increase in size, pronounced cytoplasmic basophilia and fluffy nuclear structure; c) lympho- or immunoblast, 2-3 times enlarged, when compared to the normal lymphocyte; note the basophilia of the cytoplasma, nucleoli are clearly visible within the cell nucleus (PAPPENHEIM STAINING, X1250).

1.1.4.3. Urea and creatinine levels

As parameters of kidney function after renal transplantation, serum urea and creatinine levels were measured in group II three times weekly. Any increase of the serum urea above 30 mmol/l and of the serum creatinine above 200 mmol/l was considered significant and a sign of impaired graft function.

1.1.4.4. Transmyocardial and renal core biopsies

At weekly intervals, transmyocardial biopsies were performed in group I and renal core biopsies in group II. For this procedure, the animals were briefly anaesthetised (Ketamine 5 mg/kg b.w., halothane 1%, oxygen 4 l/min and N_2O 6 l/min) and a small skin incision was made above the transplanted organs. The biopsies were performed using a trucut biopsy needle. Using this method, relatively large samples were obtained by cutting through the whole left ventricular myocardial wall or through half the renal core. The biopsy samples were stored in formaldehyde and evaluated histologically.

Acute rejection was graded according to the University of Cape Town grading system (30). This is a semi-quantitative scoring system which gives the clinician an easily understood guide as to the severity of rejection. Rejection is categorized as mild, moderate or severe. During mild rejection, only perivascular or interstitial mononuclear cell infiltration is observed but without myocardial cell damage. During moderate and severe rejection, cell infiltrates are present in addition to myocardial cell necrosis. The latter rejection episodes require rejection treatment after clinical heart transplantation.

Figures 4, 5, and 6 show typical examples of mild, moderate and severe rejection after cardiac allotransplantation. A similar scoring system may be used for renal allotransplantation (Figs. 7, 8, 9).

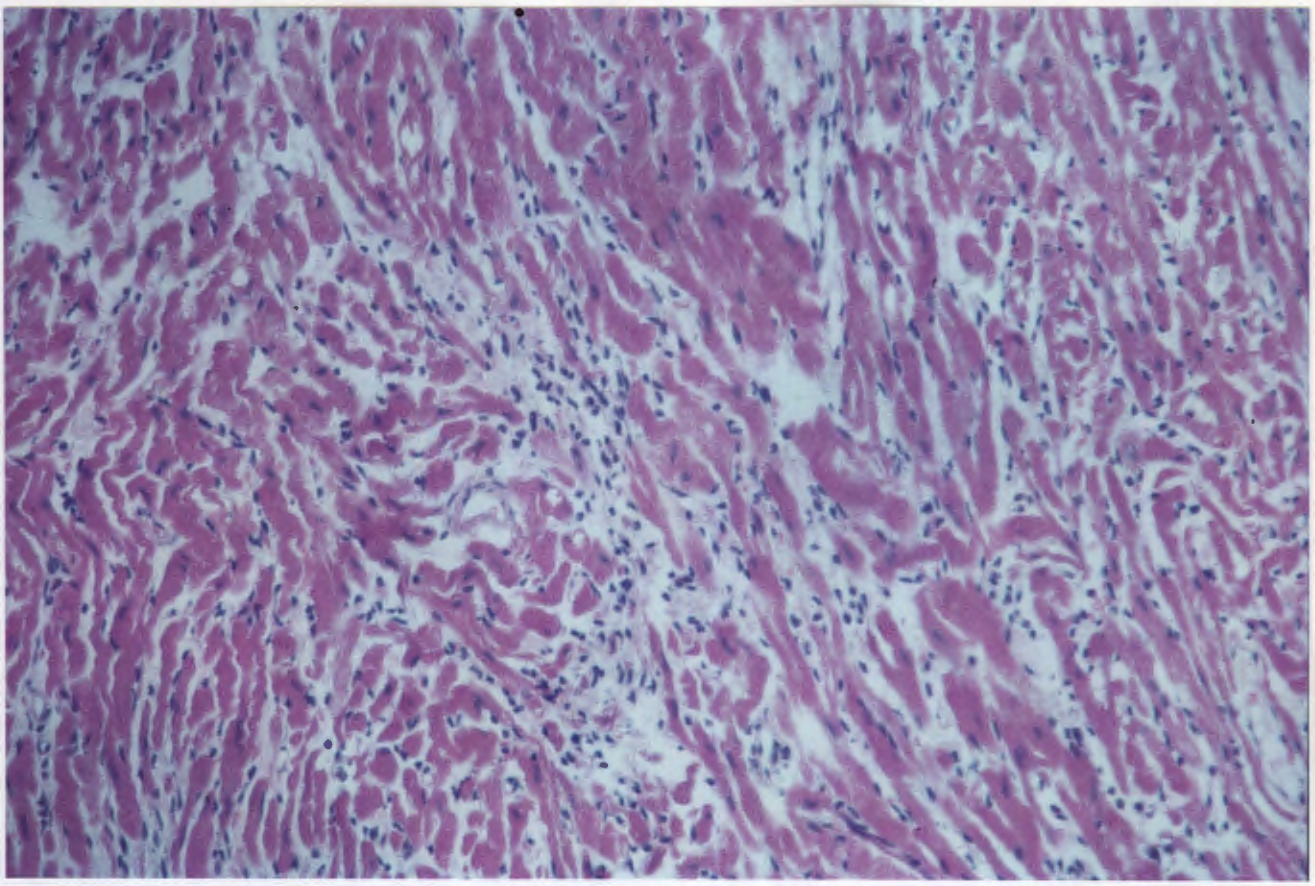


Fig. 4: Mild acute rejection episode: perivascular and scanty interstitial mononuclear cell infiltrations, slight interstitital oedema, but no sign of myocyte damage. Figs. 4-9 and 19-22 were generously supplied by Professor Alan Rose.

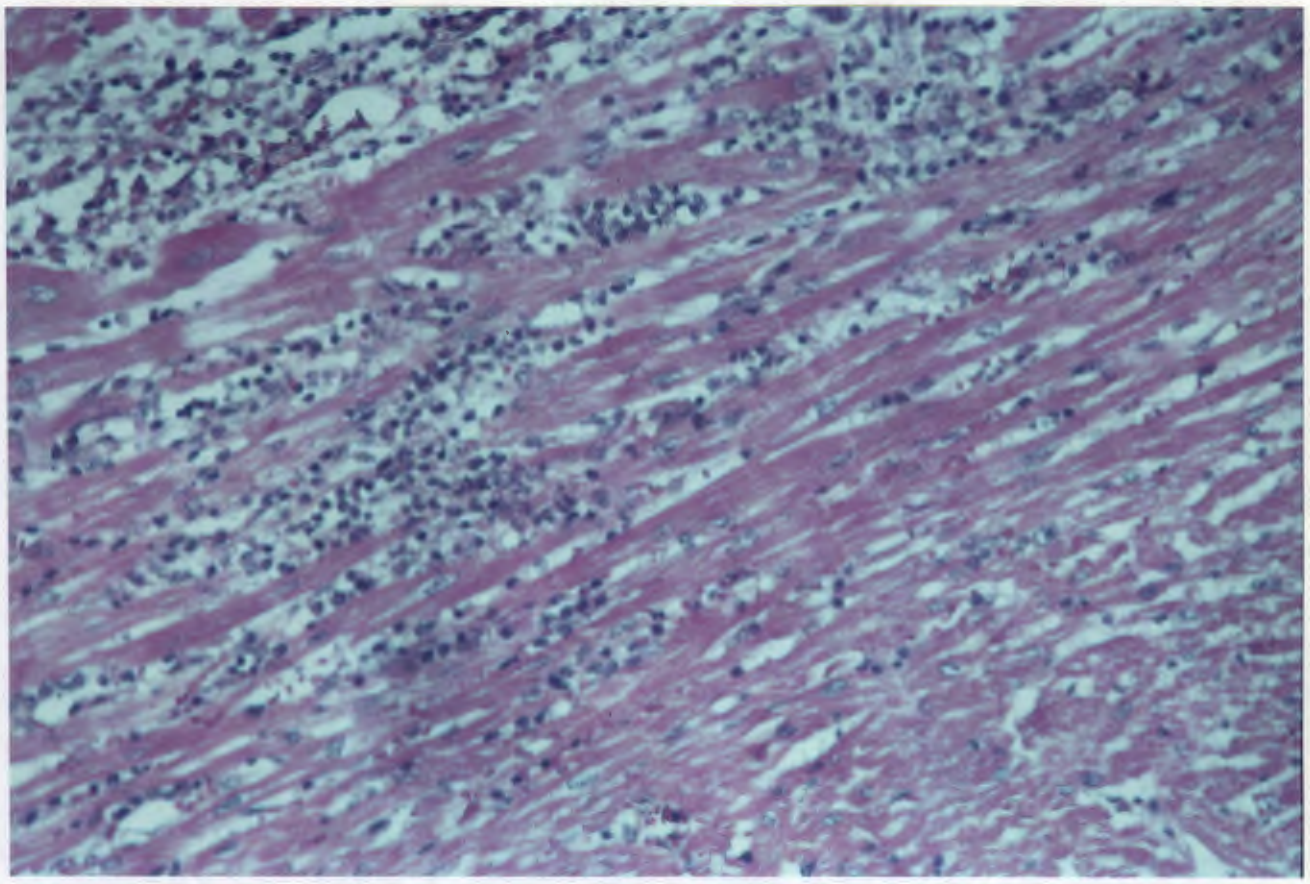
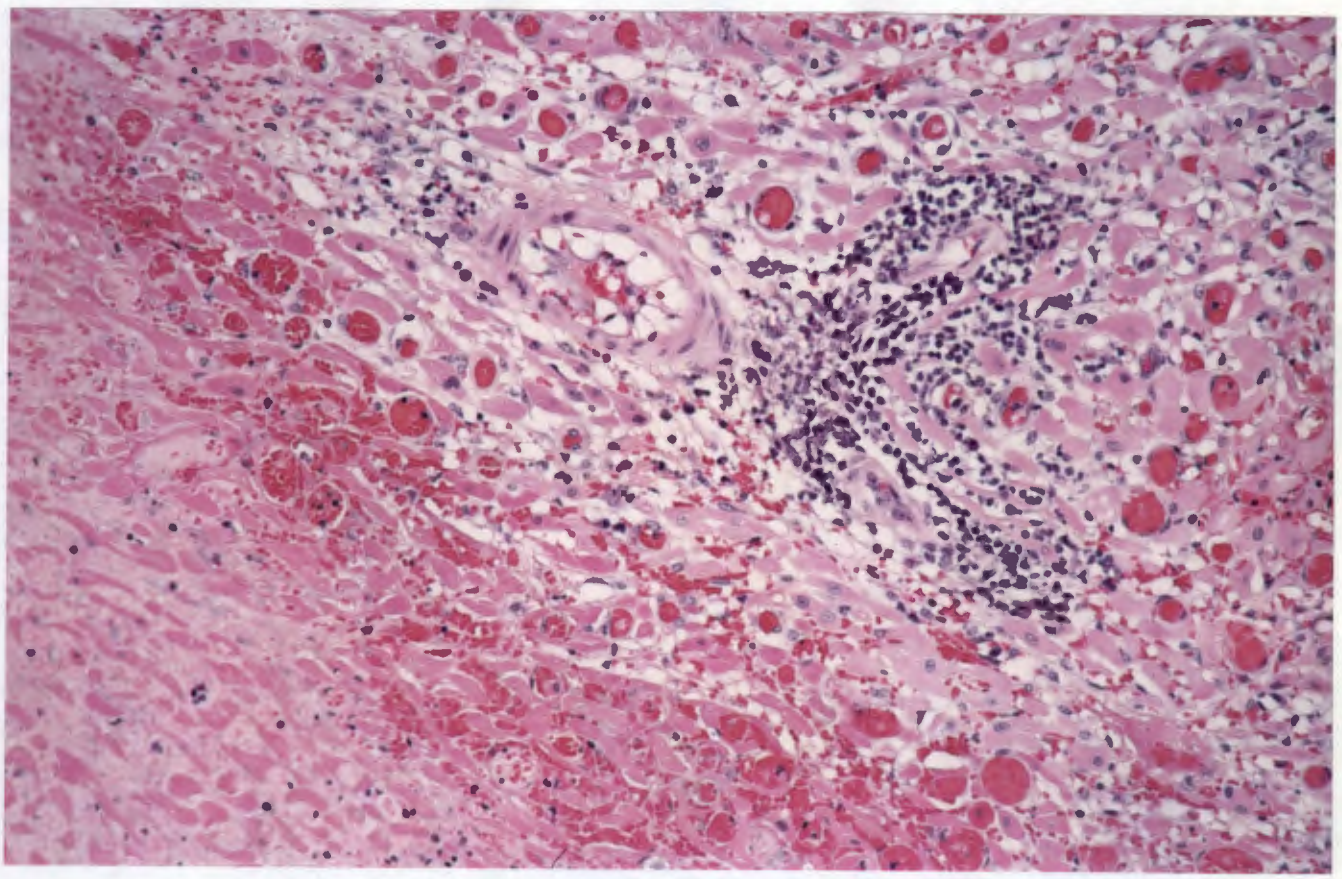


Fig. 5: Moderate acute rejection: Diffuse interstitial mononuclear cell infiltration, more pronounced interstital oedema and signs of myocyte necrosis.
Fig. 6: Severe acute rejection: In addition to the mononuclear cells, there is infiltration of neutrophile granulocytes, diffuse myocyte necrosis and haemorrhage.



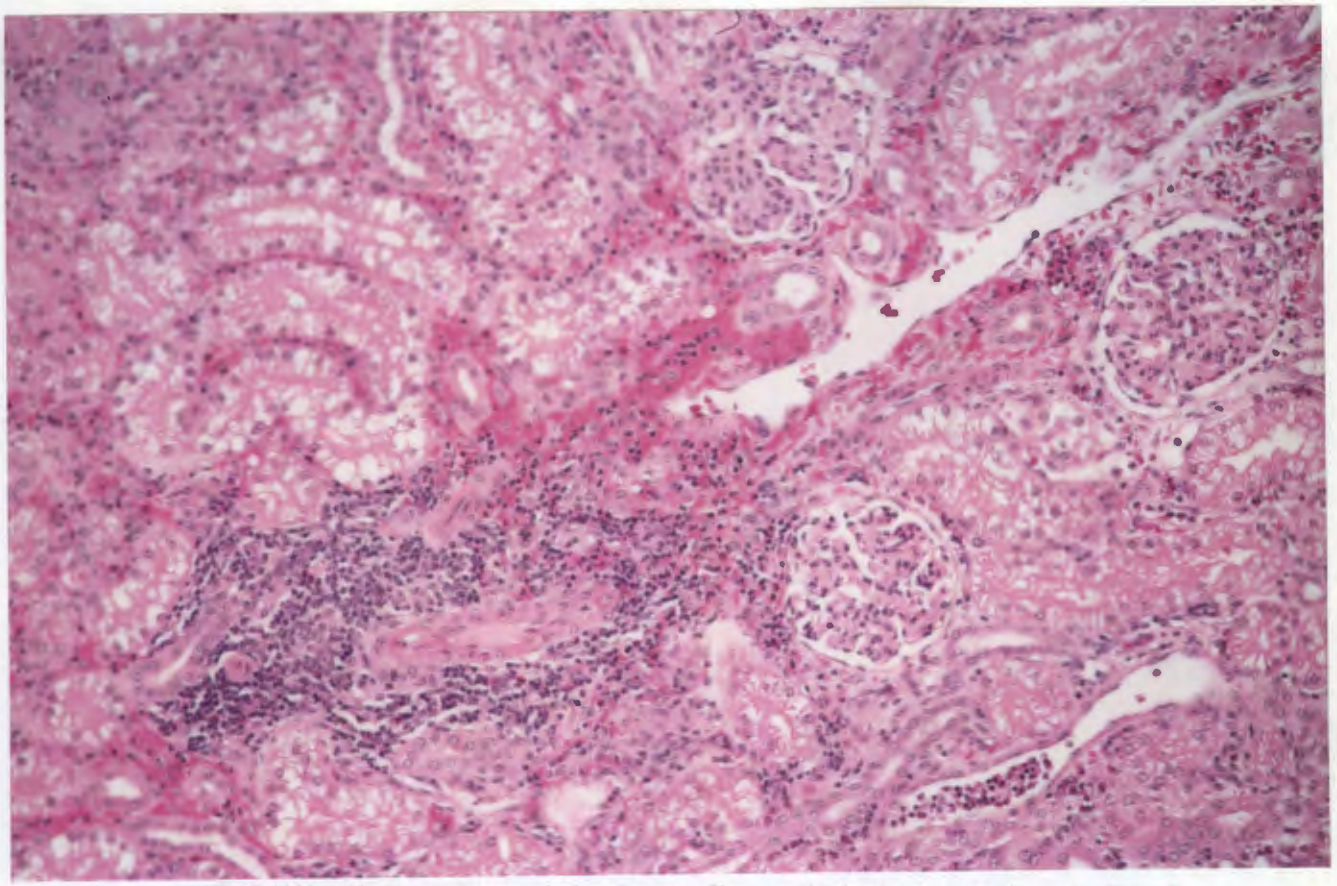
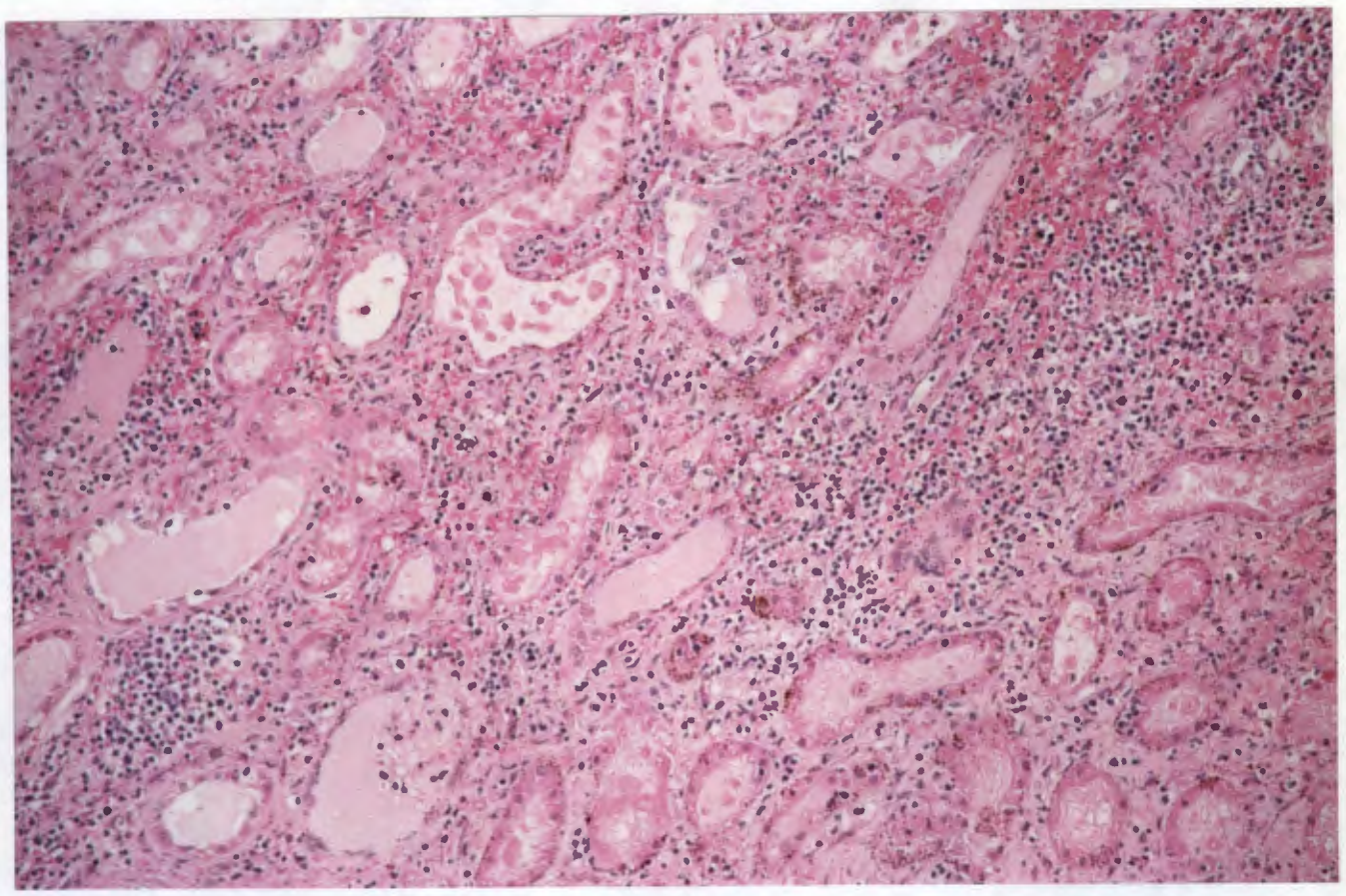


Fig. 7: Mild renal rejection: Perivascular lymphocytic infiltrates and only scanty interstitial mononuclear cell infiltrates are visible.

Fig. 8: Moderate renal rejection: There is mononuclear cell infiltration with evidence of acute tubular necrosis.



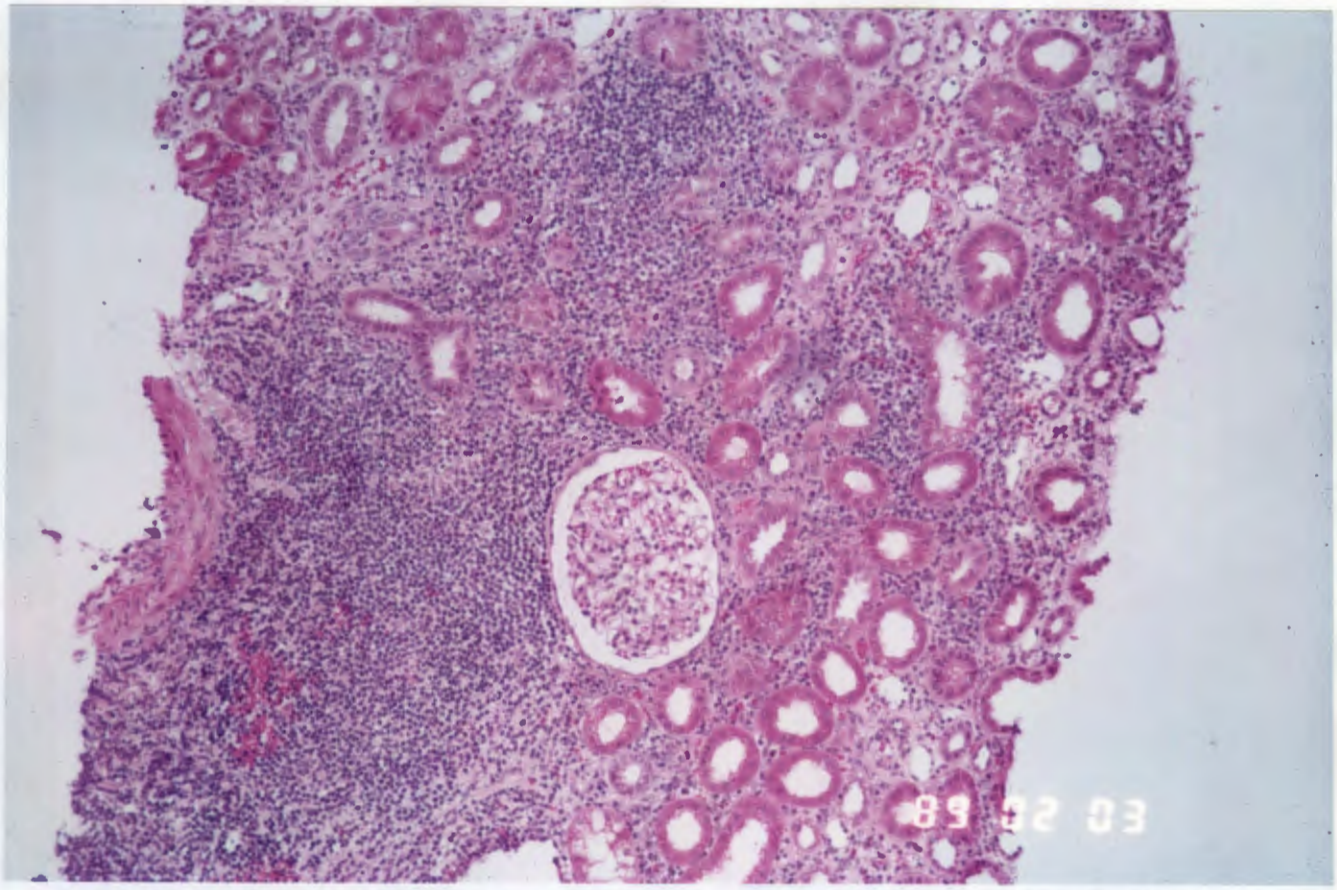


Fig. 9: Severe renal rejection: Diffuse lymphocytic and neutrophile granulocyte cell infiltration with signs of haemorrhage and diffuse acute tubular necrosis are obvious.

1.1.4.5. Autopsy

In both groups I and II, the experiments were terminated in the event of graft failure or when the animals died of any other complication. After cardiac transplantation, graft failure was evident when there was loss of palpable graft function. After renal transplantation, uraemia and hyperkalaemia terminated the survival and indicated graft rejection.

At the end of each experiment, the animals underwent full autopsy to evaluate the exact cause of death or of graft failure. In addition, samples of all organs were taken in order to look for treatment related side effects, which may be apparent at histological examination.

1.1.5. Statistics

Statistical significance was calculated using the Log Rank analysis and the Student-t-Test.

1.2. Results

1.2.1. Graft survival

1.2.1.1. Graft survival after heterotopic cardiac transplantation

The survival rates for group I are listed in Fig. 10 and Table 2 and 3.

Animal no.	Date of tx	Date of death	Survival (d)	Cause of death
GROUP IA:				
1			5	rejection
2			6	rejection
3			6	rejection
4			9	rejection
5			9	rejection
6			10	rejection
7			10	rejection
8			16	rejection
9			17	rejection
10			22	rejection
GROUP IB:				
222	29-09-88	17-10-88	18	rejection
180	20-06-88	12-07-88	22	graft ischaemia*
152	09-05-88	06-06-88	28	rejection
186	04-07-88	04-08-88	31	rejection
173	06-06-88	18-07-88	42	Diarrhoea + emaciation
GROUP IC:				
313	16-05-88	21-05-88	5	rejection
316	16-05-88	04-06-88	18	rejection
317	23-05-88	05-07-88	43	organized thrombus
319	23-05-88	06-07-88	47	rejection
323	26-05-88	14-07-88	49	rejection
GROUP ID:				
277	09-02-89	14-02-89	5	rejection
254	10-11-88	27-11-88	17	unknown
236	27-10-88	05-12-88	39	rejection
262	24-11-88	04-01-89	41	graft ischaemia*
224	13-10-88	12-12-88	60	rejection
257	17-11-88	25-01-89	67	rejection
289	07-03-89	19-05-89	73	rejection

Table 2: Number of experimental animal, date of transplantation, date of death, survival time and cause of death after cardiac allotransplantation (* see text).

After heterotopic cardiac transplantation, the control group which received no immunosuppression (group IA), showed a mean graft survival rate of 11.0 ± 1.8 days. Treatment with 15-DS alone (group IB) for postoperative days 0-9 improved the mean graft survival rate to 28.2 ± 4.1 days; this was statistically significant when compared to the control group ($p < 0.05$).
Therapy with CyA (group IC) for postoperative days 0-30 led to a graft survival rate of 32.4 ± 8.8 days on average ($p < 0.05$ vs. control).

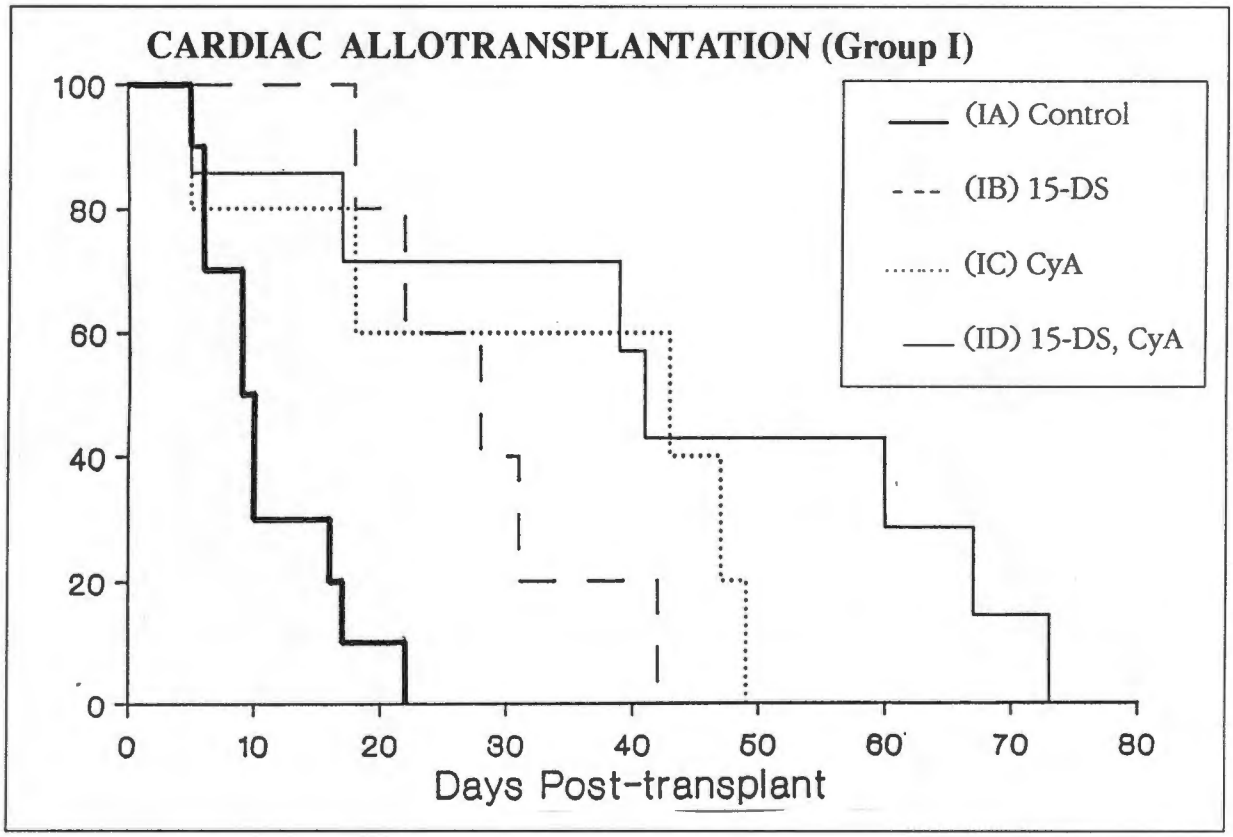


Fig. 10: Survival rates for the different treatment groups after heart transplantation. Increased graft survial is already apparent with 15-DS treatment alone (group IB). The best graft survival was achieved in group ID (15-DS and CyA), when compared to the control group IA.

The combination of 15-DS (for postoperative days 0-9) with CyA (for postoperative days 0-30, group ID) prolonged graft survival further to 43.1± 9.6 days on average ($p < 0.025$ vs. control).

Group	IA	IB	IC	ID
GSR (d)	11.0 ± 1.8	28.2 ± 4.1*	32.4 ± 8.8*	43.1 ± 9.6**
			* $p < 0.05$ vs. control ** $p < 0.025$ vs. control	

Table 3: Mean graft survival rate (GSR) in days after heterotopic cardiac (group I) allotransplantation.

1.2.1.2. Graft survival after renal transplantation

The graft survival rates after renal transplantation are shown in Fig. 11 and Tables 4 and 5.

Animal no.	Date of tx	Date of death	Survival (d)	Cause of death
GROUP IIA:				
150	09-05-88	16-05-88	7	organized thrombus
189	04-07-88	12-07-88	8	uraemia + hyperkalaemia
174	06-06-88	17-06-88	11	uraemia + hyperkalaemia
211	11-08-88	24-08-88	13	uraemia
160	23-05-88	06-06-88	14	uraemia + hyperkalaemia
179	20-06-88	11-07-88	21	uraemia
GROUP IIB:				
291	07-03-89	12-03-89	5	uraemia + hyperkalaemia
268	09-02-89	15-02-89	6	uraemia
223	29-09-88	08-10-88	9	uraemia + hyperkalaemia
160	23-05-88	02-06-88	10	ureamia
150	09-05-88	19-05-88	10	diarrhoea + emaciation
174	06-06-88	17-06-88	11	uraemia + diarrhoea
GROUP IIC:				
329	30-05-88	05-06-88	7	uraemia
315	23-05-88	10-07-88	19	uraemia
320	23-05-88	20-07-88	29	unknown
326	30-05-88	16-07-88	48	uraemia
314	16-05-88	04-07-88	49	uraemia
GROUP IID:				
263	24-11-88	04-12-88	10	uraemia
265	24-11-89	05-12-89	11	unknown
249	10-11-88	28-11-88	18	uraemia
226	13-10-88	10-11-88	28	uraemia
225	13-10-88	25-04-89	194	uraemia
292	07-03-89		274	alive
279	23-02-89		286	alive
267	01-12-88		370	alive

Table 4: Number of experimental animal, date of transplantation, date of death, survival time and cause of death after renal allotransplantation.

The control group which received no immunosuppression (group IIA) showed a mean graft survival rate of 12.3 ±2.1 days. Treatment with 15-DS alone (group IIB) did not influence graft survival positively when compared to the control (8.5 ±1.0 days).

Group	**IIA**	**IIB**	**IIC**	**IID**
GSR (d)	12.3 ± 2.1	8.5 ± 1.0	30.4 ± 8.2	148.9 ± 52.7**
				** p < 0.025 vs. control

Table 5: Mean graft survial rate (GSR) in days after renal (group II) allotransplantation.

Treatment with CyA alone (group IIC) improved graft survival to 30.4 ± 8.2 days on average (p = n.s. vs. control).
However, the combination of 15-DS (for postoperative days 0-9) and CyA (for postoperative days 0-30, group IID) again prolonged graft survival even further to 148.9 ± 52.7 days on average with 3 animals still alive 274, 286 and 370 days after transplantation (p < 0.025 vs. control) at the time of writing.

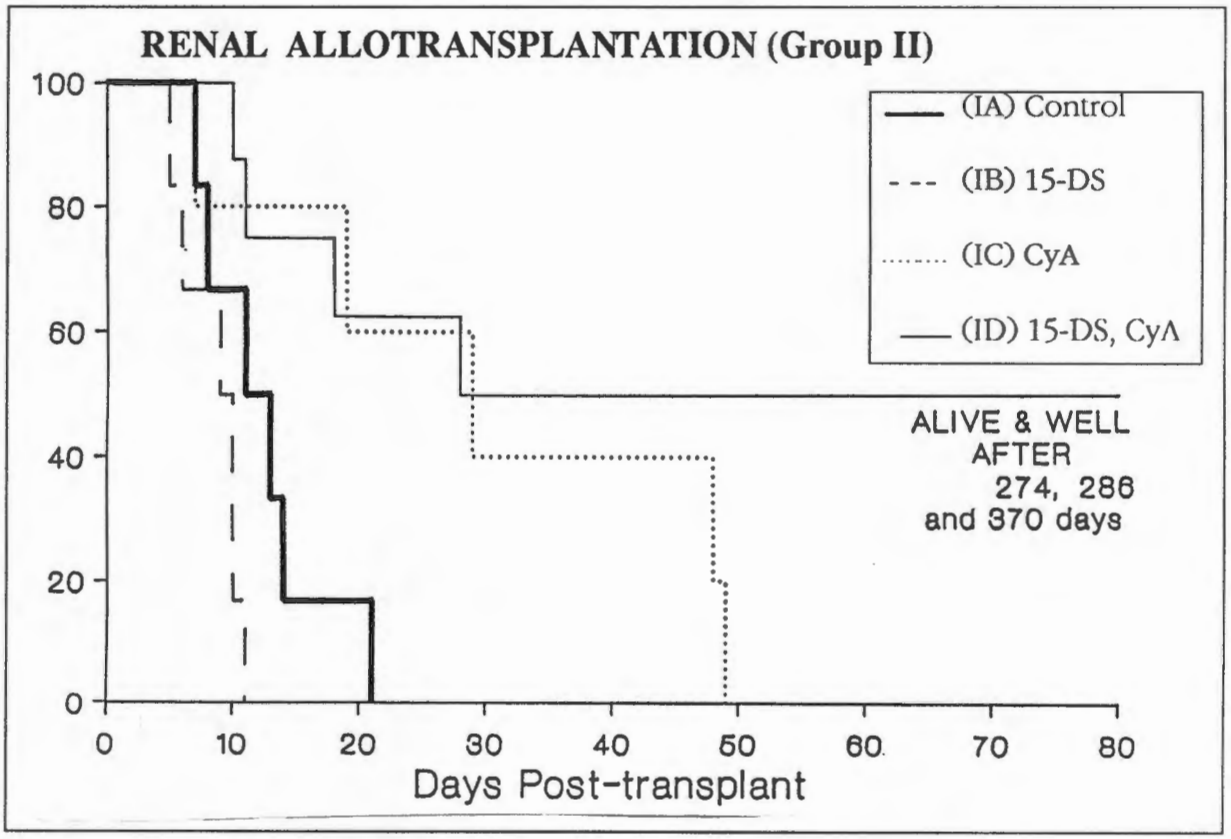


Fig. 11: Survival rates after renal transplantation. Only group IID (15-DS and CyA) showed a significant increase in survial time when compared to the control group IIA. 3 primates are still alive 370, 286 and 274 days after surgery; 1 baboon died 194 days after transplantation.

1.2.2.1. Causes of experiment termination and side effects after heterotopic cardiac transplantation

The experimental endpoints in the different treatment subgroups after cardiac transplantation are listed in Fig. 12.

In the control group IA, acute rejection was the cause of graft failure in all cases.

In group IB, one of 5 animals died of severe diarrhoea and emaciation, probably the result of 15-DS treatment. The remaining grafts stopped functioning due to acute rejection in 3 cases and graft ischaemia in 1 case. At histology, graft rejection as the cause of the described graft ischaemia could not be excluded.

In group IC, acute rejection again was the dominant cause of graft failure. In 1 case, an organized thrombus near the venous anasto-

mosis led to termination of graft function.
In the combination treatment group ID, acute rejection was responsible for graft failure in 4 out of 7 cases. One unexplained cause of death was noted 60 days after surgery. In the 1 case with graft ischaemia, graft rejection as the cause could also not be excluded.

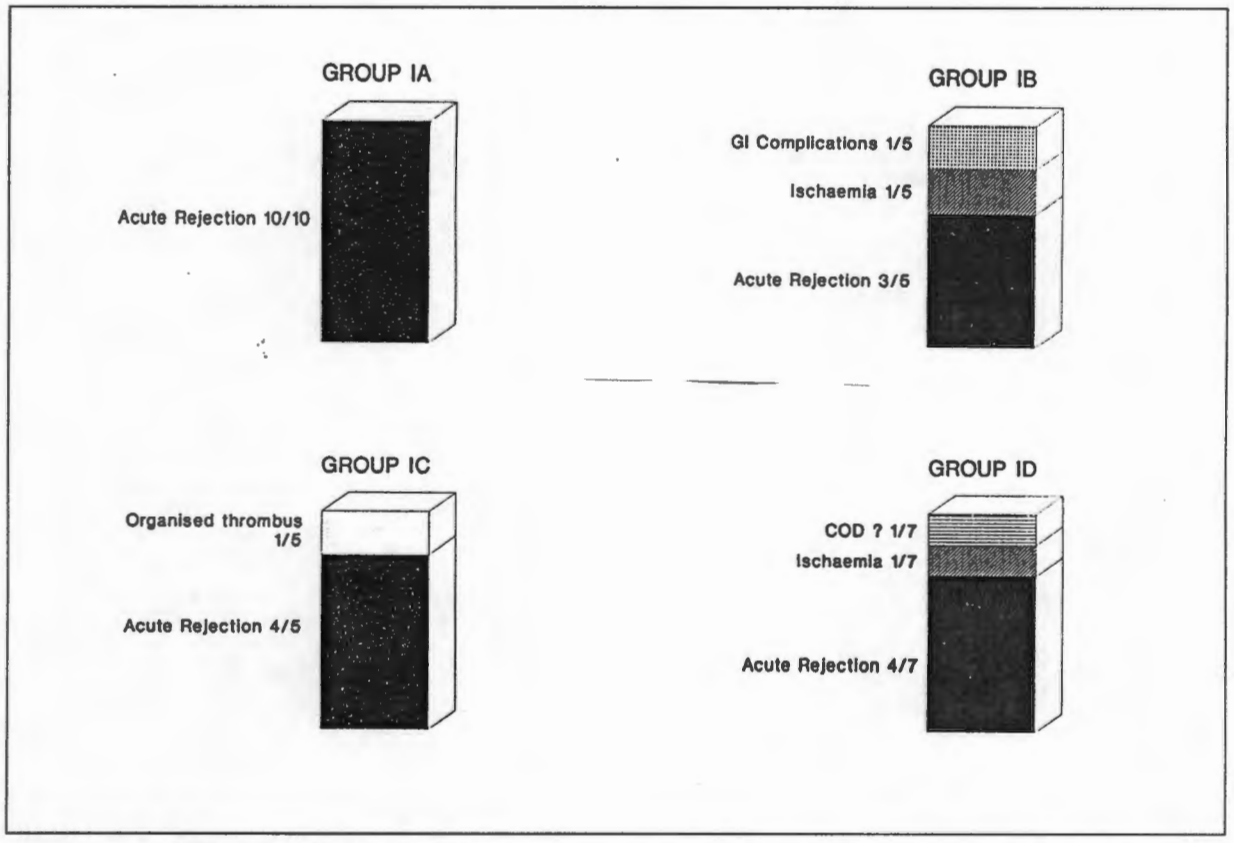


Fig. 12: Experimental endpoints after heart transplantation. Most grafts stopped functioning due to acute graft rejection. One animal in group IB died from gastrointestinal complications probably due to 15-DS therapy.

Regarding treatment related side effects, except for the 1 animal with diarrhoea and emaciation, no lethal treatment related side effect was seen. No major infections occurred among the treated animals, minor wound infections being excluded. Postoperative weight loss, which was noted in all treated animals, was not different when 15-DS treated animals were compared with CyA treated baboons.

1.2.2.2. Causes of experiment termination and side effects after renal transplanation

The causes of experiment termination after renal transplantation are shown in Fig. 13.

In the control group IIA, uraemia and hyperkalaemia as a result of acute graft rejection was the cause of death in most cases.

In group IIB, 2 out of 6 animals died of gastrointestinal complications associated with diarrhoea and emaciation, probably as a result of 15-DS treatment; one animal was not uraemic at this time. Uraemia and hyperkalaemia were the cause of death in the remaining 4 cases.

In group IIC, graft rejection was again the dominant cause of graft failure in 4 out of 5 cases. One animal died 48 days after transplantation of unknown cause.

In group IID 50% of the animals (4 out of 8) died due to graft rejection. One animal died 194 days after surgery and no cause of death was found clinically or at autopsy. Three out of the 8 recipients are still alive at the time of writing 274, 286 and 370 days after transplantation.

In common with observations found after cardiac transplantation, treatment related side effects occurred only in animals treated with 15-DS alone (group IIB). These lethal gastrointestinal complications of diarrhoea and emaciation were probably the result of 15-DS treatment. The autopsy showed a nonspecific enterocolitis in these animals. In the combination group IID no such side effects were seen. Again in the whole experimental group, no major infection was seen, minor wound infections were excluded.

With regard to weight loss after transplantation, animals treated with 15-Deoxyspergualin did not differ from baboons treated with CyA.

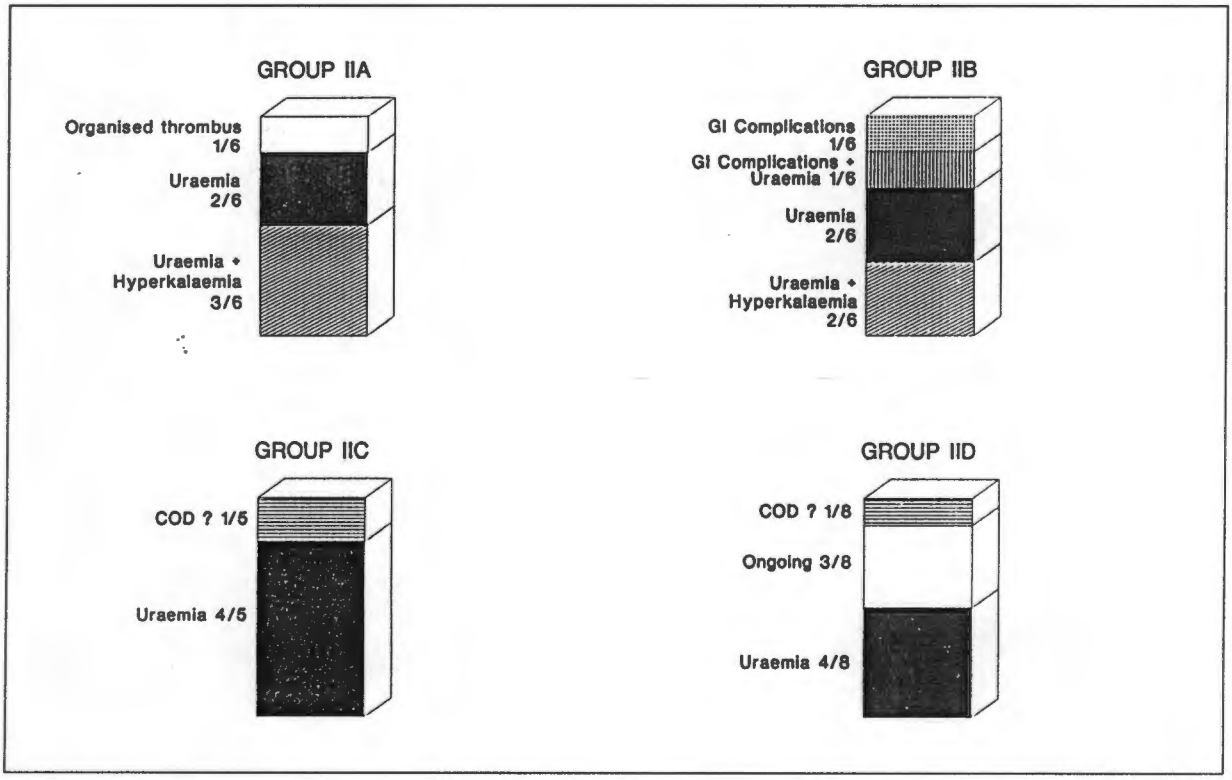


Fig. 13: Experimental endpoints after renal allografting. Uraemia and hyperkalaemia as a result of acute graft rejection were the dominant causes of death in groups IIA and IIB. 2 animals in group IIB died from gastrointestinal complications. 3 out of 8 animals of group IID are still alive (COD? = unknown cause of death).

1.2.3. Number of acute rejection episodes

A rejection episode was defined as such by the histological result of the myocardial or renal biopsy.

In order to compare the figures, the number of acute rejection episodes per animal was calculated in relation to every biopsy as well, because the animals had different survival periods (n = nAR/animal x biopsy).

A difficulty arose in defining the rejection episode because the biopsy was taken routinely at weekly intervals. It is possible that the first biopsy may have shown evidence of acute rejection and that histological evidence of rejection one week later may in fact reflect on-going rejection rather than sequential episodes of rejection. It would have been impossible without taking biopsies every day (which was not feasible) to differentiate between on-going rejection and different rejection episodes. It is believed that the demonstrated calculation allows best the comparison of frequency and evaluation of rejection within the different groups.

1.2.3.1. Number of acute rejection episodes after heart transplantation

For this calculation, only moderate and severe rejection episodes were taken into consideration. In this situation, myocardial cell damage will be seen at histological examination and would lead to rejection treatment in a clinical situation. No biopsies were taken in the control group (Table 6).

As detected by myocardial biopsies, the number of rejection episodes were significantly reduced in the cardiac transplant group ID treated with 15 DS and CyA (0.08 vs. 0.17 acute rejections /biopsy/animal, p = 0.013, group ID vs. IB, Table 4). The single treatment groups IB and IC did not differ with regard to the number of acute rejections/animals and biopsy

Group	IA	IB	IC	ID
n AR/animal biopsy	—	0.17	0.14	0.08*
		* p = 0.013 vs. IB		

Table 6: Number of acute rejection episodes per animal and biopsy after cardiac (group I) transplantation. The number of rejection episodes per animal was calculated in relation to every biopsy, because the animals had different survival periods.

1.2.3.2. Number of acute rejection episodes after renal transplantation

After renal transplantation the number of acute rejection episodes per animal and biopsy between the different treatment groups was not significantly different (Table 7). Again, only moderate and severe rejection episodes were taken into consideration.

Group	IIA	IIB	IIC	IID
n AR/animal biopsy	—	0.23	0.18	0.15

Table 7: Number of acute rejection episodes per animal and biopsy after renal (group II) transplantation.

1.2.4. Cyto-immunological monitoring

In order to compare the different groups, absolute numbers of activated lymphocytes were calculated per mm^3.
The number of total lymphocytes/mm^3 is calculated by using the total white blood cell count/mm^3 and the percentage of lymphocytes among the white blood cells. The number of activated lymphocytes per mm^3 is then calculated by using the percentage of activated lymphocytes/100 lymphocytes and the total number of lymphocytes/mm^3.

1.2.4.1. Cyto-immunological monitoring after heart transplantation

Figure 14 shows the absolute number of activated lymphocytes before and during acute rejection as detected by myocardial biopsies. Cyto-immunological monitoring was only done in the 15-

DS treated groups; thus, no such monitoring was done in the control group IA and group IC.
In both 15-DS treated groups IB and ID a significant increase of activated lymphocytes during rejection was noted ($p < 0.005$).

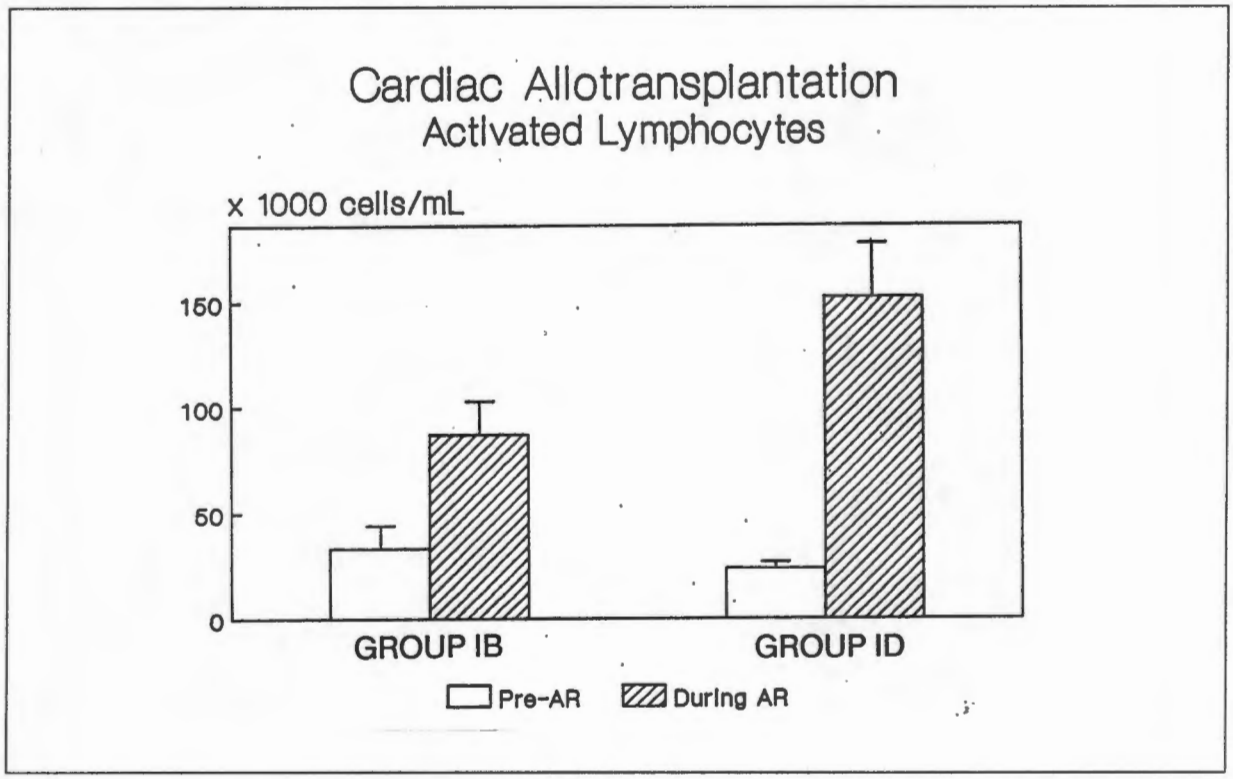


Fig. 14: Cyto-immunological monitoring after cardiac transplantation. In the treatment groups IB and ID, a significant increase of activated lymphocytes within the mononuclear concentrate of the peripheral blood was noted during acute rejection ($p<0.05$).

The increase of activated lymphocytes during rejection under CyA immunosuppression has been shown in earlier studies (31).

1.2.4.2. Cyto-immunological monitoring after renal transplantation

Similarly, after renal transplantation an increase in the absolute number of activated lymphocytes was noted in the control group and 15-DS treated groups during rejections proved by biopsy. However, this increase turned out to be significant only in the combination treatment group IID ($p = 0.023$; Fig. 15). No cyto-immunological monitoring was done in the CyA-treated group IIC.

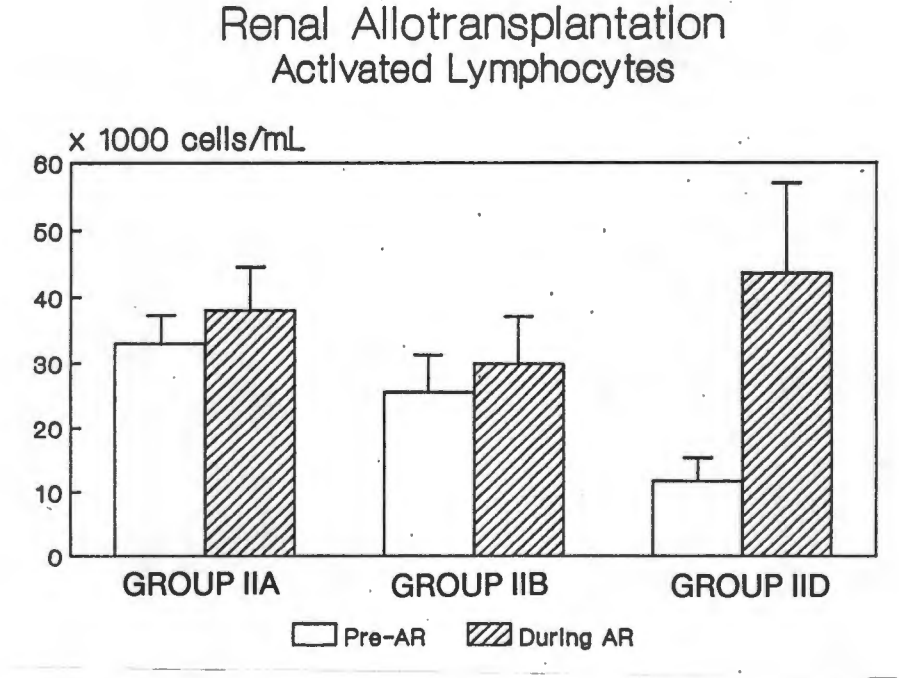


Fig. 15: Cyto-immunological monitoring after renal transplantation. During acute rejection an increase of activated lymphocytes within the mononuclear concentrate of the peripheral blood was also noted. However, the increase of these activated cells proved not to be significant except for group IID ($P<0.05$).

1.2.5.1. Urea levels after renal transplantation

The serum urea levels of all treatment subgroups after renal transplantation are shown in Figure 16.

An initial rise of serum urea within the first 6 days after transplantation was seen in all treatment groups. In the control group IIA and the 15-DS treated group IIB, the levels increased even more during the later follow-up.

In the CyA-treated group IIC, and particularly in the combination treatment group IID, the levels returned to normal again within the first 10 days and stayed low for the whole postoperative period in the long-term survivors. These data show a normal renal function in the long-term survivors after renal transplantation.

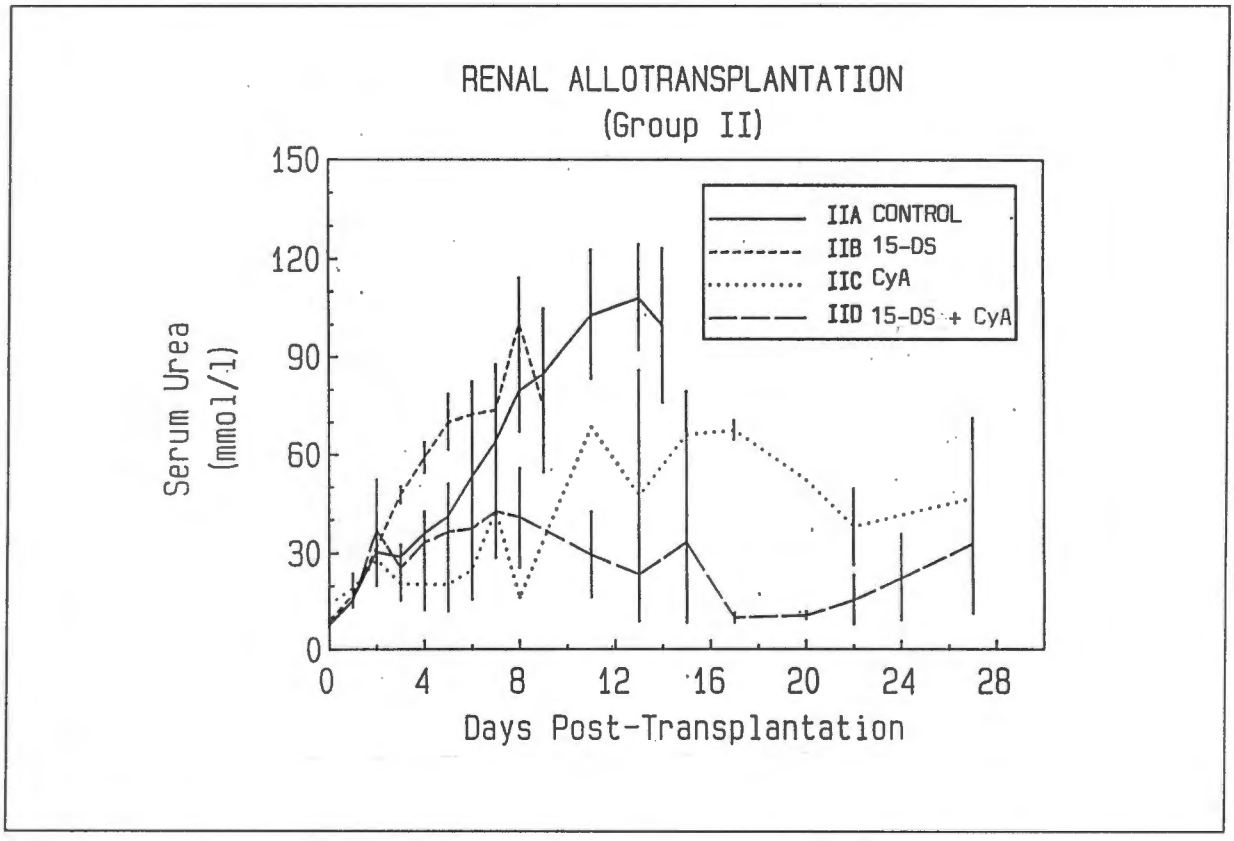


Fig. 16: Urea levels after renal transplantation. After an initial postoperative rise, the levels returned to acceptable levels again in groups IIC and IID.

1.2.5.2. Creatinine levels after renal transplantation

The creatinine levels are demonstrated in Figure 17. Like the urea levels, there was an initial rise of serum creatinine in all treatment groups within the first 4 postoperative days after transplantation.

Once again the control group IIA and the 15-DS treated group IIB showed a further increase in serum creatinine, demonstrating ongoing graft rejection and failure.

Within the CyA treated group IIC and the combination treatment group IID, the creatinine levels returned to nearly normal levels within the first 10 days after transplantation. In the long-term survivors, these levels stayed low, representing normal renal function.

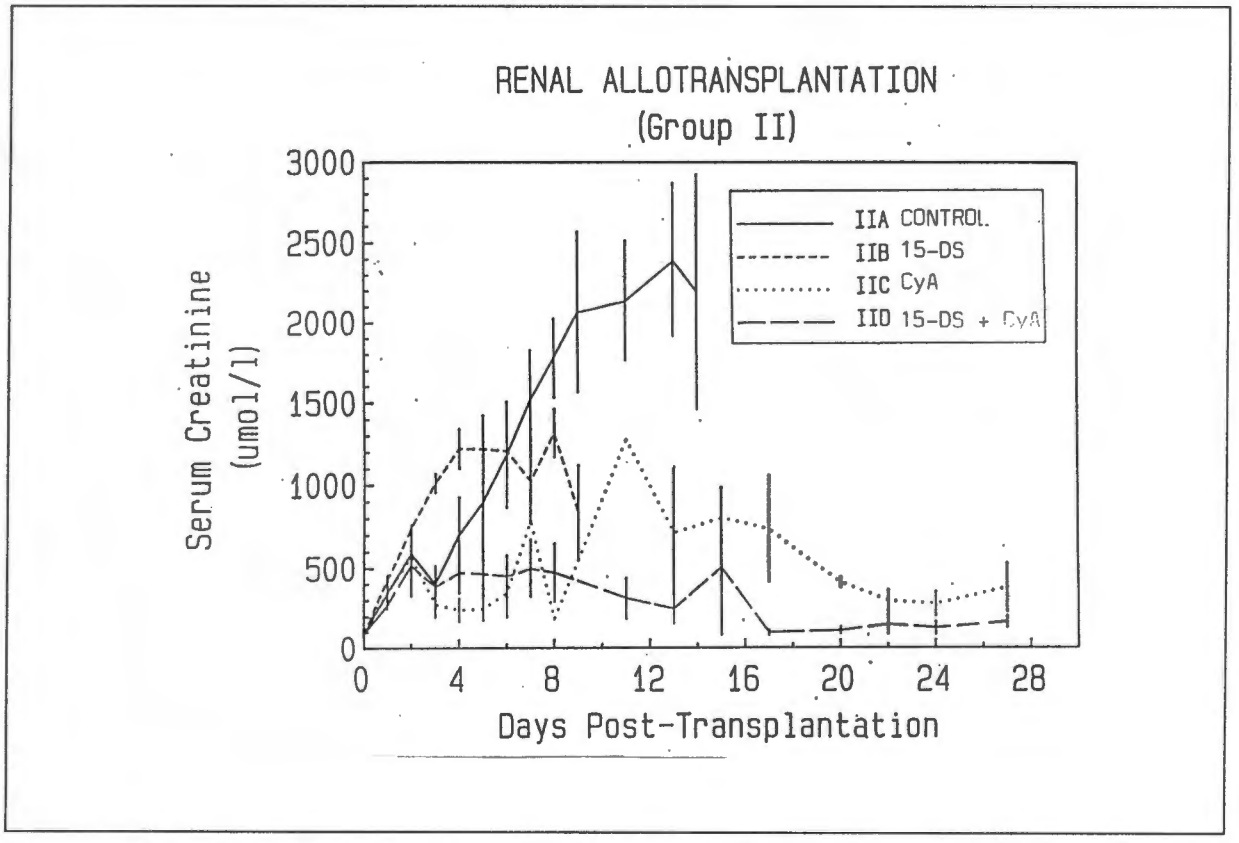


Fig. 17: Creatinine levels after renal allografting. In groups IIC and IID, these levels dropped again after an initial postoperative rise.

A comparison of serum creatinine levels before and during acute rejection episodes was done in the control group IIA and the 15-DS treated groups IIB and IID (Fig.18). A rise of serum creatinine was noted in groups IIA and IIB during acute rejection. Relatively high creatinine levels as a sign of impaired renal function may indicate acute rejection episodes even before rejection is evident within the renal biopsies.

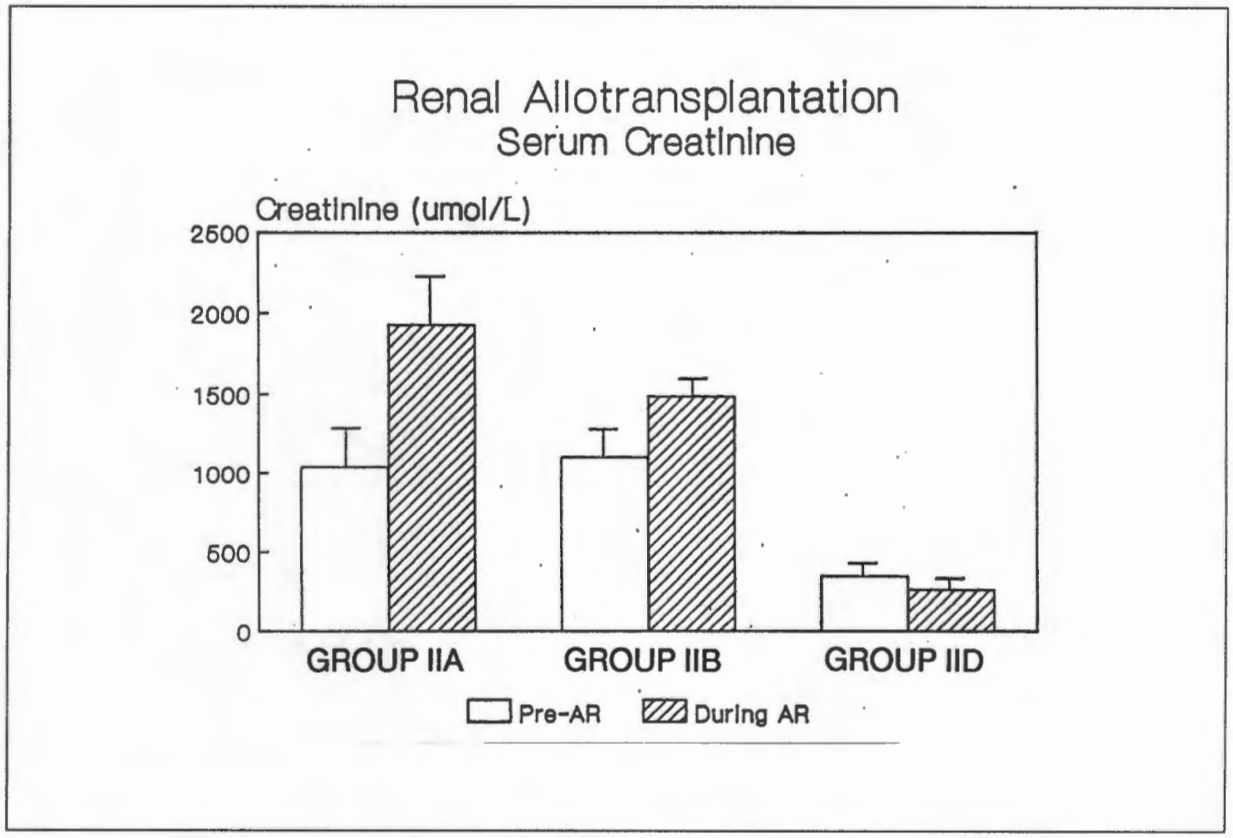


Fig. 18: Creatinine levels before and during acute rejection after renal transplantation. Except in the combination treatment group IID, a rise of creatinine was noted during acute renal rejection episodes.

1.2.6. Induction of graft nonreactivity

After cardiac transplantation, long-term graft non-reactivity was not achieved in either of the subgroups IB, IC and ID. However, the occurrence of a delayed rejection was noted in group ID. Four out of 6 animals in this group lived with no sign of rejection up to a mean of 21.8 days, counted from the time treatment was discontinued. The best survivor lived 40 days with no sign of rejection after therapy was stopped (Table 8).

After renal transplantation, a 9-day treatment period with 15-DS alone did not show any short or long term graft tolerance.

Graft nonreactivity was observed only in group IID, which was treated with a combination of 15-DS and CyA. In this group, 4 out of the 8 animals were graft tolerant up to 340, 256, 244 and 164 days after immunosuppression was discontinued. The first 3 of those animals were still alive at the time of writing, 1 animal died due to unknown reason 194 days after transplantation.

	GRAFT NON-REACTIVITY
Group ID	4/6 animals with no sign of rejection up to a mean of 21.8 days after treatment discontinuation
Group IID	4/8 animals were graft tolerant up to 340, 256, 244 and 164 days after treatment discontinuation

Table 8: Induction of graft nonreactivity after heart (group I) and kidney (group II) transplantation.

In order to explore the nature of this graft tolerance, the 3 long-term survivors received a third party skin graft in the later follow-up

(1st baboon: 252 days, 2nd baboon: 214 days, 3rd baboon: 133 days after the first transplantation). All animals rejected these skin grafts within 1 week after implantation (5, 6 and 7 days). The histopathology showed evidence of acute cellular rejection, such as mononuclear and polymorphnuclear cell infiltrations and tissue necrosis.

At the same time they did not reject their transplanted kidneys.

Thus, these data are suggestive of donor graft specific nonreactivity after short-term treatment using a combination of 15-DS and CyA.

1.3. Discussion

Current research in the field of organ transplantation should aim at a reduction of the incidence of acute rejection and of treatment related side effects without increasing the risk of infection.

Various new immunosuppressive drugs are being currently tested for their potency and side effects (11,32,33).

Remarkable results have been achieved when using the new immunosuppressive drug 15-DS after various transplant procedures in rodents (11,22,23). 15-DS, given in a dosage of 2.5 mg/kg/day, increased graft survival significantly after allogeneic heart, kidney and pancreas islet cell transplantation in rats. Little information exists concerning its efficacy in larger animals. In dogs, for example, it has been reported that equivalent dosages of 15-DS cause severe side effects such as diarrhoea and emaciation (34,35). Reduction of dosage and/or combination with other drugs, such as steroids or CyA, improved the efficacy of 15-DS and reduced its side effects (34,36).

In a project on skin transplantation in monkeys, however, these primates seemed to tolerate dosages of 15-DS in a similar way to rodents (37); survival rates superior to those achieved with CyA were observed in this model.

Stimulated by these results, 15-DS was tested for its potency, efficacy and side effects for the first time in a primate model after heart and renal allotransplantation, as an ideal preclinical experiment.

The number of animals in each group is obviously small, being limited by the availability of primates. However, care was taken to

perform enough experiments in order to compare the different groups statistically.

In our study, 15-DS was given alone or in combination with CyA after cardiac (group I) and renal (group II) allotransplantation in Chacma baboons.

15-DS was used as an immunosuppressive agent in a dosage of 4 mg/kg/day for 10 days starting on the day of operation, as recommended by DICKNEITE et al., who used the same drug after skin transplantation in monkeys (37). Exact dosage and toxicity investigations with 15-DS exist so far only for the rodent model (6). Reduction of the 15-DS dosage below 2.5 mg/kg/d led to a significant decrease of graft survival time after skin and renal transplantation. The duration of treatment also seems to be of importance. Studies in rodents revealed that treatment with 15-DS for less than 10 days resulted in inferior survival rates after skin and renal transplantation (6). This was confirmed by WALTER et al. who recommended a minimum duration of at least 10 days after kidney transplantation in rats (38).

Groups IA and IIA served as control groups and received no immunosuppression. In groups IB and IIB, 15-DS was used for postoperative days 0-9 (4mg/kg/day) only and then stopped.

In groups IC and IIC, CyA was administered alone (10-40 mg/kg/day) for the first 30 postoperative days. In groups ID and IID, 15-DS from postoperative day 0-9 was combined with CyA from postoperative day 0-30; thereafter, no further immunosuppression was given.

After cardiac transplantation, 15-DS alone led to a significant prolongation of graft survival from 11.0 days to 28.2 days on average ($p < 0.05$ vs. control). This confirms a previous report which descri-

bes an increase of transplant survival after heart transplantation in rodents using 15-DS (23). CyA alone also achieved an improved survival rate of 32.4 days on average ($p < 0.05$ vs. control).

When 15-DS was combined with CyA, mean graft survival improved to 43.1 days on average ($p < 0.025$ vs. control), with the longest animal living up to 75 days after transplantation, 45 days without any immunosuppression. The additive effect of 15 DS and CyA is also described in recent studies after pancreatic transplantation in rats; this drug combination allowed even lower dosages of both drugs to be used (39).

A recent study performed at the University of Cape Town compared two new immunosuppressive agents, FK 506 and Rapamycin, in a similar model after heterotopic cardiac allotransplantation in primates (33). FK506 and Rapamycin are both microlide antibiotics with immunosuppressive properties. Their success has also been reported after various transplant procedures also in primates (32). The primates in this study received either FK506 or Rapamycin for 14 days, either intravenously or intramuscularly. The mean survival rates were 86.8 days on average using FK506 i.m. and 49.7 days using FK506 i.v. Intramuscular Rapamycin achieved an average survival of 17.7 days on average and 37.7 days when given i.v. These results demonstrate that Rapamycin, when adminstered i.v., achieved similar results to those which were obtained using 15-DS. FK506 treated animals seemed to have a superior outcome. However, one must emphasize that the animals in the mentioned study were treated for acute rejection in contrast to our study using 15-DS, where rejection episodes were not treated.

Following renal transplantation, 15-DS alone did not improve survival when compared to the control group. This confirms studies after renal transplantation in dogs, when no clear benefit of 15-DS alone was discernible (35). The exact reason for this phenomenon

is not yet known. When used as a sole immunosuppressive agent, the effect of 15-DS is definitely dose-dependant (36). A higher dosage of 15-DS could thus eventually have improved the results. But when 15-DS is combined with other immunosuppressive agents, like CyA, the additive effect of both drugs allows lower dosages to be used (39).

CyA alone led to an expected improved survival rate of 30.4 days on average; however, this proved not to be significant when compared to the control group.

The combination of 15-DS with CyA again showed a strong additive effect. A mean graft survival of 148.9 days was achieved ($p < 0.025$ vs. control), with 3 animals still alive at the time of writing. This is the first time, such excellent survival has been reported using the described drug combination in a primate model. Previous studies confirming the additive effect of 15-DS and CyA were performed only in rodents (39). These results in primates are far superior to those described using the same drug combination of CyA and 15-DS after renal transplantation in dogs (35).

Concerning the question of severe treatment related side effects, one of the primates in group IB and 2 of the animals in group IIB, died due to gastrointestinal complications. Stool specimens were sent for microbiological examination with no positive results. The autopsy showed an unspecific enterocolitis in those animals, possibly as a result of drug toxicity. Similar effects have been observed when using 15-DS after transplantation in dogs (34,35). General side effects (nausea, diarrhoea and weight loss) were noticed after renal transplantation in dogs using a dosage of 1.2 to 4.0 mg/kg/d (35,36,40) and after renal xenotransplantation (fox to dog) with 2.5 mg/kg/d given intravenously (41). A delayed start of 15-DS treatment and further dose reduction has been described as being able to reduce the occurrence of these side effects even further (37).

Measuring blood levels of 15-DS would certainly be desirable, but such a test was not available yet at the time of the study.

Further haematological studies revealed that 15-DS induced leucopenia and anaemia (42,43), an inhibition in the maturation of bone marrow cells (44) and a decrease in number of bone marrow cells (44) after transplantation in rodents. Toxicity studies in these animals showed that these haematological side effects of 15-DS appeared only when a dosage of more than 10mg/kg/day was used (6). In our own experiments, no leucopenia or anaemia was observed in the long term follow up; no bone marrow studies were performed for this reason.

When 15-DS was combined with CyA no general side effects were observed, although the same dosage of 15-DS was used in both groups. The combination of both drugs showed not only a strong synergism with regard to graft survival, but apparently also prevents the animals from developing the previously described nonspecific enterocolitis; this was observed after cardiac and renal transplantation. No major infection occurred among our experimental animals.

15-DS in combination with CyA influenced the frequency of acute rejection after heart transplantation: In group ID the number of acute rejection episodes per animal was significantly reduced when compared to the 2 groups treated with either CyA or 15-DS alone. As explained above, the term rejection episode does not always reflect single episodes of short duration, but might well represent longer ongoing rejections. Thus the described results allow comparison of frequency and duration of acute rejection.

In another study, to be described later, after xenogeneic heart transplantation in primates, we were also able to decrease the num-

ber of severe acute rejection episodes using a combination of 15-DS and CyA (45).

However, since rejection was not treated in our protocol, the observed rejection episodes did not resolve spontaneously, but caused termination of graft function at some stage in all experimental groups.

Cyto-immunological monitoring of the peripheral blood was used as a noninvasive parameter to detect acute rejection episodes. In both15-DS-treated groups IB and ID, a significant increase of activated lymphocytes in the mononuclear concentrate of the peripheral blood was noted during acute rejection episodes after cardiac transplantation. This confirms earlier studies using CyA immunosuppression, when cyto-immunological monitoring was also able to diagnose acute rejection episodes after heart transplantation (31). In our study using 15-DS treatment, the acute inflammatory process during acute cardiac rejection episodes was also visible within the mononuclear concentrate of the peripheral blood.
This increase of activated lymphocytes during rejection was also noted after renal transplantation in groups IIA, IIB and IID, but did not gain statistical significance in all these groups. This phenomenon may be based on the fact that lymphocytes which are activated within the graft do not all recirculate into the blood, but are excreted into the urine as well. This phenomenon has been used to detect renal rejection episodes - activated lymphocytes are noted at urine cytology (46).

Postoperative urea and creatinine levels were monitored to assess the function of the transplanted kidney in group II. In the control group and the group treated with 15-DS alone, levels never reached normal values, but increased further until final graft rejection and failure occurred.

However, in the CyA treated group and particularly in the combination treatment group IID, these levels returned to nearly normal levels within a few days after transplantation and stayed low for the whole postoperative period in the long-term survivors, representing normal renal function after transplantation.

During acute rejection, a rapid increase of serum creatinine was noted. This confirms that monitoring of creatinine levels is a useful diagnostic hint for renal graft rejection.

The most important finding in this study, however, was the induction of graft specific nonreactivity after renal transplantation.

This nonreactivity may be defined as the indefinite survival of an allograft with normal function and without the necessity for the permanent administration of immunosuppressive drugs. At an immunological level, tolerance should be specific. This means that the recipient must be unresponsive to the relevant allograft alone, and immunological reactivity to other foreign antigens or third party grafts must be maintained.

Using 15-DS treatment, the phenomenon of graft tolerance has so far been reported only in the rat model after heart, kidney and liver transplantation (25,26,47); after renal transplantation, all transplanted rats remained graft tolerant after initial treatment with 2.5 mg/kg/day 15-DS given for the first 10 postoperative days (26). The combination of 15-DS and CyA also achieved tolerance after rat-tail skin transplantation, a model which is very susceptible to acute rejection (48).

In our primate heart transplant model, long-term graft nonreactivity was not achieved in any of the treatment groups. However, delayed rejection occurred in group ID within a mean of 21.8 days after treatment discontinuation. The occurrence of graft nonreacti-

vity has recently been reported when using FK506 after heterotopic cardiac transplantation in primates (33). When FK506 was administered intramuscularly for 14 days after transplantation, 2 out of 5 grafts still showed excellent function 138 and 110 days after immunosuppression had been stopped.

Using the combination of 15-DS and CyA after renal transplantation, however, long-term non-reactivity was achieved in 4 out of 8 animals (50%). These 4 animals remained graft tolerant up to 340, 256, 244 and 164 days after immunosuppression had been discontinued completely.

In order to explore the nature of this graft tolerance, the 3 long term survivors received a third party skin graft in the later follow up. The animals rejected these skin grafts within one week without affecting their transplanted kidneys. From these observations, one may assume evidence of donor graft specific nonreactivity after initial treatment with 15-DS and CyA. An ideal proof of this specific graft nonreactivity would be the re-acceptance of grafts from the original donor animal. This, however, was not possible in our experiments, because the original donor animals served as heart donors as well at the time of their explantation.

An immunological explanation for this phenomenon of graft nonreactivity in vivo has not yet been worked out in vitro, since the exact mode of graft tolerance after transplantation is still not fully understood.

One theory of tolerance induction is the elimination of a particular cell clone by the tolerogenic substance (49).

Other authors advance their theories according to the phenomenon of neonatally induced tolerance and showed that this early form of non-reactivity is mediated by suppressor cells (50). These

suppressor cells abolish rejection of grafts bearing specific donor antigens with high efficiency. In addition, suppressor-cell mediated graft tolerance was induced by various immunosuppressive agents like ALG according to THOMAS and CARVER (51) and even by CyA according to KUPIEC-WEGLINSKI (52). Confirmation that these suppressor cells are T-cells resulted from surface labelling studies which showed that they carried the T-cell antigen (53). However, these cells are not marked by the usual suppressor/cytotoxic cell marker and this specific suppressor action is not removed by depletion of the suppressor/cytotoxic cell population (54). These data clearly differentiate the suppressor cells of transplantation tolerance from suppressor T-cells operating in responses to conventional (non-transplantation) antigens.

It is known that 15-DS affects macrophage function and inhibits clone expansion of lymphocytes during acute rejection (55); however, 15-DS does not inhibit the suppressor cell function and these cells may be responsible for maintenance of immunologic nonreactivity (56). The synergistic effect of CyA may be explained by its relatively sparing action on suppressor cells (57).

The exact mode of graft nonreactivity was discussed in a study after renal transplantation in rats, where 15-DS treatment induced graft tolerance in 100% of the transplanted animals (6). Specific nonreactivity was achieved in this study as well, while third party grafts were rejected. However, this long lasting graft survival was not achieved after skin transplantation in the same model. This may reflect the different immunological response to various transplanted organs, since in our study we also achieved different results after cardiac transplantation.

As described before, 15-DS plays an essential role in antigen presentation and/or recognition of target antigens after transplantation (see chapter I.2.). Using immunohistological studies, the de-

crease of MHC class I-expression after 15-DS therapy could well explain the nonreactivity after renal transplantation in rats (6); since MHC class I antigens are the most important target antigens for cytotoxic T-cells, non-expression of these structures limits the antigen recognition by T-effector cells.

In addition, 15-DS inhibits the release of Interleukin 1 from the antigen presenting cells. Allospecific T-helper cells are able to recognize the alloantigen, but fail to respond due to the lack of the signal IL-1. One of the consequences may be "clonal anergy" as a mechanism of specific nonreactivity (58).

Suppressor MLC-studies after renal transplantation in rats using 15-DS treatment demonstrated that unspecific suppressor cells are responsible for functional graft tolerance in the early phase after transplantation and donor-specific suppressor cells in the late phase (6). This analysis of cell mediated nonreactivity was confirmed in various transplantation models in rats using CyA after liver transplantation (59) and 15-DS after renal (60), skin (17) and liver transplantation (61).

These suppressor cells influence the induction of the immune response with effect against MHC class I and class II antigens (62, 63). In addition to the effect on the immune response, the activation and differentiation of effector T-lymphocytes is suppressed (64).

Since the early nonreactivity is maintained by unspecific suppressor cells, one may conclude that these cells are responsible for the inhibition of the immune response; the specific suppressor cells, however, would account for the effect on effector T-lymphocytes. Both effects may, if permanent absence of the immunological reaction results, lead to graft tolerance. The nonreactivity just described can develop spontaneously, without the use of an immuno-

suppressive agent (65). In case of stronger histoincompatibility, this is only achieved by the use of drugs like CyA (59) or 15-DS (60).

Similar results with regard to graft nonreactivity following renal transplantation in primates have also been described when total lymphoid irradiation has been applied (66). In this model using irradiation, evidence for suppressor cell mechanisms seems to have been obtained; so called "natural suppressor cells" inhibit the generation of cytotoxic T-cells, but allow the emergence of antigen-specific suppressor T-cells (66). The authors were able to demonstrate a donor-specific T-cell mediated suppression in appropriate coculture experiments. As in our experiments, third party grafts were rejected in an unmodified acute fashion, while the original kidney allografts remained unaffected. Further data on allograft acceptance have been published by THOMAS et al., who used the combination of posttransplant antithymocyte globulin and donor bone marrow infusion after renal transplantation in rhesus monkeys (67). In Thomas' series the percentage of long-term nonreactive primates was 38% (57). A possible explanation offered for this graft acceptance is the presence of apparent bone marrow suppressor/cytotoxic cells of donor origin in the long term functioning kidneys (68).

The described incidence of 50% graft non-reactivity after renal transplantation following initial treatment using 15- DS and CyA is very encouraging. These findings justify further experimental evaluation using 15-DS, particularly in combination with CyA in order to achieve the goal of potential graft nonreactivity.

2. 15-Deoxyspergualin after cardiac xenotransplantation

2.1. Introduction

The issue of xenotransplantation has become more and more prominent because of the lack of suitable human donor organs. This scarcity of donors is compounded further by the fact that transplantation of the heart may be necessary as an emergency procedure. There is a clearly defined need for heart transplanation in neonates and infants. Severe cardiac defects, such as hypoplastic left heart syndrome, have a poor prognosis after conventional cardiac surgery, but organ procurement for neonates remains a major problem. The use of anencephalic infant donors raises additional ethical and legal restrictions (69). For these children, the only biological option to an allogeneic graft is therefore a xenogeneic donor heart.

In the case of adults, there is little evidence, as yet, that transplant laws or specific regulations have done anything to improve the supply of donor organs (70). Due to the increased demand for donor organs, there is no doubt that there is a need for xenotransplantation. Cooperation with ethic commitees and the media is important in order to persuade the public to accept the fact that the lives of animals will be sacrificed in order to save human lives.

2.1.1. Concordant-discordant xenotransplantation

The occurrence of acute rejection episodes is largely dependent on the genetic homogeneity between donor and recipient. With greater donor-recipient genetic disparity, the onset of such acute rejection mechanisms becomes more acute, often occurring within

minutes of transplantation - predominantly when donor and recipient are members of different orders. Such early hyperacute rejection involves humoral responses (74). The terminology of concordant and discordant xenotransplantation was proposed by CALNE as a means of distinguishing between potentialy successful grafts (concordant model, e.g. Vervet monkey - baboon), in which rejection is largely cellular, and unsuccessful grafts (discordant model, e.g. pig - baboon), in which antibody-dependent, early hyperacute rejection occurs (72). In addition to this definition, there seems to be a restriction of hyperacute rejection to specific organs, since the liver may not be hyperacutely rejected in a given species combination, while the kidney and heart are (73).

In distinguishing between concordant and discordant models, the presence of preformed natural cytotoxic antibodies, as determined by a cytotoxic crossmatch, as well as high mixed lymphocyte reaction, is of greater importance than assumptions based on phylogenetic comparisons (72). Unfortunately, the target antigens of hyperacutely rejected grafts are not known. There may be a cross-reaction with donor MHC antigens or with entirely different antigen systems that may be species specific or shared between related species in the "concordant" system.

2.1.2. Rejection mechanisms

The distinguishing feature of graft rejection in xenotransplantation as opposed to allotransplantation is the action of preexisting humoral antibodies: graft destruction results from immunoglobulin binding to vascular endothelium and subsequent activation of both the complement and platelet aggregation system. This, in turn leads to thrombosis and severe ischaemic damage (74).

This may result in early hyperacute rejection with features of oedema, myocyte necrosis, microvascular thrombi and interstitial haemorrhage due to vascular disruption.

The exact pathways are described as follows: During hyperacute rejection, the vaso-occlusive and -destructive changes precede the appearance of neutrophil granulocytes, which then progressively accumulate (75) and the cellular infiltrate is completed by the appearence of macrophages.

Complement activation by preformed humoral antibody is the essential prerequisite for initiation of hyperacute rejection.

Graft binding of C3 is a consistent immunohistological concomitant of hyperacute rejection (76). Complement activation could induce marked vasoconstriction, directly lyse endothelial targets and generate vasoactive related agents (77).

Microvascular blockage by platelet-fibrin thrombi has been emphasized as an early and cardinal feature of hyperacute rejection (78). Vascular occlusion with resultant hypoperfusion and graft ischaemia could be the central determinant of this kind of graft rejection. Thus, although hyperacute rejection is clearly initiated by antibody-dependent complement mediated mechanisms, the possibility remains that immune-mediated activation of platelets and

the coagulation cascade within the grafts could be the proximate effector mechanism of graft rejection (74).

In concordant xenograft models, like the Vervet monkey/baboon model, levels of species specific preformed natural antibodies are observed less often than in discordant models. In earlier experiments at the University of Cape Town, it has been shown that the presence of preformed natural antibodies did not necessarily correlate with early hyperacute rejection (71).

However, accelerated graft rejection is characteristically observed in concordant xenotransplantation (73). In the hamster to rat model, low levels of heterophile immunoglobulin molecules do not usually give rise to early hyperacute rejection but, instead, induce specific features of the rejection process (79):By 3 days, interstitial oedema and focal infarction occur together with a neutrophilic infiltrate. Late rejection was associated with a mixed cellular infiltrate, prominent arteritis and arteriolitis with fibrinoid necrosis and arteriolar thrombosis, progressive interstitial haemorrhage, and widespread myocardial cell necrosis; microvascular thrombi are missing.

Accelerated rejection appears to be associated with a certain humoral response, although cellular immune mechanisms may be involved to a variable degree (73).

Concordant xenotransplantation between closely related species, such as interprimate combinations, appears to most closely simulate allograft rejection (80,81). There is, as yet, no evidence that the cellular part of the response is quantitatively or qualitatively different from allograft rejection. In addition, however, a distinctive arteritis was observed, occuring as early as 1 day post transplantation. This vasculitis seems to be primarily induced by humoral immune mechanisms. Later, myointimal proliferation and obliterative

arterial sclerosis complete the picture of vascular damage. This, also, is not very different from allogeneic heart transplantation, since obliterative coronary artery disease is the major cause of late graft failure (82).

These studies demonstrate that there is a chance of successful immunosuppression in the concordant xenotransplant model, since the rejection mechanisms are not that different to the allogeneic response, when compared to the discordant system.

2.1.3. Design of the experimental study

In order to avoid early hyperacute rejection, as it occurs in discordant xenograft models, a concordant xenograft model was chosen by using Vervet monkeys as donors and Chacma baboons as recipients.

In order to do research relevant to the clinical situation, primates were used as experimental animals in this study. E. KEMP has recommended the primates as an ideal model for xenotransplantation, since the apes are phylogenetically and immunologically the species nearest to man and it seems possible to extrapolate the results to the clinical situation (83). They have ABO antigens which are easily assayed and are large enough to enable repeated graft biopsies to be taken.

The genetic relationship between different species is based on the similarity in structural and organizational development of the DNA (84). According to RAKE, it was shown in reassociation experiments and DNA-DNA-hybridisation that 98% of human DNA can be found in the genes of chimpanzees, 92% in other old world mon-

keys (e.g. vervet monkeys and baboons) and 85% in asiatic primates (85). While the numeric differences in nuclein acids may be small, differences in genes and genetic control mechanisms are so obvious that they clearly distinguish between such closely related species such as man and ape (86,87).

With regard to evolution studies, the genetic disparity of vervet monkeys and baboons is comparable with the system homo sapiens - chimpanzee.

Another very important point is that baboons and Vervet monkeys are still plentiful in Southern Africa, and these animals are relatively easy to maintain and breed under laboratory conditions.

The major aim of this research protocol was to optimize immunosuppressive therapy after xenogeneic transplantation. CyA, in addition to its wellknown use in allogeneic transplantation, has also been used in order to prolong graft survival after xenogeneic transplantation (88,89,90). For example, MICHLER et al. demonstrated a 12-fold prolongation of mean cardiac xenograft survival to 77 days using parenteral CyA and steroids (91).

The present study shows the influence of different immunosuppressive drug combinations with CyA on xenograft survival, as well as on occurrence and number of hyperacute and acute cellular rejection episodes.

A particular point of interest was the combination with a new immunosuppressive agent, 15-Deoxyspergualin with its previously described anti-macrophage activity. The success of anti-macrophage agents after xenotransplantation has been described earlier (92). CHAUSSY et al. compared the efficacy of anti-macrophage serum (AMS) with ALG after allogeneic and xenogeneic skin and kidney transplantation. AMS increased xenograft survival more significant-

ly with an improved function of the renal xenografts. Histologically, the cellular infiltration of both grafts was delayed and the humoral response was decreased. The authors demonstrated indirect evidence for the importance of the macrophage in the immune response to xenogeneic tissue and the probable requirement for processing of xenogeneic antigen by host antigen presenting cells.

In addition, the possibility of clinical xenotransplantation is discussed extensively and preconditions and contraindications are mentioned.

2.2. Experimental animals, material and methods

2.2.1. Donor and recipient animals

Vervet monkeys (Cercopithecus aethiops) weighing 2 to 6 kg served as donors in our xenograft model.Chacma baboons (Papio ursinus) weighing 10 to 15 kg were used as recipients.

Vervet monkeys and baboons have human type A, B and H antigens expressed on their tissues and in secretions while their sera regularly contain reciprocally related anti-A and anti-B isoagglutinins (93). The distribution of ABO blood groups in both species are as follows: About one third of baboons tested are blood group A, one third group B, and one third group AB. In vervet monkeys, a high frequency of blood group A is observed (94). Group O is very rare in both species (95).

The donor and recipient animals were matched and compatible within the AB blood group system.

Screening for preformed antibodies was not done, since it has been shown that species specific reactions between vervet monkeys and baboons are weak or erratic (94).

In previous experimental studies using primates, no correlation was found between positive pretransplant crossmatch tests and graft survival (96). Although such testing would be desirable, particularly in the clinical situation, it involves a considerable amount of work for an immunological laboratory and, since previous studies did not show a positive correlation with the experimental results, we refrained from pretransplant crossmatch testing in this study.

All animals received care according to the "Ethical consideration in medical resarch, revised edition: 1987" set out by the South African Medical Research Council, Parow, 1989.

2.2.2. Anaesthesia and surgical technique

After premedication with Ketamine (5 mg/kg b.w.), morphine (0,25 mg/kg b.w.), pancuronium bromide (0,2 mg/kg b.w.) and atropine (0,5 mg), anaesthesia was maintained with a combination of halothane (1%), oxygen 4 l/min and N_2O 6 l/min as inhalation.

The operative technique of heterotopic heart transplantation in the neck, using the technique of MANN et al., has already been described in detail in chapter 1.1.2.1.

2.2.3. Immunosuppressive protocol

Depending on immunosuppressive regimes, the following groups were studied (Table 9):

Group I (n=8) served as a control group with no immunosuppression given. A historic control group from earlier studies in our laboratory was used for this purpose.

Group II (n=5) received CyA given in a dosage of 20 to 40 mg/kg/day administered intramuscularly, according to a whole blood trough level aimed between 400 and 600 ng/ml. CyA was combined with azathioprine (2.5 mg/kg/ day) and methylprednisolone (0.3 mg/kg/day tapered down to 0.2 mg/kg/day within 3 weeks).

Group III (n=6): In addition to the drug regimen of group II, rabbit antithymocyte globulin (RATG, Fresenius, Frankfurt, West Germany) in a dosage of 9 - 15 mg IgG/kg/day was given i.v. for postoperative days 0-4.

Group IV (n=7): In this group, the triple-drug regimen of group II consisting of cyclosporine A (CyA), azathioprine and methylprednisolone, was combined with 15-Deoxyspergualin (15-DS, 3 mg/kg/day administered intravenously for postoperative days 0-4 and 2 mg/kg/day for postoperative days 5-9). Due to severe treatment related side effects which were observed in this treatment group, the immunosuppression was later modified as follows:

In group V (n=5), 15-DS (4 mg/kg/day administered intravenously for postoperative days 0-9), was combined with CyA and methylprednisolone only, and azathioprine was omitted. In addition the infusion time of 15-DS was prolonged to 3 hours of continuous intravenous infusion.

GROUPS	IMMUNOSUPPRESSIVE PROTOCOL
I	*control, no immunosuppression*
II	*CyA 20-40 mg/kg/d, aza 2.5 mg/kg/d, mp 0.3 mg/kg/d*
III	*CyA + aza + mp + RATG 9-15 mg IgG/kg/d, p.o.d 0-4*
IV	*CyA + aza + mp + 15-DS 3 mg/kg/d (p.o.d 0-4), 2 mg/kg/d (p.o.d 5-9)*
V	*CyA + mp + 15-DS 4 mg/kg/d (p.o.d 0-9)*

Table 9: Immunosuppressive protocol in the different groups after heterotopic cardiac xenotransplantation (aza=azathioprine, mp=methylprednisolon).

Acute rejection episodes were treated with 500 mg methylprednisolone intravenously for 3 to 5 consecutive days in groups II - V.

As antibiotic prophylaxis, all animals received 500 mg of Ampicillin on the day of operation and twelve hourly on the first postoperative day.

2.2.4. Postoperative Monitoring

2.2.4.1. Graft palpation and clinical examination

Every day after transplantation, the graft function was checked and the heart was palpated by one specific person. In addition, the animals underwent a full clinical examination.

2.2.4.2. Cyto-immunological monitoring

Three times weekly, blood samples were taken for routine haematological, biochemical and cyto-immunological monitoring. The latter test was performed in groups II, III and IV.

The technique of cyto-immunological monitoring has been extensively described in chapter 1.1.4.2.

The percentage of activated lymphocytes per 100 lymphocytes was calculated and served as a diagnostic hint for acute rejection episodes.

2.2.4.3. Transmyocardial biopsies

At weekly intervals, transmyocardial biopsies were performed. For this procedure, the animals were briefly anaesthetized (Ketamine 5 mg/kg, Halothane 1%, N_2O, O_2) and a small skin incision was made above the transplanted heart. The transmyocardial biopsy was then performed using a trucut biopsy needle and a whole transmyocardial biopsy was taken through the left ventricular wall.

The biopsies were evaluated histologically. The following types of rejection reactions were seen:
Acute rejection episodes were divided into 3 groups according to the histopathology (81):

1. Acute cellular rejection: Perivascular and interstitial mononuclear cell infiltration combined with interstitial oedema and/or presence of myocyte necrosis (Fig. 19).

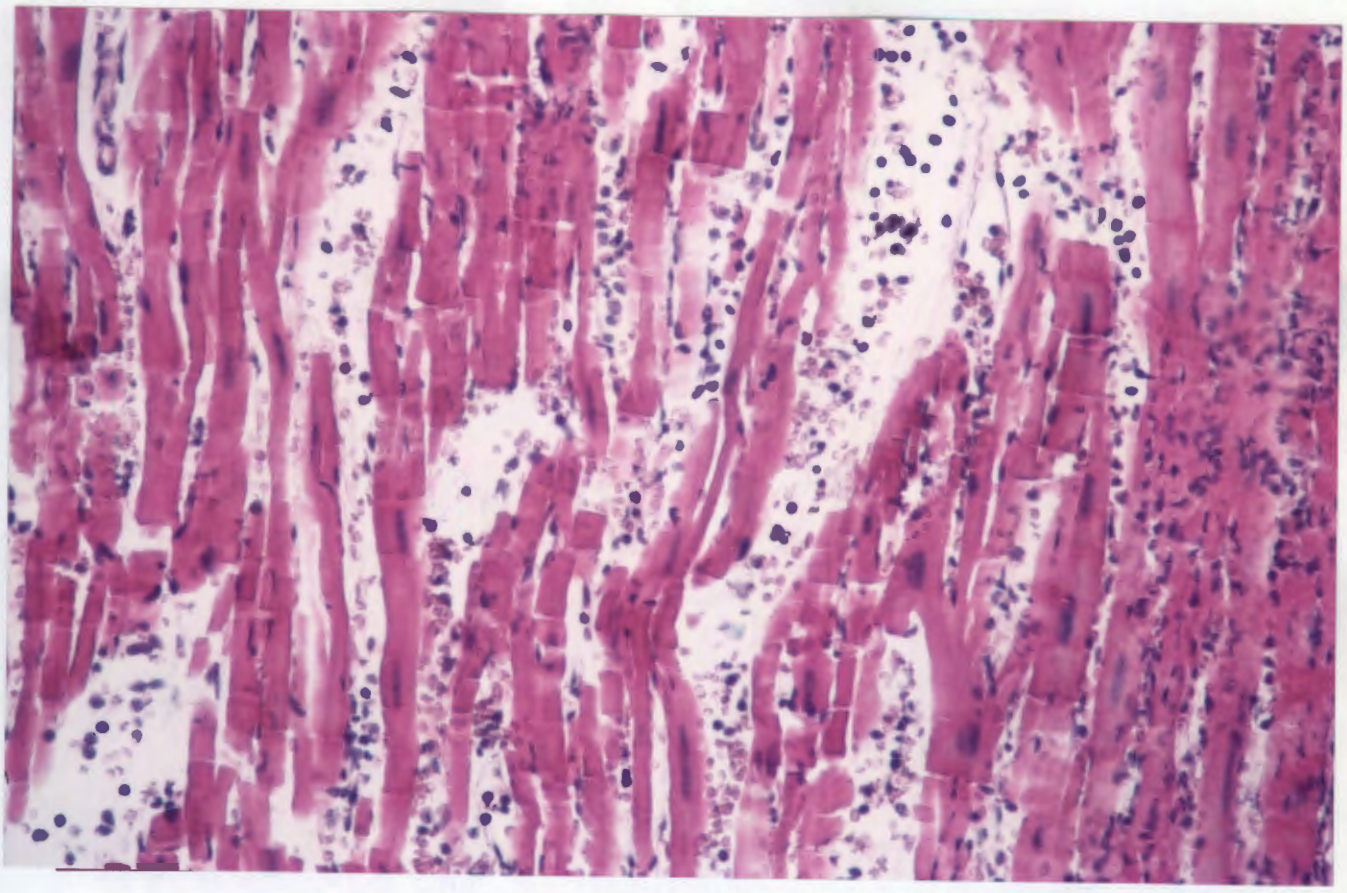


Fig. 19: Acute cellular rejection after cardiac xenotransplantation: A similar picture occurred as after allotransplantation: Mononuclear cell infiltration occurs perivascularly and in the interstitium, in combination with myocyte necrosis and interstitial oedema.

2. Hyperacute rejection: The typical morphological changes include microvascular thrombi, endothelial cell necrosis, and widespread interstitial haemorrhages throughout the graft, accompanied by a polymorphonuclear cellular infiltration (Figs. 20,21). The resulting vascular obstruction and destruction rapidly lead to serious malfunction of the graft and ischaemic changes will become recognizable in the myocytes.

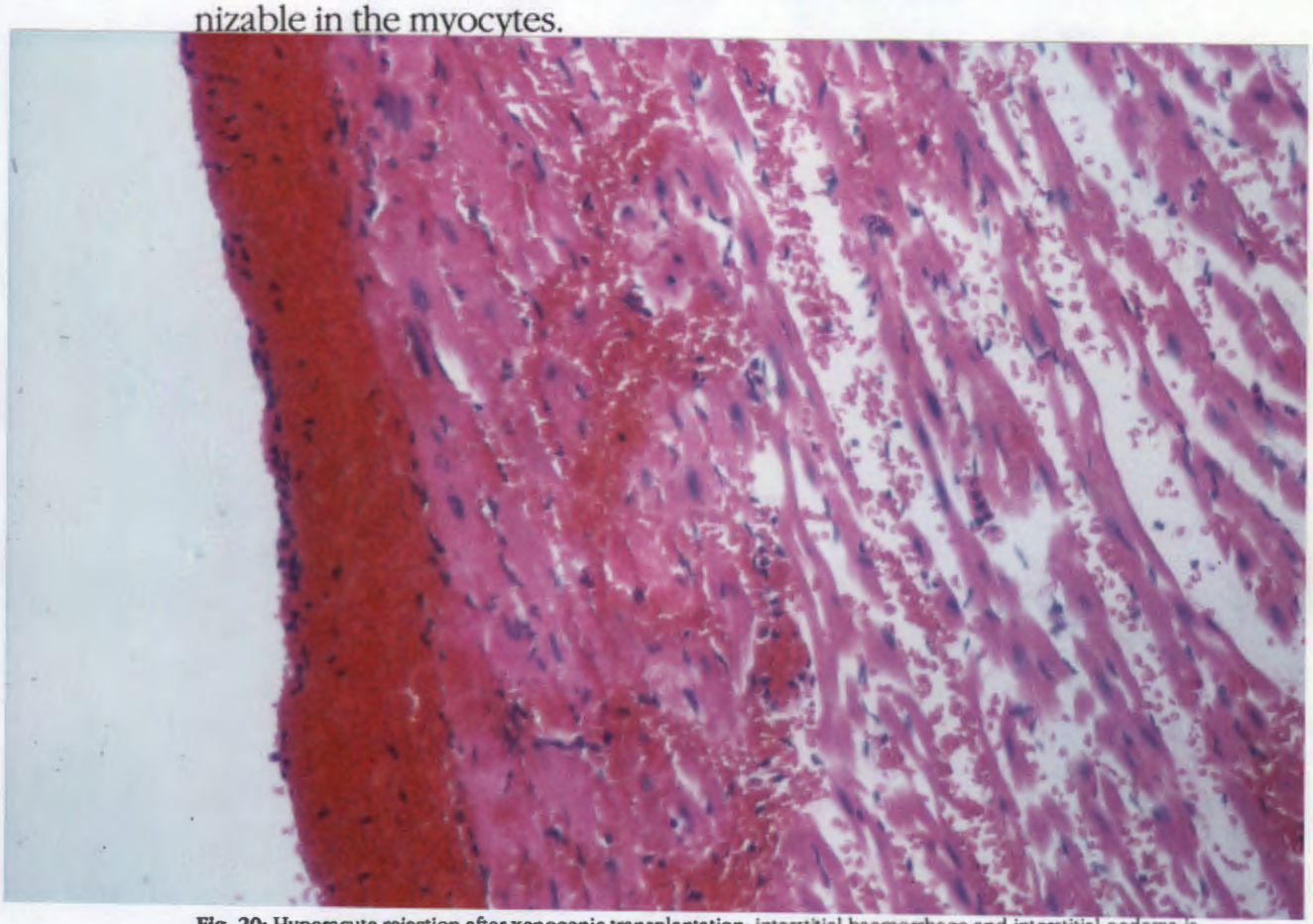


Fig. 20: Hyperacute rejection after xenogenic transplantation: interstitial haemorrhage and interstitial oedema is seen without any mononuclear cell infiltrate.

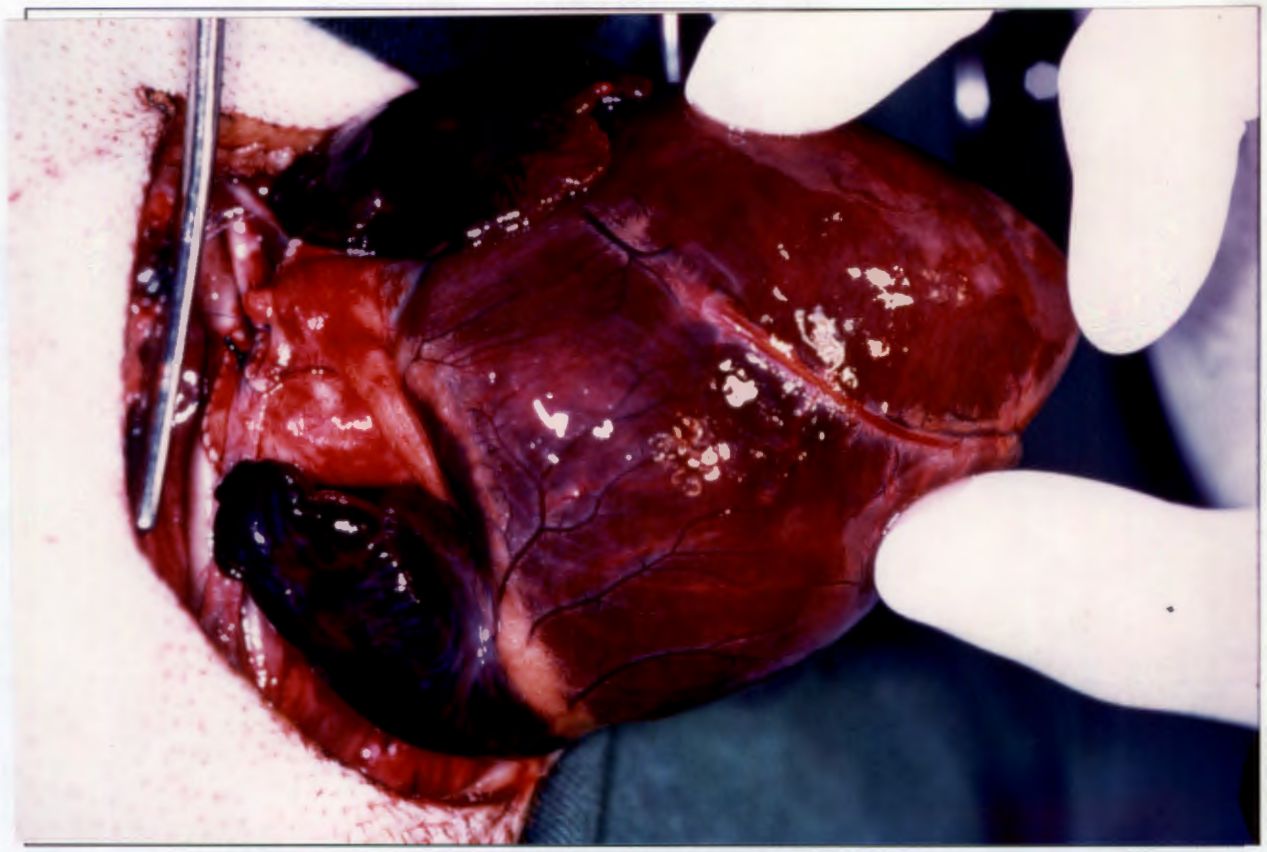


Fig. 21: Macroscopic picture of hyperacute rejection; note the swelling of the heart and the haemorrhage on the surface.

3. Mixed rejection: The biopsy findings in this group show features of both the acute cellular and hyperacute type of rejection (Fig. 22). Thus, a distinction between acute cellular and hyperacute rejection is not always clear cut, and mixed forms may occur. In our study, frequently neither form of rejection was uniform. In some microscopic fields only one form of rejection was seen, in other areas only the other form, and in some fields both forms, were seen in juxtaposition. The late onset of the hyperacute component of mixed rejection also differs from the classical concept of early hyperacute rejection.

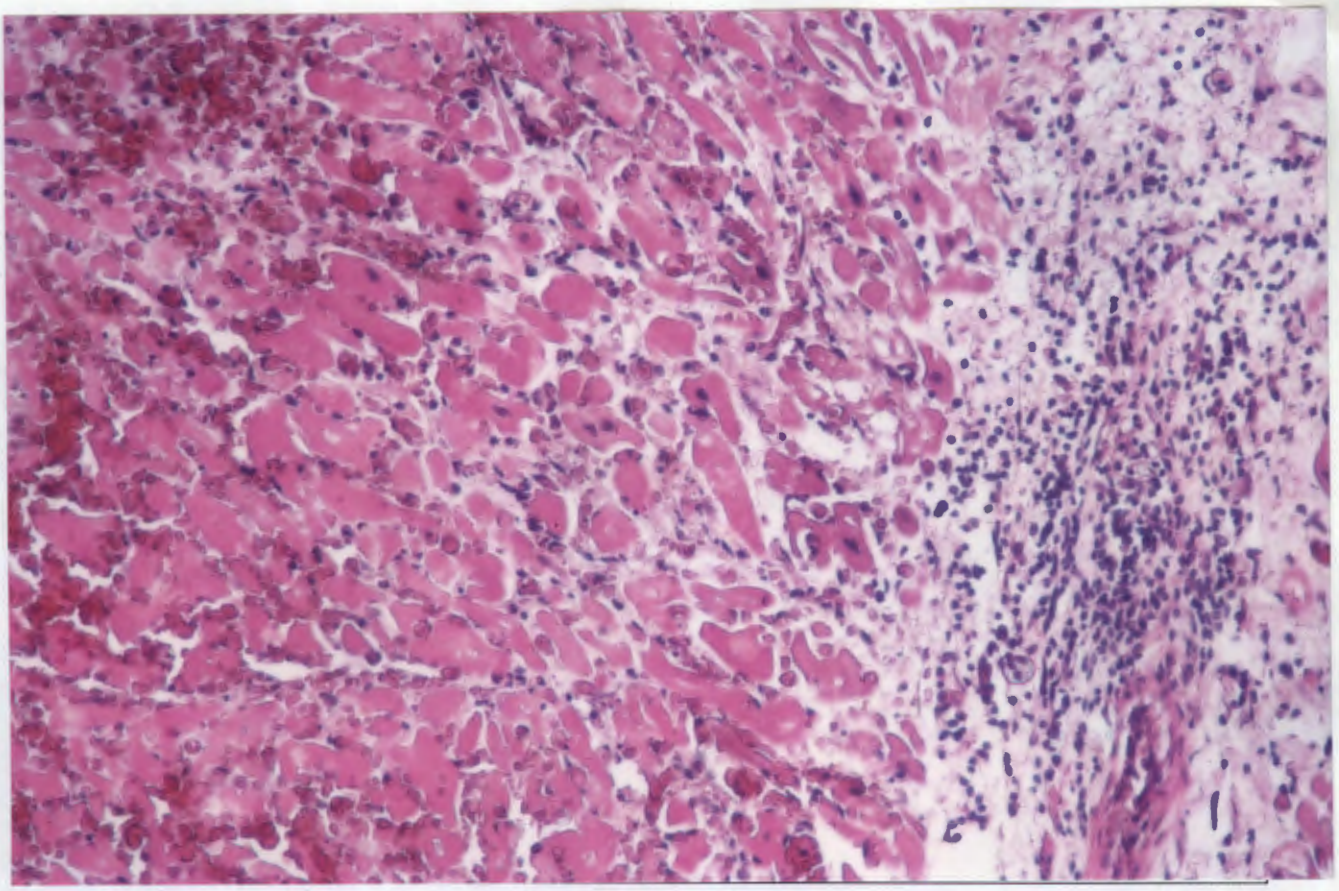


Fig. 22: Mixed type of acute rejection after xenogeneic transplantation. In addition to the features of hyperacute rejection demonstrated above, typical signs of cellular rejection such as mononuclear cell infiltrations are seen as well.

2.2.4.4. Autopsy

At the end of each experiment, the animals underwent full autopsy in order to confirm the cause of death and to look for treatment related side effects by histological examination of all organs.

2.2.5. Statistics

Statistical significance was calculated in accordance with the Log rank analysis or the Student-t-test.

2.3. Results

2.3.1. Graft survival

The experiments were terminated whenever the grafts stopped functioning or when the animal died of any other complication.

The survival rates for the different groups are listed up in Figure 23 and Tables 10 and 11.

Animal no.	Date of tx	Date of death	Survival (d)	Cause of death
GROUP I:				
844			1	hyperacute rejection
837			6	severe acute rejection
843			9	severe acute rejection
841			10	severe acute rejection
839			11	unknown
855			12	severe acute rejection
811			17	hyperacute rejection
829			18	severe acute rejection
GROUP II:				
858	05-03-87	11-03-87	6	hyperacute rejection
842	06-03-87	16-03-87	11	severe acute rejection
005	29-01-87	09-02-87	11	severe acute rejection
857	17-03-87	30-03-87	14	unknown
868	17-04-87	10-06-87	53	severe acute rejection
GROUP III:				
065	20-07-87	14-08-87	25	severe acute rejection
023	11-05-87	10-06-87	30	severe acute rejection
006	26-05-87	02-07-87	37	severe acute rejection
044	17-06-87	31-07-87	44	unknown
015	19-05-87	05-07-87	47	pulmonary infection
025	20-05-87	05-08-87	77	diarrhoea and emaciation
GROUP IV:				
103	19-10-87	20-10-87	1	hyperacute rejection
087	14-09-87	27-09-87	13	diarrhoea and emaciation
097	05-10-87	21-10-87	16	pulmonary infection
088	21-09-87	09-10-87	18	severe acute rejection
090	23-09-87	13-10-87	20	diarrhoe and emaciation
098	06-10-87	11-11-87	36	severe acute rejection
094	28-09-87	04-11-87	37	pulmonary infection
GROUP V:				
112	16-11-87	01-12-87	15	pulmonary infection
113	20-11-87	22-12-87	32	mixed rejection
116	27-11-87	31-12-87	34	mixed rejection
125	16-02-88	03-04-88	47	mixed rejection

Table 10: Number of experimental animal, date of transplantation, date of death, survival time and cause of death after cardiac xenotransplantation.

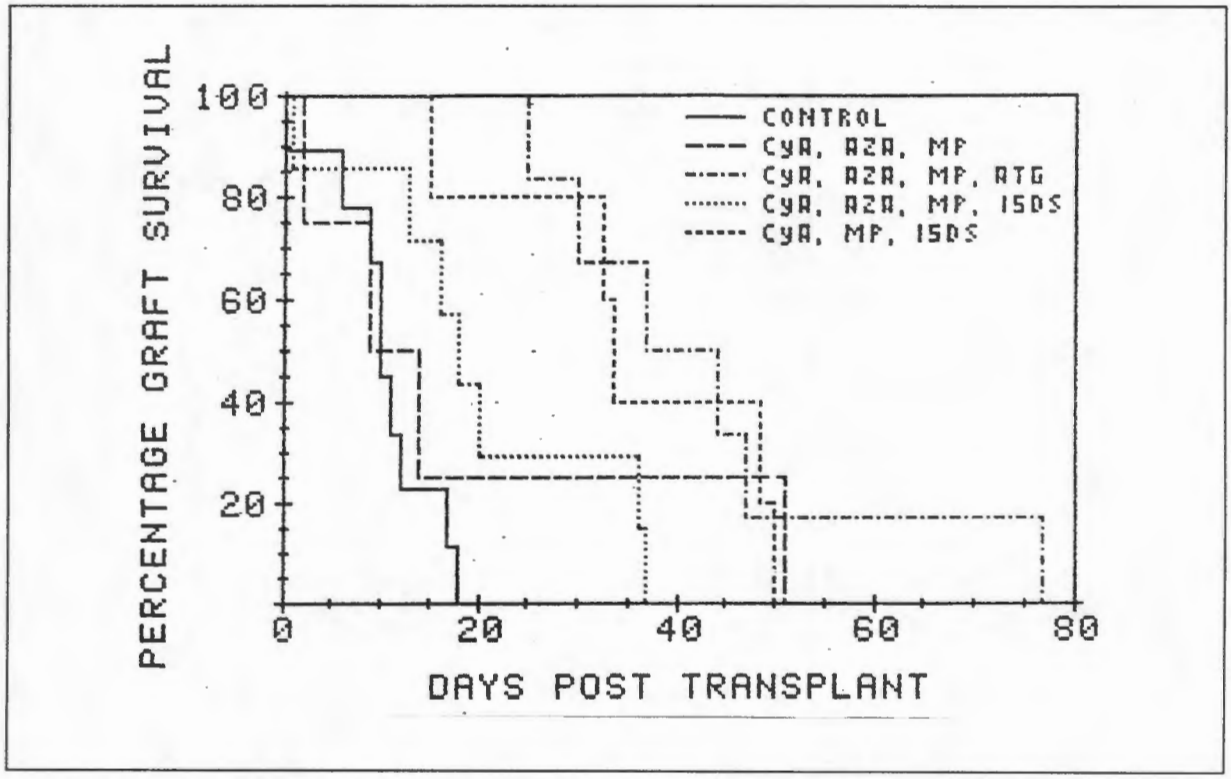


Fig. 23: Xenograft survival rates for the different treatment groups. The best graft survival rate was achieved with a combination of CyA, azathioprine, methylprednisolone and RATG (group III). The termination of graft survival within the first 2 postoperative days always corresponded to the occurrence of hyperacute rejection.

The control group I with no immunosuppression, had a mean graft survival of 10.3 ± 5.4 days.

Treatment with CyA, azathioprine and methylprednisolone (group II) led to a mean graft survival of 19.0 ± 21.8 days and showed no significant difference to the untreated control group (Table 8).

The addition of RATG to the immunosuppressive protocol (group III) led to a significantly higher survival time of 43.3 ±18.5 days ($p < 0.005$ vs. control). The most successful experimental animal lived for 77 days after transplantation.

The combination of the basic drug regimen with 15-DS (group IV) also increased the graft survival rate to 20.1 ±11.5 days ($p < 0.05$ vs. control). The omission of azathioprine in this drug regimen con-

sisting now of CyA, Methylprednisolone and 15-DS (from postoperative day 0-9) improved graft survival even more to 35.6 +- 14.2 days on average ($p < 0.01$ vs. control group, Table 11).

Groups	I	II	III	IV	V
GSR	10.3	19	43.3***	20.1*	35.6**
(days)	±5.4	±21.8	±18.5	±11.5	±14.2
	*** $p < 0.05$ ** $p < 0.01$ * $p < 0.05$				

Table 11: Graft survival rate (GSR) in days after cardiac xenotransplantation within the different groups.

2.3.2. Causes of experiment termination and treatment related side effects

The causes of experiment termination are graphically shown in Figure 24.

In groups I and II, cellular and hyperacute graft rejection were the dominant causes of graft failure in 89% and 80% of the animals respectively. When RATG was added (group III), acute rejection terminated the graft function in 50% of the cases, while infections, diarrhoea and other complications such as thrombosis of the graft anastomosis or renal failure were the cause of death in the remaining 50%.

The combination of the triple-drug regimen with 15-DS led to a high incidence of lethal complications, such as infections and diarrhoea in 57% of the animals. The remaining 43% of the experiments were terminated due to graft rejection.

Omission of azathioprine (group V) showed fewer treatment related side effects; 80% of the animals were sacrificed due to the graft rejection and only 20% died of infections.

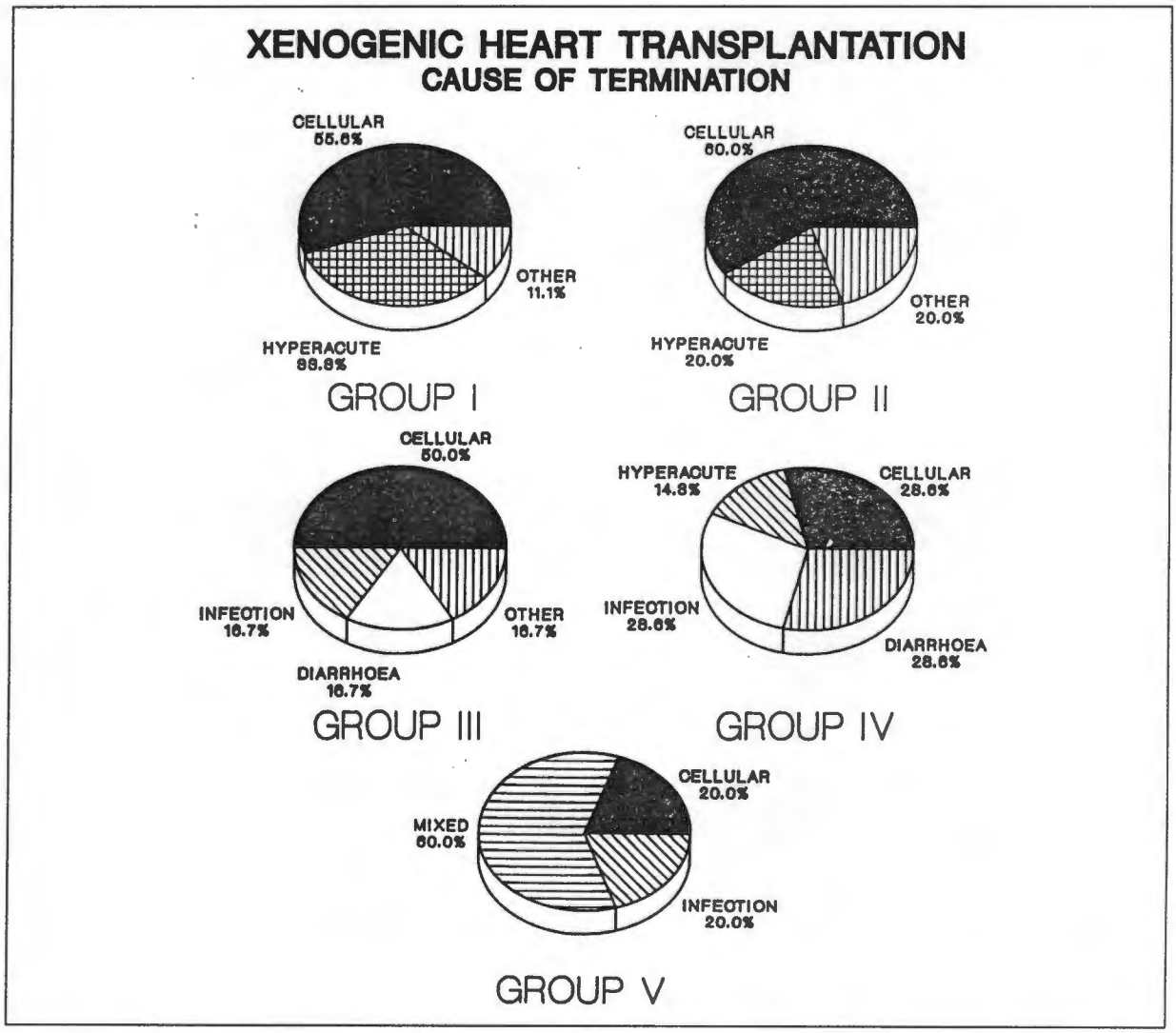


Fig. 24: Causes of experiment termination shown graphically in a pie diagram. Rejection (hyperacute, cellular or mixed) was the dominant cause in groups I and II, while infections and gastrointestinal complications occured mainly in groups III and IV. In group V, less lethal treatment related side effects were seen.

2.3.3. Number of acute rejection episodes

The number of rejection episodes per animal and biopsy was calculated for each group and is listed in Table 12. In order to compare the figures, the number of acute rejection episodes per animal was calculated in relation to every biopsy as well, because the animals had different surival periods (n = nAR/animal x biopsy).

In group II, an average of 0.53 rejection episodes per animal and biopsy was observed. In group III, the group with the longest survival rate, still 0.4 acute rejections per animal and biopsy were nevertheless observed and had to be treated.

The results were significantly different in the 15-DS treated groups. In group IV only 0.14 graft rejection episodes occurred per animal and biopsy ($p < 0.05$ group IV vs. group III). In group V, also only 0.14 rejections per animal were observed, although survival and observation period is similar to group III ($p < 0.05$ group V vs. group III).

Groups	I	II	III	IV	V
nAR	—	0.53	0.40	0.14*	0.14*
		* $p < 0.05$ vs. group III			

Table 12: Number of acute rejections in each treatment group. In group IV and group V only 0.14 rejections per animal and biopsy were observed. These numbers differed significantly from the results obtained in group III ($p < 0.05$, AR=acute rejection episode).

2.3.4. Histopathology of acute rejection

In the control group I, hyperacute rejection was seen in 33.3%, mixed rejection in 55.6% and cellular (acute) rejection in 11.1% of biopsies (Fig. 25). This means, in nearly 90% of all biopsies, there were at least some features of rejection in the biopsy findings.

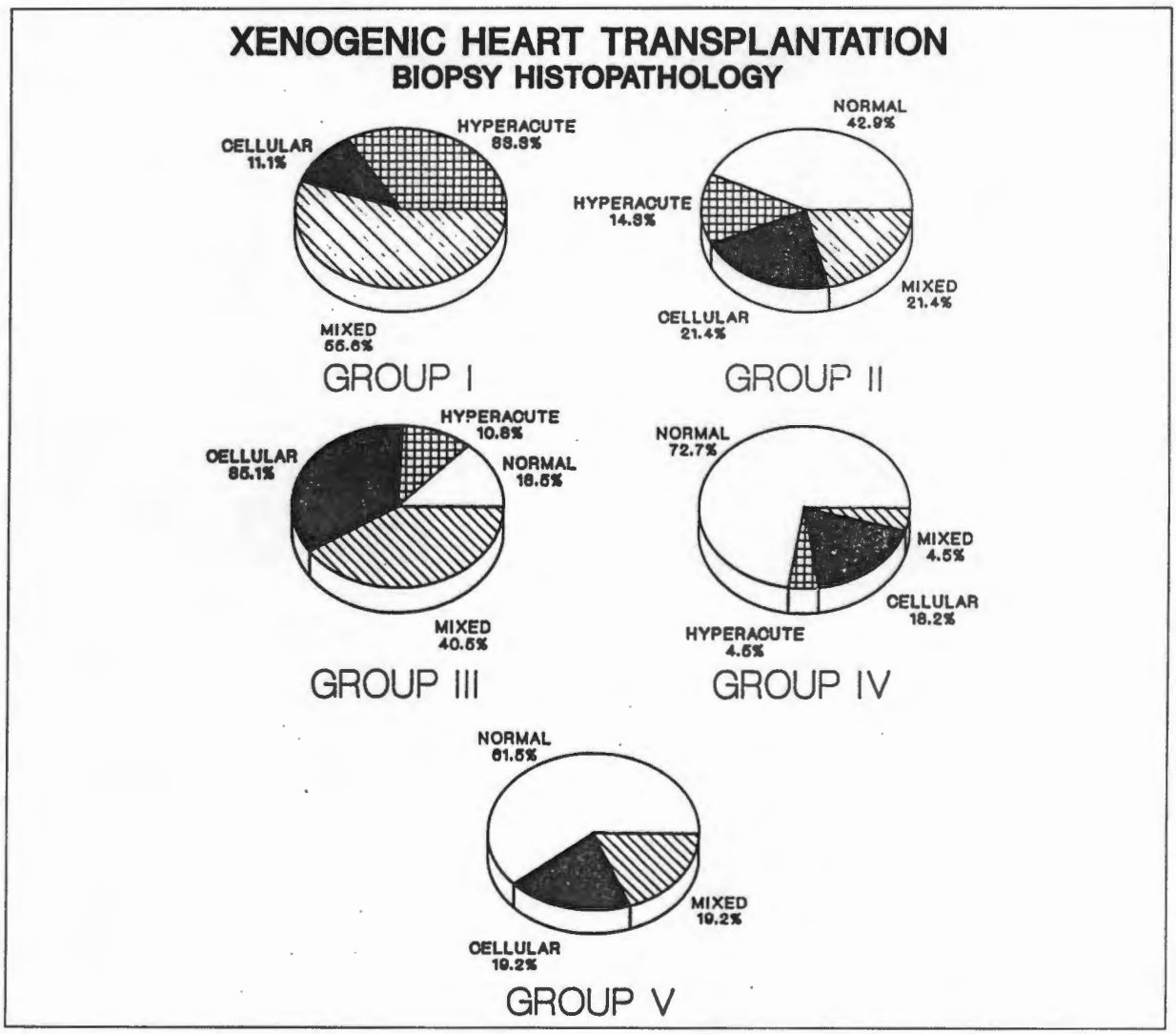


Fig. 25: Histopathology of the myocardial biopsies. Most of the biopsy findings in groups I, II and III showed evidence of rejection; in groups IV and V, 72.7% and 61.5 % of the results were normal with no sign of acute rejection.

In the treated groups II, III and IV, hyperacute rejections occurred less frequently. In group II, only 14.3% of the rejections were

hyperacute and 21.4% of mixed type. Twenty-one point four percent of rejections were of the cellular type and 42.9% of the biopsies turned out to be normal. In group III, hyperacute rejection occurred in only 10.8% of biopsies, cellular rejection in 35.1% and mixed rejection in 40.5%. Despite the longest survival rate, only 18.5% of the biopsies showed normal myocardium with no evidence of rejection.

In both 15-DS treated groups 4 and 5, the majority of all biopsy samples were normal and showed no sign of rejection. In group IV, 72.7% of the biopsies showed normal myocardium, and only 4.5% hyperacute rejection occurred. 18.2% of the rejections were of cellular and 4.5% of mixed origin. In group V, 61.5% of the biopsies were normal, while 19.2% showed rejections of the cellular type and 19.2% rejections of the mixed type (Fig.25).

2.3.5. Cyto-immunological monitoring

The results of the cyto-immunological monitoring showing the percentage of activated lymphocytes per 100 lymphocytes before, during and after rejection episodes are shown in Figure 26. As mentioned above, this test was performed in the treated groups II, III and IV.

In those groups, a significant rise of activated lymphocytes per 100 lymphocytes was noted whenever an acute rejection episode was diagnosed by biopsy; in group II from 2.5% to 4.6% ($p=0.0125$), in group III from 3.2% to 5.9% ($p=0.0014$), in group IV from 2.0% to 4.4% ($p=0.0071$). The decrease in the number of activated lymphocytes after successful rejection therapy was clearly evident and did

not show any statistical difference when compared to the pre-rejection level.

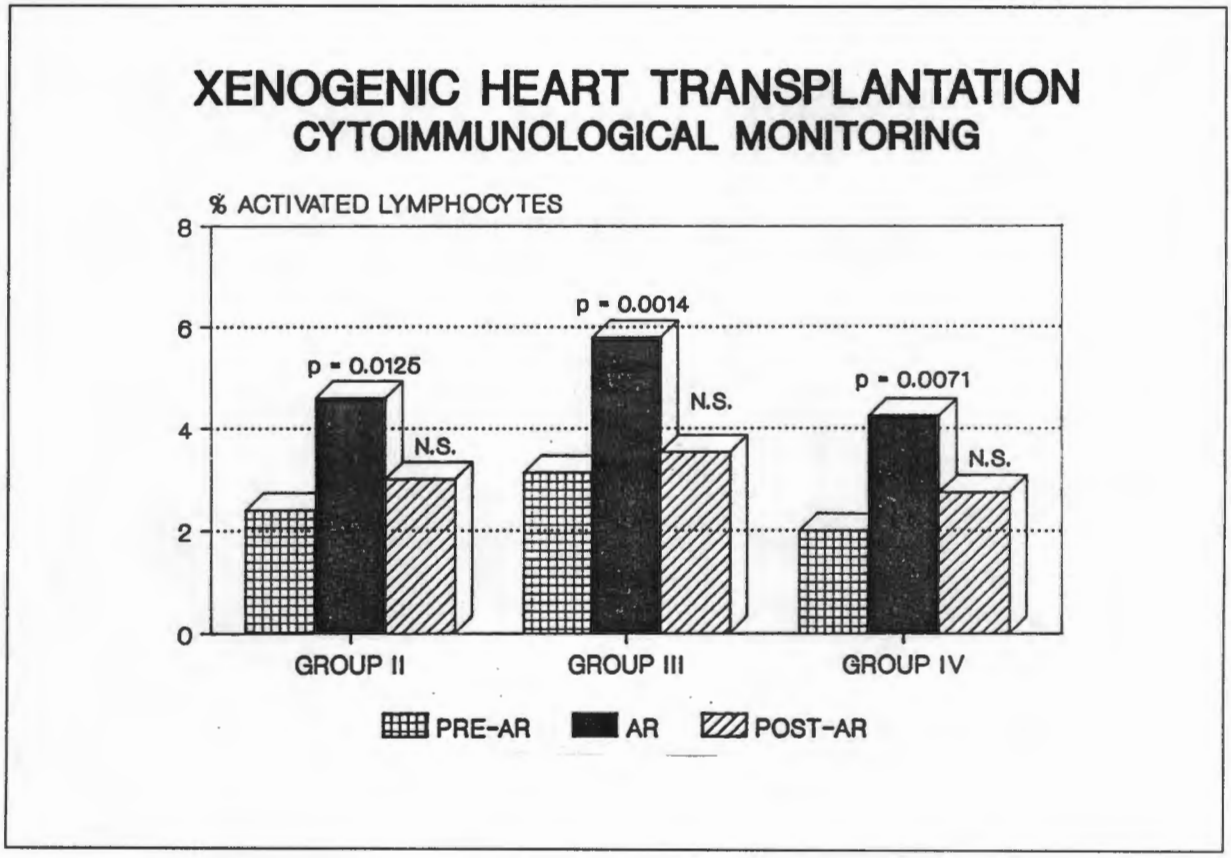


Fig. 26: Results of the cyto-immunological monitoring after xenogenic transplantation. All acute rejection episodes in the treated groups II, III and IV were accompanied by a significant rise of activated lymphocytes within the mononuclear concentrate of the peripheral blood (AR = acute rejection episode).

These data confirm that cyto-immunological monitoring is a reliable method to diagnose acute rejection after xenotransplantation irrespective of which drug regimen is used as immunosuppressive therapy.

2.4. Discussion

During the last 30 years, xenogeneic transplantation has become a more and more interesting field in research, the aim being to optimize immunosuppressive therapy.

2.4.1. Experimental xenotransplantation

Cyclosporine, in addition to its well known use in allogeneic transplantation, has also been described as prolonging graft survival in the xenogeneic model (88,89,90).

Before CyA became available, conventional immunosuppressive drugs like azathioprine, steroids or antilymphocyte globulin (ALG) were used to avoid graft rejection.

DUBERNARD and DONAWICK described ALG being able to prolong renal,cardiac and skin xenograft survival in an experimental model (97,98). The ability of ALG to prolong survival of renal xenografts in large animals was described by BRENDEL et al. who used fox-to-dog and wolf-to-dog xenograft combinations (99). The best results with equine-ALG were achieved, when the recipient was first desensitized against horse serum proteins. PERPER et al. studied the ability of ALG to prolong pig renal xenografts in goat recipients (100). Of great interest was the histological evidence suggesting that ALG substantially alters the mechanism of graft rejection. Without ALG treatment, the features of xenograft rejection consisted of cellular and humoral components. ALG was able to ablate the mononuclear cell response, while the humoral components, like interstitial haemorrhage and vascular disruption, remained uninfluenced.

The effect of CyA on xenograft survival has been described controversially. HOMAN et al. were able to extend the survival of hamster hearts, transplanted heterotopically into rats, only by applying toxic dosages of CyA (88). In the hamster to rat xenograft model, suppression of mainly humorally mediated rejection episodes was only achieved by CyA in combination with splenectomy of the recipient (101).

In contradistinction, BAILEY et al. have described significant increase in survival of lamb hearts in newborn goats with the use of CyA (102). MICHLER et al. reported a 12-fold prolongation of graft survival after xenogeneic cardiac transplantation in primates using parenteral CyA and steroids (91). Although both cellular and humoral mechanisms were involved in the rejection process, these episodes were reversible with increased immunosuppression. The histological appearances were notable for the absence of accelerated transplant atherosclerosis. The same group has published a comparative study between heterotopic and orthotopic cardiac grafts (114). The good results achieved with CyA and particularly with the addition of RATG in the heterotopic could not be repeated in the orthotopic group. This could mean that in closely related species, minimal or moderate rejection does not really affect the function of a heterotopic graft, but could lead to failure of the orthotopic heart in its function to support the entire circulation. Thus, results achieved in heterotopic heart transplant models should only with caution be transfered to the orthotopic situation.

Experiments carried out in the fox/dog model have shown an improvement in heterotopic heart xenograft survival time using immunosuppression with CyA in combination with methylprednisolone (89). In several other experimental studies, the positive effect of CyA has not been confirmed and it has been suggested that the success of CyA is dependent on the various animal species being used (103).

As another form of immunosuppressive therapy, selective lymphoid irradation (TLI) was reported to be effective after cross-species transplantation, particularly when combined with ALG (104).

During TLI treatment, there is a significant decrease of the total lymphocyte count. After completion of a TLI course the absolute lymphocyte count begins to recover (105). It has been clearly demonstrated, however, that the T-cell count remains diminished for many years after TLI. Suppressor/cytotoxic cells will slowly reach pretreatment levels again whereas helper/inducer T-cells remain decreased (106). Thus TLI creates a reversed helper/suppressor cell ratio. TLI has been successfully used after allotransplantation and excellent results have been achieved by MYBURGH et al. after renal transplantation also in humans (107).

The mechanism of action also explains the possible effect of TLI after xenotransplantation. TLI as monotherapy, however, did not show a positive influence on xenograft survival in rodents (104).

The combination of CyA and TLI led to a prolongation of heart xenograft survival in the hamster/rat model (108); graft survival was greater than 100 days in most of these animals. In our own laboratory experience, when the combination of CyA and TLI was used in primates, the occurrence of rejections was decreased significantly, but severe treatment related complications were the major cause of death in the experimental animals (109).

In the present study, cross-species heterotopic heart transplantation was performed in the concordant model using Vervet monkeys as donors and Chacma baboons as recipients. A concordant model was chosen due to the disappointing results after discordant xenotransplantation (110). Using pigs as donors and baboons as recipients, early hyperacute rejection occurred within minutes after

perfusion of the transplanted organs. Absorption of the donor specific preformed natural antibodies by perfusion of donor kidneys on the recipient circulation, resulted only in temporary delay of early hyperacute rejection, with termination of graft function occurring 4 or 5 days subsequently. In order to avoid this early complication, a concordant model was chosen in this study.

In our Vervet monkey/Chacma baboon model, levels of species specific preformed natural antibodies were observably less than with the pig/baboon model, with notable posttransplant increases in antibody titre being exhibited in only a small fraction of the recipients (71). This preformed natural antibody presence did not correlate with early hyperacute rejection, although hyperacute rejection can still be observed in this model, occurring as early as within the first 24 hours after implantation.

A further precondition of our study was AB blood group compatibility between donor and recipient. The Vervet monkey and baboon species share the AB blood groups with the human race and reciprocal natural anti-A and anti-B antibodies are regularly present. As mentioned before, the proximity in genetics and immunology of these primates justify their use for a preclinical research project (111). The A and B antigens of the AB genetic system in the Chacma baboon and Vervet monkey are not expressed on the erythrocyte, but are present on the vascular endothelium and can thus be identified as a target antigen by the immune system (112). In another study performed at our own laboratory, heterotopic cervical xenografts were performed between AB-compatible and -incompatible pairs (113). Survival was increased in the compatible group and the incidence of hyperacute rejection was also decreased in the compatible group compared to the AB-incompatible group. However, these results did not gain statistical significance. Because of these results, AB-compatibility was a precondition in our experimental model.

In this study, CyA was used in different drug combinations to enhance optimal immunosuppressive therapy.
A particular point of interest was the effect of 15-DS on xenograft outcome.
Graft survival rate, frequency and histopathological features of rejection episodes and the occurrence of treatment related side effects were taken into consideration.

The combination of CyA with Azathioprine and Methylprednisolone did not show a significant increase in xenograft survival rate in this model. These findings confirm those by SADHEGI et al. who used a similar experimental model in primates (114).

When CyA was used as monotherapy after xenogeneic skin transplantation in rodents, no significant prolongation of graft survival was achieved using three different dosages of CyA (115). From these experiments one may conclude that CyA will have a positive effect only when combined with other immunosuppressive agents.

In our primate experiments, the triple-drug regimen with the addition of rabbit antithymocyte globulin (RATG), however, led to a highly significant prolongation of experiment duration of 43.3 days on average as compared to 10.3 days in the untreated control group ($p < 0.005$ vs. control). A recent publication by MICHLER et al. confirms the success of this drug regimen including RATG after xenotransplantation in primates (111). The previously mentioned study by SADHEGI et al. (114), did not confirm this finding particularly after orthotopic transplantation. However, the main difference between the two experimental models is that in our study acute rejections were treated with steroids to make the experiment as close to clinical transplantation as possible.

The success of RATG therapy after xenotransplantation has already been described by THOMAS et al who used this drug after mouse-to-rat skin transplantation (115). Graft survival was prolonged significantly and equally impressive was the low level of toxicity of this drug. The degree of inflammatory reaction and the vigour of rejection was diminished, resulting in prolongation of graft survival (115).

The ability of RATG and CyA to synergize as immunosuppressive agents after allotransplantation has been described previously (116). One of the important findings by the THOMAS' group was that RATG was able to prolong xenografts more effectively than even toxic doses of CyA (115). One possible explanation is that RATG may act to ablate antibody-dependent cell-mediated cytotoxicity (ADCC) which has been shown to be operative in xenografts (117). The effector cells mediating ADCC are suppressed by RATG because these cells express surface antigen determinants in common with T-lymphocytes and are thus destroyed or blocked by RATG (118). In addition, RATG has some anticomplementary activity which may contribute particularly to the suppression of hyperacute xenograft rejection (115). Thus, it is not astonishing that RATG particularly in combination with other immunosuppressive agents is able to prolong xenograft survival.

The use of 15-DS instead of RATG showed a less significant increase in survival of 20.1 days on average ($p < 0.05$ vs. control).

In the THOMAS series after xenogeneic skin transplantation in rodents, 15-DS as monotherapy given in three different dosages was not able to prolong graft survival significantly (115). 15-DS, however was very toxic; the overall death rate from toxicity was 66% when given in a dosage from 5-10 mg/kg/d. However when combined with RATG in this model, the combination was very successful (119). Interestingly enough, this drug combination was the most

successful regimen used in the THOMAS series (115). There exist even other positive reports using 15-DS after xenogeneic heart transplantation in rodents, where this drug was able to increase transplant survival significantly (120). Also in the fox/dog model, 15-DS was able to prolong graft survival after renal transplantation (41). Thus, these controversial reports may indicate that the effect of 15-DS after xenotransplantation may be organ and/or species specific. Several effects of 15-DS, such as the suppression of IL1 secretion and expression of MHC class I antigens on macrophages, and the decrease of the plaque-forming response may easily explain its effect also after xenotransplantation (121).

Omission of azathioprine and modification of the dosage of 15-DS resulted in an improved survival time of 35.6 days in group V ($p < 0.01$ vs.control). Graft survival in this group was not significantly different from results achieved in the group with RATG (group III).

Graft rejection was the main cause of experiment termination in the untreated control group I and in group II treated with CyA, azathioprine and methylprednisolone (89% and 81% resp.), which confirms the latter regimen as being insufficient immunosuppression after xenogeneic transplantation (103).

In group III (33%) and particlarly in group IV (53%), infections and severe treatment related side effects, such as diarrhoea and emaciation, were the main reasons for termination of the experiments. As after cardiac and renal allotransplantation, autopsy revealed nonspecific enterocolitis in these animals. The stool specimen sent for microbiological examination, were all negative apart from the usual enteral flora. This high rate of complications occurring in group IV led to a modification of the 15-DS protocol in group V. Omission of azathioprine and combination of 15-DS with CyA and methylprednisolone only, resulted in a decrease of treatment rela-

ted side effects and thus provided a significant prolongation of animal survival time. Diarrhoea is a well known side effect of azathioprine; therefore the combination of 15-DS and azathioprine increases the incidence of this fatal side effect. In the clinical situation, in contrast to the experimental setup, this complication may be treated successfully by parenteral hyperalimentation and enteral abstinence.

The most prominent finding in group IV and group V was the fact that 15-DS was able to reduce the number of hyperacute and acute cellular rejection episodes significantly to only 0.14 rejection episodes per animal and biopsy ($p < 0.05$, groups IV and V as compared to group III). These results confirm our studies using 15-DS and CyA after allogeneic cardiac transplantation in primates. In this study, 15-DS in combination with CyA was also able to reduce the number of acute rejection per animal significantly when compared to the other groups which were treated with either CyA or 15-DS alone. In the same model after renal transplantation, even long-term graft non-reactivity was achieved after initial treatment with 15-DS and CyA, as mentioned previously.

The histopathology of the rejecton episodes also showed different results for the examined groups. In the control group, hyperacute and mixed (hyperacute and acute cellular) rejection were mainly present (89%).

In the treated groups II, III and IV, the acute cellular type of transplant reaction was predominant besides the mixed type. In these groups only one hyperacute rejection episode occurred in each group.

In the 15-DS treated group V, no hyperacute rejection episode occurred, and 61.5% of all histology results were negative with regard to acute rejection. Also in the 15-DS treated group IV over 70%

of the biopsy results were normal with no evidence of rejection features.

Thus, 15-DS treatment led not only to a decreased number of acute rejection episodes, but also to a lowered incidence of severe hyperacute rejections.

This is particularly important, because it has been shown in earlier studies after xenogeneic transplantation that the acute cellular type of rejection can be treated with additional anti-rejection therapy in contrast to vascular or hyperacute rejection episodes (89).

As a new immunosuppressive agent, FK 506 has also been described to be effective after cardiac xenotransplantation in rats (122). FK 506 is thought to exert its effect by inhibiting IL 2 production and IL 2 receptor expression on lymphocytes. One of the major problems of this drug, however, is its toxicity. Used as monotherapy and in combination with CyA, there was a high incidence of toxic side effects after xenogenic skin transplantation in rodents (115). In combination with RATG, HSU et al. have described a synergism of both drugs after xenotransplantation with a lowered incidence of toxic side effects (119). FK 506 in combination with 15-DS also prolonged hamster-to-rat cardiac xenograft survival with reduced toxic side effects due to a lowered dosage being administered (123).

The use of monoclonal antibodies has also been studied in the xenotransplant model. The combination of anti-CD4 and anti-CD8 prolonged both allo- and xenogeneic skin grafts (124). Anti-CD4 alone successfully prolonged xenografts only, while anti-CD8 alone showed no effect after allo- and xenotransplantation. The combination of anti-CD4 with very low doses of CyA produced significant prolongation of both xeno- and allografts. This report confirms the

central role of CD4-positive cells in the reponse to xenogeneic tissue.

The results of cyto-immunological monitoring showed a significant increase in the percentages of activated lymphocytes within the mononuclear concentrate of the peripheral blood during acute rejection episodes in all treatment groups. This also confirms earlier studies that cyto-immunological monitoring is a reliable method of detecting acute rejections after xenogeneic cardiac transplantation (89).

2.4.2. Clinical xenotransplantation

Pioneering work has been performed by Reemtsma et al. in the field of xenogeneic kidney transplantation using chimpanzee donors in human patients (125), followed by STARZL et al. using baboons as donors (126). However, due to the relatively poor results compared with allogeneic transplantation, these projects were discontinued. In the field of cardiac transplantation, HARDY et al. reported the first clinical xenogeneic heart transplantation, in which a chimpanzee heart was transplanted into a 68 year old man with cardiogenic shock (127). This was the first clinical heart transplantation. The heart failed, 1 hour after implantation, probably as a result of gross weight mismatch between donor and recipient. Further clinical reports derive from COOLEY et al. who used a sheep heart (128) and from BARNARD (129). The latter used a chimpanzee heart in the heterotopic position in 1 patient, and a baboon heart in another, while awaiting a suitable human heart. Both patients, however, died within a short space of time due to severe hyperacute rejection.

The most recent case reported by BAILEY and coworkers is also the most successful one (90). A neonate with hypoplastic left heart syndrome who received a baboon heart died after 20 postoperative days from multiorgan failure and ongoing haemolysis. The donor heart was ABO-incompatible and the phenomenon of ongoing haemolysis was probably caused by humoral anti-ABO antibodies. Should this single clinical finding be representative of donor/recipient ABO-incompatibility, the role of the ABO-blood groups systems become especially significant. Due to the relative scarcity of 0-type non-human primates, as mentioned before, this would therefore exclude 0-type recipients from xenotransplantation. Although pretransplant splenectomy of the recipient may eliminate the deleterious effects of ABO-incompatibility (130), the ef-

ficacy of this procedure in cardiac xenotransplantation is doubtful (131).

Clinical preconditions for clinical xenotransplantation:

Before clinical application, the following preconditions must be considered in accordance with BAILEY and coworkers (90):
Pretransplant immunological tests should include at least 6 individual animals: A mixed lymphocyte culture is neccessary in which the responsiveness of recipient lymphocytes to the cells of the potential donor is examined in order to select the most suitable donor animal. In addition, the usual direct lymphocytotoxic crossmatch should also be performed. The presence of maternal antibodies must, however, be considered and excluded before final evaluation. This testing, however, requires a lot of work done by technicians in an immunological laboratory and can therefore only be done in a clinical situation.

ABO-compatibility is essential and a conditio sine qua non. Since primates such as baboons or Vervet monkeys do rarely have the blood group O, neonates and infants with blood group 0 cannot be considered for xenotransplantation.

Postoperatively, every blood transfusion administered to the transplant recipient should be tested for lymphocytotoxic antibodies against the donor animal and for CMV-seronegativity. Only irradiated packed cells, after 4 repeated washings should be used.

Finally, non-human primates are known to harbour a substantial population of virus species that could cause serious disease or death, especially in the immunosuppressed transplant recipient (132). Prior to their utilization as organ donors in the clinical setting, therefore, these animals must be screened for different virus species such as the Herpes simplex type, cytomegalovirus, the simian im-

munodeficiency retrovirus and other human immunodeficiency virus species related to the retroviral strains (132).

The most important precondition is the adequate suppression of the immune response to xenogeneic transplantation. Concordant xenografts would appear to be more suitable for clinical application in the near future, because several immunosuppressive agents showed some potential in overcoming rejection between concordant species. All reports are consistent with regard to the superiority of combination therapy when compared to monotherapy.

As discussed before, the most effective immunosuppression presently available involves the combination of CyA, azathioprine, methylprednisolone and either polyclonal or monoclonal antilymphocyte antibodies. The use of 15-DS showed promising results experimentally, particularly with regard to the incidence and number of acute rejection episodes. In view of the evidence implicating macrophage activity in the xenogeneic response, further studies of anti-macrophage agents, like 15-DS seem to be extremely important. First clinical trials using 15-DS after allotransplantation must, however, be awaited, before using this new drug in clinical xenotransplantation.

The use of non-human primates as concordant donors for humans poses several problems. The baboon does not grow to a size large enough to make it a suitable heart donor for adult humans, although their use might well be possible for paediatric transplantation. Other higher primates, such as chimpanzees are very rare and are therefore not suitable for transplantation purposes. In addition, there are moral and ethical objections to the use of such animals (133).

Thus, the ultimate goal in the field of xenotransplantation would be the use of discordant species as donors. Despite impor-

tant advances in chemical, radiation and mechanical immunosuppression, significant problems are yet to be solved. The presence of preformed natural antibodies remains a formidable obstacle in discordant xenotransplantation. Combination therapy is necessary safely reducing antibody production and at the same time suppressing cell-mediated immunity. For this aim, further work is necessary to characterize the naturally occurring xenoantibody as well as the antigens it recognizes.

In the final analysis, transplant research in this field should aim at modulation of antigen recognition rather than influencing the immune response.

LEGENDS

Fig.1: Operative technique of heterotopic heart transplantation in the neck according to the technique of MANN (CCA = common carotid artery, IJV = internal jugular vein, Ao = ascending aorta, PA = pulmonary artery).

Fig.2: Operative situs after classical renal transplantation. The renal artery is anastomosed to the common iliac artery and the renal vein to the common iliac vein. The ureter is anastomosed to the bladder tunnelled under bladder muscle.

Fig.3: A typical mononuclear concentrate of the peripheral blood during an acute inflammation process:
a) normal lymphocyte, b) activated lymphocyte, identifiable by the noticeable increase in size, pronounced cytoplasmic basophilia and fluffy nuclear structure; c) lympho- or immunoblast, 2 - 3 times enlarged, when compared to the normal lymphocyte; note the basophilia of the cytoplasm, nucleoli are clearly visible within the cell nucleus (Pappenheim staining, x1250).

Fig.4: Mild acute rejection episode: perivascular and scanty interstitial mononuclear cell infiltrations, slight interstitial oedema, but no sign of myocyte damage. Figs. 4-9 and 19-22 were generously supplied by Professor Alan Rose.

Fig.5: Moderate acute rejection: Diffuse interstitial mononuclear cell infiltration, more pronounced interstitial oedema and signs of myocyte necrosis.

Fig.6: Severe acute rejection: In addition to the mononuclear cells, there is infiltration of neutrophile granulocytes, diffuse myocyte necrosis and haemorrhage.

Fig.7: Mild renal rejection: Perivascular lymphocytic infiltrates and only scanty interstitial mononuclear cell infiltrations are visible.

Fig.8: Moderate renal rejection: There is mononuclear cell infiltration with evidence of acute tubular necrosis.

Fig.9: Severe renal rejection: Diffuse lymphocytic and neutrophile granulocyte cell infiltration with signs of haemorrhage and diffuse acute tubular necrosis are obvious.

Fig.10: Survival rates for the different treatment groups after heart transplantation. Increased graft survival is already apparent with 15-DS treatment alone (group IB). The best graft survival was achieved in group ID (15-DS and CyA), when compared to the control group IA.

Fig.11: Survival rates after renal transplantation. Only group IID (15-DS and CyA) showed a significant increase in survival time when compared to the control group IIA. 3 primates are still alive 370, 286 and 274 days after surgery, 1 baboon died 194 days after transplantation.

Fig.12: Experimental endpoints after heart transplantation. Most grafts stopped functioning due to acute graft rejection. One animal in group IB died from gastrointestinal complications probably due to 15-DS therapy.

Fig.13: Experimental endpoints after renal allografting. Uraemia and hyperkalaemia as a result of acute graft rejection were the dominant causes of death in groups IIA and IIB. 2 animals in group IIB died from gastrointestinal complications. 3 out of 8 animals of group IID are still alive (COD? = unknown cause of death).

Fig.14: Cyto-immunological monitoring after cardiac transplantation. In the treatment groups IB and ID, a significant increase of activated lymphocytes within the mononuclear concentrate of the peripheral blood was noted during acute rejection ($p < 0.05$).

Fig.15: Cyto-immunological monitoring after renal transplantation. During acute rejection an increase of activated lymphocytes within the mononuclear concentrate of the peripheral blood was also noted. However, the increase of these activated cells proved not to be significant except for group IID ($p < 0.05$).

Fig.16: Urea levels after renal transplantation. After an initial postoperative rise, the levels returned to acceptable levels again in groups IIC and IID.

Fig.17: Creatinine levels after renal allografting. In groups IIC and IID, these levls dropped again after an initial postoperative rise.

Fig.18: Creatinine levels before and during acute rejection after renal transplantation. Except in the combination treatment group IID, a rise of creatinine was noted during acute renal rejection episodes.

Fig.19: Acute cellular rejection after cardiac xenotransplantation: A similar picture occurred after allotransplantation: Mononuclear cell infiltration occurs perivascularly and in the interstitium, in combination with myocyte necrosis and interstitial oedema.

Fig.20: Hyperacute rejection after xenogeneic transplantation: interstitial haemorrhage and interstitial oedema is seen without any mononuclear cell infiltrate.

Fig.21: Macroscopic picture of hyperacute rejection; note the swelling of the heart and the haemorrhage on the surface.

Fig.22: Mixed type of acute rejection after xenogeneic transplantation. In addition to the features of hyperacute rejection demonstrated above, typical signs of cellular rejection such as mononuclear cell infiltrations are seen as well.

Fig.23: Xenograft survival rates for the different treatment groups. The best graft survival rate was achieved with a combination of CyA, azathioprine, methylprednisolone and RATG (group III). The termination of graft survival within the first 2 postoperative days always corresponded always to the occurrence of hyperacute rejection.

Fig.24: Causes of experiment termination shown graphically in a pie diagram. Rejection (hyperacute, cellular or mixed) was the dominant cause in groups I and II, while infections and gastrointestinal complications occurred mainly in groups III and IV. In group V, less lethal treatment related side effects were seen.

Fig.25: Histopathology of the myocardial biopsies. Most of the biopsy findings in groups I, II and III showed evidence of rejection; in groups IV and V, 72.7% and 61.5% of the results were normal with no sign of acute rejection.

Fig.26: Results of the cyto-immunological monitoring after xenogeneic transplantation. All acute rejection episodes in the treated groups II, III and IV were accompanied by a significant rise of activated lymphocytes within the mononuclear concentrate of the peripheral blood (AR = acute rejection episode).

Table 1: Immunosuppressive protocol after cardiac (group I) and renal (group II) transplantation.

Table 2: List of experimental animals, date of transplantation, date of death, survival and cause of death after cardiac allotransplantation.

Table 3: Mean graft survival rate (GSR) in days after heterotopic cardiac (group I) allotransplantation.

Table 4: List of experimental animals, date of transplantation, date of death, survival and cause of death after renal allotransplantation.

Table 5: Mean graft survival rate (GSR) in days after renal (group II) allotransplantation.

Table 6: Number of acute rejection episodes per animal and biopsy after cardiac (group I) transplantation. The number of rejection episodes per animal was calculated in relation to every biopsy because the animals had different survival periods.

Table 7: Number of acute rejection episodes per animal and biopsy after renal (group II) transplantation.

Table 8: Induction of graft nonreactivity after heart (group I) and kidney (group II) transplantation.

Table 9: Immunosuppressive protocol in the different groups after heterotopic cardiac xenotransplantation.

Table 10: List of experimental animals, date of transplantation, date of death, survival and cause of death after cardiac xenotransplantation.

Table 11: Graft survival rate (GSR) in days after cardiac xenotransplantation within the different groups.

Table 12: Number of acute rejections in each treatment group. In group IV and in group V only 0.14 rejections per animal and biopsy were observed. These numbers differed significantly from the results obtained in group III ($p < 0.05$, AR=acute rejection episode).

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