THE EFFECT OF DONOR-SPECIFIC TRANSFUSION 24 HOURS PRE-TRANSPLANT AND CYCLOSPORIN ON ALLOGRAFT SURVIVAL: A CLINICALLY RELEVANT INDUCTION PROTOCOL FOR CADAVERIC SMALL BOWEL TRANSPLANTATION

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Donor-specific transfusion and cyclosporin in small bowel transplantation.

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Abstract

The combination of pretransplant donor specific transfusion (DST) and cyclosporin (Cys) has proven to be an effective mode of immunomudulation in numerous allograft models. Our experiments were designed to study the effect of clinically applicable protocols using DST and low-dose cyclosporin in an heterotopic, fully allogenic model of small bowel transplantation in the rat.

A 1 ml systemic DST 24 hours pretransplant with Cys (10 mg/kg day -1, 5 mg/kg POD 0 to 7, 2.5 mg/kg POD 8 to 14) was shown to be more effective than DST or Cys alone in prolonging graft survival ($p<0.05$). Adding successive post-transplant DST (POD 7,14,21) had no effect on graft survival. Portal transfusion and Cys was the most effective mode of antigen presentation ($p=0.01$ vs systemic DST), with 33% of the animals having prolonged survival. Adding successive post-transplant DST was deleterious to the portal DST effect. The adjunct of anti-lymphocyte serum to the DST-Cys combination was ineffective.

Sommaire

La transfusion spécifique au donneur (TSD) en combinaison avec la cyclosporine (Cys) ont été prouvées efficaces dans de nombreux modèles de greffes allogéniques. Nos expériences ont été construites pour tester différents protocoles cliniquement applicables utilisant ces TSD et Cys.

Nous avons démontré qu'une TSD de 1 ml à moins 24 heures avec Cys (10 mg/kg -24 heures, 5.0 mg/kg jours 0 à 7, 2.5 mg/kg jours 8 à 14) étaient plus efficace à prolonger la survie du greffon que la TSD ou Cys seules. Transfuser 3 TSD après la greffe n'aidait pas la survie. Nous avons démontré que la transfusion portale avec Cys étaient le mode testé d'immunomodulation le plus efficace (p=0.01 vs TSD systémique): 33% des rats survécurent de façon prolongée. Transfuser après la greffe diminuait l'effet de la TSD portale. L'ajout du sérum anti-lymphocytaire à la combinaison TSD-Cys était inéfficace.

INTRODUCTION

Short bowel syndrome is a state of malnutrition and malabsorption following the loss of a major portion of the small bowel and may also include part of the large intestine. In infants, volvulus, severe necrotizing enterocolitis, and intestinal atresias are the most common conditions requiring major resections. Older children may need resection because of a variety of congenital, acquired, or traumatic disorders. Adults may be affected by Crohn's disease and radiation enteritis, while in elderly patients mesenteric vascular disease is the most prominent cause of major resection.

Total parenteral nutrition (TPN) can maintain the nutritional state of these patients with short bowel syndrome for long periods of time. TPN therapy is subject to complications such as infections, lack of venous access site and cholestasis, which increase with duration of treatment[1]. These problems are accentuated in treatment of children with TPN, because of increased nutritional requirements, difficulty with patient compliance and the risk of associated liver damage, especially in very young infants[2]. Definitive therapy for short bowel syndrome would be transplantation of a healthy small intestine.

Small bowel transplantation was first proposed by Carrel in 1901[3] but it was not seriously considered before 1959, when Lillehei proved that it was technically feasible[4]. The

introduction of cyclosporin and other potent immunosuppressive medications in the 1980s, has renewed the interest in small bowel transplantation due to the impressive results obtained in other clinical organ transplantation.

Numerous models have been used in the study of small bowel transplantation. The inbred rat model has an economical advantage, but is also ideal to study rejection, and graft versus host disease, separately, using hybrids. The microsurgery involved renders this model technically difficult. The dog and swine model have also been used as they closely mimic the human physiology. Those large animal models have been utilized more as a preclinical evaluation stage for immunosuppressive protocols.

Small bowel transplantation is already a clinical reality. Three centres, Pittsburg, Paris, and London, Ontario, have well established clinical programs. Other European centres, Innsbruck, Kiel, and Uppsala, for example, have also transplanted human bowel. In the first years of human bowel transplantation (1967-70), no long surviving graft was reported. All grafts had to be removed for rejection or uncontrollable sepsis. It is only in recent years that successful small bowel transplantation has been reported either as isolated grafts or as combined liver and small bowel grafts. The postoperative course of these patients is still very complicated: those patients face long months in the intensive care unit fighting numerous episodes of rejection

and sepsis. In the Pittsburg group's experience, the average cost of a small bowel transplant is around half a million US dollars (personal communication).

As Watson and Lear[5] point out in their review article, there are two major problems that hinder successful small bowel transplantation from becoming common reality: first, small bowel transplantation triggers a series of complex immunological phenomena, which include both rejection and graft versus host disease, that are not well controlled by current immunosuppressive regimens and require elaborate treatment of both the recipient and the graft. Second, the physiological functions of the graft are severely deranged by the process of transplantation and further immunological damage will not only hamper recovery but destroy the barrier functions so vital to the recipient's survival. The gut is special among vascularized graft as it carries its own immune system known as the gut associated lymphatic tissue, GALT [6].

The goals of this project are to study new combinations of immunosuppression and immunomodulation that would be practical for future clinical use, both in matched living related donor and cadaveric transplantation. Induction of immunological tolerance by perioperative manipulation would be ideal for the control of rejection and would also reduce the risks of cyclosporin toxicity and overimmunosuppression resulting in lymphoproliferative disorders and other sepsis related complications. In recent years, the pretransplant

presentation of antigen with blood transfusion specific to the donor has markedly improved the outcome of clinical renal transplantation[7]. The beneficial effects of this active pretransplant conditioning with blood transfusion on allograft survival has been well established in several animal models. Donor specific antigen presentation induces specific unresponsiveness to organ allograft but in a much weaker fashion than when combined with the administration of an immunosuppressive drug, especially cyclosporin[8]. The mechanisms underlying this phenomenon are still not fully elucidated but suppressor cells[9], antiidiotypic antibodies[10], clonal deletion of cytotoxic T cell precusors[11] and clonal anergy have been suggested as possible mechanisms.

Few studies have investigated the use of donor specific transfusion (DST) in small bowel transplantation (SBT). Martinelli et al.[12] found significant improvement on survival when they administered one DST 8 days prior to transplantation and cyclosporin at the peritransfusion and post-transplantation period. On the other hand, De Bruin et al.[13] did not improve graft survival when they administered three pretransplant DSTs (day -21,-14,-7) and post-transplant cyclosporin. Only one group attempted to ameliorate graft-versus-host disease giving a DST to the donor: the transfusions induced a more severe graft-versus-host disease[14].

The underlying goal of this project was to find a

specific immunosuppressive regimen that would be clinically applicable to both cadaveric and living related donor transplants. All the experiments were designed with antigen presentation given within 24 hours of transplant. Previous studies have shown that such a regimen combined with cyclosporin was effective at prolonging allograft survival[15],[16] in both the kidney and cardiac allograft model.

We have designed experiments using a fully allogenic model of heterotopic SBT in the rat. We studied the effect of DST in several protocols differing in dosage, timing, route of administration but always in combination with one immunosuppressive agent (cyclosporin) or a combination of immunosuppressive agents (cyclosporin and anti-lymphocyte serum).

Material and method

Animals.

A fully allogenic donor-recipient model was employed using adult inbred male Brown-Norway (BN,$RT1^n$) donors and Lewis (Lew, $RT1^l$) recipient rats. The Lew rats were obtained from Charles River Canada (St-Constant,Que) and the BN rats from Harlan Sprague Dawley (Indianapolis, IN). Animals weights ranged between 175-300 gm. All animals were housed in conventional animal facilities accredited by the CCAC, being fed rat chow and tap water ad libitum. Both the donors and the recipients were fasted the day prior to surgery. All experiments were carried in accordance with CCAC guidelines and were approved by the institutional animal ethics committee.

Surgery.

Donor Surgery:The donor rat was anaesthetized with pentobarbital 50mg/kg intraperitoneally or with halothane. An heterotopic transplantation was performed by a modification of the technique described by Monchik and Russell[17]. After a total colectomy the entire small bowel from the ligament of Treitz to the terminal ileum was harvested with its vascular pedicle consisting of the superior mesenteric artery on a cuff of proximal aorta, and of portal vein up to the porta hepatis.

After systemic heparinization (100-200 U) the bowel lumen was flushed with 10 ml of a chilled 0.5% neomycin sulfate solution. The bowel and its vascular pedicle were then quickly resected and the arterial lumen was immediately perfused with 3 to 6 ml of chilled heparinized saline (1: 10 000). The graft was kept in a saline solution placed on ice until the recipient was ready.

Recipient surgery:The recipient rat was anaesthetized in the same rashion as the donor rat. After prophylactic administration of gentamicin 6mg/kg and ampicillin 200mg/kg IM, the infrarenal aorta and inferior vena cava of the recipient rat were isolated and cross-clamped both proximally and distally. An end-to-side aorto-aortic and portocaval continuous anastomosis were performed with 10-0 nylon suture(either 10-0 Ethilon BV75-4, Ethicon or 10-0 Dermalon TE-75, Davis-Geck). After removal of the clamps the reperfused bowel was inspected and areas of poorly vascularized bowel were resected. Bcth the proximal and distal ends of the graft were brought out as Brook's stomas on the right flank. The recipient's bowel was left intact. The rats were given a total of 10-15 ml of Ringer's Lactate IV or by clysis for the entire operative time. The recipient rat was left to fully recover from anaesthesia in an incubator.

Postoperative monitoring.

After transplantation, the rats were placed in individual

cages and received standard rat chow and water ad libitum. The rats received no antibiotics in the postoperative period. The animals were examined daily for signs of both rejection and GVHD, and were weighed three times a week. Rejection was clinically defined as the appearance of a palpable mass and loss of weight. Animals were sacrificed when judged to have rejected their graft. The severity of GVHD was clinically assessed and was graded as previously described: grade 1, light redness of ears, snout, and paws; grade 2, moderate redness of ears , snout, and paws, with light hair loss and diarrhea; grade 3 severe redness of ears, snout, and paws, with alopecia, generalized dermatitis, and profuse diarrhea.

All rats that died within 4 days were considered technical failures. All rats surviving longer than 150 days without any signs of rejection were considered permanent survivors.

Blood transfusions.

After the removal of the allograft, a phlebotomy was performed on the previously heparanized donor at the inferior vena cava. Two to five mililiters of blood were obtained for future donor-specific transfusions. A 1 ml DST was administered under anaesthesia to the recipient rat via the penile vein or the portal vein according to the protocol, the day before transplantation.

Cyclosporin.

Cyclosporin A (Sandimmune ,Sandoz) was dissolved in olive oil at a concentration of 5 mg/ml and was injected subcutaneously daily according to the protocol.

Anti-lymphocyte serum.

Lyophilized rabbit anti-rat lymphocyte serum (ALS) (CL015A, Cedarlane,Hornby,Ont) was reconstituted with 1.0 ml of ice cold sterile water and then diluted to a concentration of 1:20 with a PBS solution. One ml of that solution was injected systemically via the penile vein.The ALS dilution of 1:20 had been chosen because of a reported in vitro cytotoxic index of 98% for thymic cells, 94% for splenic cells, 99% for lymph node cells, and 70% for bone marrow cells (Cedarlane product information data).

Pathology.

At the time of rejection,the grafted small bowel and mesenteric lymph nodes, the native small bowel and mesenteric lymph nodes, and the spleen were excised and fixed in 3.9% buffered formalin and prepared for histological analysis by hematoxylin-eosin staining.

The pathology specimens were graded by a pathologist (E. Rosenmann, Hadassah Hebrew University Medical Centre) blinded to the treatment and the origin of the bowel, according to the following scale.

Grade 0: Normal intestine.

Grade 1: Mild distortion of the villous architecture, with normal amount of mucous cells; slough of epithelial cells in the lumen of intestine; occasional apoptotic cell in the base of crypts and evidence of crypt hyperplasia.

Grade 2: Partial effacement or blunting of the villous architecture; depletion of mucous cells; slough of epithelial cells within the lumen; marked apoptosis and crypt hyperplasia; mononuclear cell infiltrate within the lamina propria and cryptitis.

Grade 3: In addition to changes in grade 2 there is also patchy or total necrosis of the mucosa or ulcerations;in some instances also thrombosed vessels , and in all cases suppurative peritonitis.

Data analysis.

Survival data were analyzed using the actuarial survival curves as calculated by the Kaplan-Meir statistical analysis method. Statistical significance was tested by the log rank test and the Mann-Whitney test and a $p<.05$ was considered significant.

Experimental groups.

Group 1: Control group;Total SBT no pre or postoperative treatment.(n=10)

Group 2: Systemic DST day -1, no other treatment. (n=5)

Group 3: Cyclosporin 10mg/kg on day -1, 5mg/kg day 0 to 7, 2.5mg/kg day 8 to 14.(n=12)

Group 4: Systemic DST day -1 and cyclosporin as in Group 3.(n=11)

Group 5: Cyclosporin 10mg/kg on day -1, 5mg/kg day 0 to 7, 2.5mg/kg day 8 to 28.(n=6)

Group 6: Systemic DST day -1,7,14,and 21, with cyclosporin as in Group 5.(n=11)

Group 7: Cyclosporin 5mg/kg on day -1 to 7, 2.5mg/kg day 8 to 28.(n=8)

Group 8: Systemic DST day -1,7,14,and 21, with cyclosporin as in Group 7.(n=9)

Group 9: Portal DST day -1, no other treatment.(n=5)

Group 10: Portal DST day -1, cyclosporin as in Group 3.(n=9)

Group 11: Portal DST day -1, systemic DST day 7,14,21, and cyclosporin as in Group 5.(n=5)

Group 12: ALS to the recipient at the time of surgery, cyclosporin as in Group 3.(n=6)

Group 13: Systemic DST day -1, ALS to the recipient at the time of surgery, and cyclosporin as in Group 3.(n=7)

Group 14: ALS 4 hours before the DST day -1,and cyclosporin as in Group 3.(n=5)

Group 15: ALS to the donor day -2, portal DST to the recipient day-1, and cyclosporin as in Group 3.(n=6)

Research design.

We designed our research to study the effectiveness of the immunomodulation with pretransplant donor-specific transfusion in a context that would be relevant to clinical cadaveric donor transplantation, i.e. within 24 hours of transplant. All DST were administered 24 hours pretransplant as this was felt to be the earliest time at which donor blood would be available in the cadaveric donor situation.

Even though there is some conflicting results, enteral Cys seems to be absorbed via the lymphatic vessels[18,19,20]. This cause a major problem in small bowel transplantation as the lymphatics are totally disrupted by the surgery inherent to the harvesting of the graft. Oral administration of cyclosporin after small bowel transplantation presents another unique problem in organ transplantation, inasmuch as the drug must be absorbed through the transplanted organ itself.The absorption depends therefore on the functional state of the graft which may be influenced by an ongoing rejection process.

Wassef et al. have studied other routes of administration in the rat and came to the conclusion that between subcutaneous, intramuscular and intraperitoneal the last two routes had a greater bioavailibility but the subcutaneous route resulted in a more steady state over 24 hours with little variation over time and was easier to perform[21]. Thus, even though all our experiments were performed in an heterotopic manner, so that enteral absorption of cyclosporin would not have been affected, it was decided to administer the

cyclosporin subcutaneously. The cyclosporin was started 24 hours pre-transplant so that the recipient would be exposed to the drug at the time of the pretransplant DST, to reduce sensitization, and so that a steady state would be achieved as soon as possible after transplant. Experiments carried out in an heterotopic heart transplant model from ACI to Lewis rats have also shown a prolonged survival when cyclosporin was started 24 hours pretransplant compared to starting the cyclosporin at the time of transplant[22].

In all experimental protocols DST was used in combination with at least one immunosuppressor, cyclosporin, to benefit from the known synergistic effect of this medication when used with DST. The protocols were grouped into five different experiments, exploring the route of administration of the DST, multiple DSTs, different dosage of cyclosporin and the combination with another immunosuppressor, anti-lymphocyte serum.

The first experiment (Groups 1,2,3,4) compared both cyclosporin and DST alone to the two in combination. We wanted to verify the hypothesis that a day-1 DST in combination with cyclosporin would be more effective than cyclosporin alone in preventing rejection as was a similar day-1 protocol in a cardiac transplant experiment[16].

In the second experiment (Groups 4,5,6,7,8) tested whether three additional DSTs post-transplant would reinforce the immunomodulatory effect of the pretransplant DST.

Additional DSTs pretransplant or pre and post transplant have been shown to improve allograft survival in renal and heart allograft models. As shown by Fabre, multiple IV injections of donor blood before grafting proved to be a better regimen than a single injection. He was able to induce indefinite survival, in a semi-allogenic model of renal transplantation, with bi-weekly pretransplant DSTs for 4 weeks[23]. Later Cofer et al. showed that pretransplant DST and multiple post-transplant DSTs where more effective than a single pretransplant DST at prolonging survival in a fully allogenic cardiac transplantation model[24].

Because clinical experience seems to show that the liver has a tolerogenic effect when transplanted in conjunction with the small bowel, and that the liver seems to play a major role in the immune reaction following SBT, we hypothesised in the third experiment that presenting the donor antigen to the liver primarily, via portal infusion, would lead to an improved survival of the graft (Groups 1,3,4,9,10,15). The liver is an immunological organ with its own antigen presentating cells, the Kupffer cells. Portal transfusions of donor specific cells presented directly to antigen presentating cells of recipient origin may allow for a more effective antigen presentation. Intra-portal vein transfusions have been shown by several investigators to be effective at prolonging survival of allogenic grafts. For example, using a renal transplantation model, Yoshima et al. showed that

infusion of splenocytes on day 0 produced statistically significant prolongation in allograft survival(28.6 $\pm$ 7.0 for portal transfusion versus 10.4 $\pm$ 1.1 for systemic transfusion)[25]. Kenick et al. have demonstrated a similar effect in a cardiac allograft model with infusion of donor mononuclear cells into the mesenteric portal system of recipient 7 to 10 days prior to transplantation[26]. None of these studies have used day -1 intraportal transfusion with cyclosporin in the small bowel transplantation model.

The fourth experiment was conceived as experiment two (Groups 5,10,11), that is, adding three successive systemic DSTs post-transplant to determine if further benefits the portal DST effect.

In the last experiment (Groups 3,4,12,13,14), the hypothesis was tested that additional immunosuppression the recipient with anti-lymphocyte serum, at different times relative to the transfusion and surgery, would improve the DST effect in rat SBT. Partial lymphocyte depletion with ALS can transiently reduce circulating lyphocytes in the recipient, producing a state of immature or embryonic immune system that may be more prone to tolerance induction with antigen presentation.

Hypothesis

Question 1: Is day -1 DST combined with low dose cyclosporin superior to cyclosporin alone at

inducing enhanced graft survival?

Question 2: Does the addition of successive post-transplant DSTs further enhance small bowel allograft survival when compared to pretransplant DST only?

Question 3: Is the portal route of antigen presentation superior to the systemic route in small bowel transplantation, and can intraportal day-1 DST combined with low dose cyclosporin induce long term survival?

Question 4: Does the addition of successive post-transplant systemic DST further enhance the effect of a pretransplant intraportal DST?

Question 5: Does additional immunosupression with ALS improves the effect of pretransplant DST in combination with low dose cyclosporin?

Results

Experiment 1

Table 1. Graft survival time experiment 1.

Groups	Graft survival days	MST ± SEM	Median
1-no treatment	6,6,8,9,9,10,11,12,14,16	10.1 ± 1.0	9.5
2-s.DST, no CyS	7,7,8,8,14	8.8 ± 1.3*	8.0
3-Cys alone	9,9*,9*,9*,9,10,13,13,13,17,26,42	14.9 ± 2.9†	11.5
4-s.DST + Cys	8,8*,9*,9*,9,10,13,14,22,30,71	18.4 ± 5.6‡	10.0

* deaths post-biopsy
* NS vs Gr.1; † NS vs Gr.1; ‡ NS vs Gr.1 & 3, $p<0.05$ vs Gr.2

Group 1, the control group, receiving no pre or post-transplant treatment, survived an average of 10.1 ± 1.0 days, which is concordant with survival results for control groups in the literature. Group 2, which received a one ml DST 24 hours pretransplant without any cyclosporin, had a mean survival time of 8.8 ± 1.3 days. Even though no significant difference was found with Group 1 ($p=0.38$), the administration of one DST pretransplant without concordant immunosuppression seems to have a slight deleterious effect on graft survival. This may be related to the sensitization effect seen in kidney recipients who had received a donor transfusion before immunosuppressive drugs were administered concomitantly [27,28]. Group 3 received cyclosporin only at 10 mg/kg the day prior to the transplantation, 5 mg/kg/day from day 0 (transplantation day) to day 7 inclusive, and 2.5 mg/kg/day

from day 8 to 14 inclusive and had a mean graft survival time of 14.9 ± 2.9, which was not significant when compared to group 1 ($p=0.20$). In this group three animals died at day 9 post-biopsy. If these rats are excluded, the mean graft survival time is then increased to 16.9 ± 3.6, with a median of 13.0. These results still do not attain significance when compared to group 1 ($p=0.071$ MW or $p>0.05$ log rank). This cyclosporin regimen must then be subtherapeutic. Group 4 received a one ml DST 24 hours pretransplant and the same cyclosporin regimen as Group 3 and had a mean survival time was 18.4 ± 5.6, with a median of 10.0. In this group also, animals died following biopsies. When those animals were excluded, the mean survival time is raised to 22.1 ± 7.5 and a median of 13.5. This group then becomes significantly different from Group 1 and Group 2 at a $p<0.05$ (log rank test). There thus seem to be a synergism between the cyclosporin and DST, as neither of them were effective when used alone, but seem to have an immunomodulatory effect when used in combination.

Experiment 2

Table 2. Graft survival time experiment 2.

Groups	Graft survival days	MST ± SEM	Median
4-s.DST + CyS	8,8*,9*,9*,9,10,13,14,22,30,71	18.4 ± 5.6	10.0
5-Cys 10 X 28d	7,10,11,11,23,26	14.7 ± 3.2	11.0
6-Xs.DST+CyS 10	8,10,10,12,12,13,13,14,14,14154	24.9 ± 12.9	13.0
7-Cys 5 X 28d	6,7,8,9,10,14,14,>150	27.3 ± 17.5	9.5
8-Xs.DST+Cys 5	10,10,10,14•,14,14,14,15,>150	29.6 ± 17.2	14.0

* deaths post-biopsy
• GVHD grade 1 POD 8 & 9
None of the groups attained significance.

In experiment 2, Group 4 (one pretransplant DST and low-dose cyclosporin) was used as one of our control. Group 5 received a longer regimen of cyclosporin to control for the administration of cyclosporin with the 3 post-transplant DSTs: the recipient received 10 mg/kg the day before transplant, 5 mg/kg/day from day 0 to 7 inclusive, and 2.5 mg/kg/day from day 8 to 28. Group 5 had a mean survival time of 14.7 ± 3.2. Changing the induction dose to 5 mg/kg was also ineffective in prolonging graft survival. Group 7, which received 5mg/kg on day -1, had a mean survival time of 27.2 ± 17.5 which was not significantly different from Group 5 by either Mann-Whitney or log-rank test. On the other hand one animal seem to have been tolerant to his graft showing no sign of rejection even when sacrificed at 150 days post-transplant.

The animals in Group 6 were given a 1 ml DST 24 hours pretransplant and on day 7, 14, 21 post-transplant. They

received the same cyclosporin regimen as in Group 5. They had a mean survival time of 24.9 ± 12.9 with a median of 13.0 (p=0.53 vs Group 4). Adding three post-transplant DSTs had no effect on mean graft survival except that one rat in this group had a prolonged graft survival to 154 days. This graft showed chronic rejection on histologic examination. Decreasing the induction dose to 5 mg did not improve Group 8's survival time compared to Group 6 (p=0.435). Group 8 reached a mean survival time of 29.6 ± 17.2 and a median of 14.0. In this group also, one animal showed prolonged graft survival (150 days) with no sign of rejection.

Experiment 3

Table 3. Graft survival time experiment 3.

Groups	Graft survival days	MST ± SEM	Median
1-no treatment	6,6,8,9,9,10,11,1214,16	10.1 ± 1.0	9.5
3-Cys 10 X 14d	9,9*,9*,9*,9,10,13, 13,13,17,26,42	14.9 ± 2.9	11.5
4-s.DST + Cys 10	8,8,9,9,9,10,13,14,22,30, 71	18.4 ± 5.6	10.0
9-p.DST,no Cys	6,7,7,8,11	7.8 ± 0.9⁑	7.0
10-p.DST + CyS 10	13,14,17,17,22,26, 41,87,>150,>150	53.7 ± 17.5†	26.0
15-dALS+p.DST+Cys	7,8,9,12,12,12	10.0 ± 0.9‡	10.5

* deaths post-biopsy
⁑ NS vs Gr. 1;
† p<0.001 vs Gr. 1, p<0.005 vs Gr.3 & 9, p<0.02 vs Gr.4
‡ p<0.002 vs Gr.10

Group 1 (no treatment), Group 2 (cyclosporin only), and

Group 4 (systemic DST and cyclosporin) were used as control. The animals in Group 9 received a 1 ml portal DST 24 hours pretransplant without cyclosporin. The survival data of Group 9 showed that a portal DST alone had no influence on graft survival. The results were no different from Group 1 (p=0.20 MW). The rats in group 10 were given a 1 ml portal DST 24 hours pretransplant with the same cyclosporin regimen as Group 3. On the other hand, when one portal DST was combined to low-dose cyclosporin, the graft survival results were significantly improved. Group 10 had a mean graft survival time of 53.7 ± 17.5 days, with a median of 26.0 days (p=0.005 vs Gr.3, p=0.01 vs Gr.4, p=0.003 vs Gr.9). None of the animals died early in the post-transplantation period, all of them surviving beyond the median survival time of Groups 3 and 4. Three animals (33%) in this group survived beyond 85 days and two animals (20%) attained indefinite survival, with no sign of rejection.

Group 15 was designed to see if decreasing the immunogenicity of the graft with the systemic administration of 1 ml of anti-lymphocyte serum (ALS) to the donor 24 hours pretransplant would further increase the graft survival in the portal DST protocol. The recipients in Group 15 underwent the exact same DST-cyclosporin regimen as Group 10. Decreasing the immunogenicity of the graft in addition to the portal DST and low-dose cyclosporin had a deleterious effect on graft survival time. The mean graft survival time of Group 15 was

10.0 ± 0.9, with a median of 10.5. These animals looked sicker before their death than animals in the other groups.

Experiment 4

Table 4. Graft survival time experiment 4.

Groups	Graft survival days	MST ± SEM	Median
5-Cys 10 X 28d	7,10,11,23,26	14.7 ± 3.2	11.0
10-p.DST + Cys	13,14,17,17,22,26, 41,87,>150,>150	53.7 ± 17.5	26.0
11-p.DST+Xs.DST+Cys	6,10,13,14,15	11.6 ± 1.6†	13.0

† NS vs Gr.5, $p<0.01$ vs Gr.10 (log rank test)

We used Group 5 (cyclosporin for 28 days) as one of our control. Group 10, which received one pretransplant intraportal DST and peritransplant cyclosporin was compared to group 11 which received also one pretransplant portal DST and cyclosporin in addition to systemic post-transplant DSTs at day 7, 14, 21. Group 11 had a mean survival time of 11.6 days which was markedly reduced when compared to group 10. One animal did not received any post-transplantation transfusion and the other four animals received only one post-transplantation transfusion.

Experiment 5

Table 5. Graft survival time experiment 5.

Groups	Graft survival days	MST ± SEM	Median
3-Cys X 14d	9,9*,9*,9*,9,10,13,13,13, 17,26,42	14.9 ± 2.9	11.5
4-s.DST + Cys	8,8*,9*,9*,9,10,13,14,22, 30,71	18.4 ± 5.6	10.0
12-ALS + Cys	9,10,14,16•,24,45	16.3 ± 2.8†	15.0
13-ALS+sDST+Cys #1	10,10,10,11,11,11,14	11.0 ± 0.5†	11.0
14-ALS+sDST+Cys #2	11,12,12,13,14	12.4 ± 0.5‡	12.0

* deaths post-biopsy
• GVHD grade 1 POD 9 & 10
* NS vs Gr.3; † p<0.01 vs Gr.12 (log rank test),NS vs Gr.3 & 4; ‡ NS vs Gr. 3, 4, 12 & 13

Group 3 (cyclosporin only) and Group 4 (systemic DST plus cyclosporin) acted as two of our controls. Group 12 received a 1 ml ALS inoculation at the time of surgery and cyclosporin as in Group 3. Group 12 had a mean graft survival time of 16.3 ± 2.8 and a median of 15.0. This was not statistically significant when compared to Group 3 nor Group 4. Group 13 received a 1 ml DST day -1, ALS at the time of surgery, and cyclosporin as in Group 3. Group 13 had a mean graft survival time of 11.0 ± 0.5, with a median of 11.0. This group differ significantly from Group 12 at a p<0.01 (log rank), but not from Group 3 or 4. Group 14 received ALS 4 hours before the DST day -1 and cyclosporin as in Group 3. Group 14 had a mean survival time of 12.4 ± 0.5, with a median of 12.0. One animal

in this group was sacrificed at day 14 because of marked weight loss and what seemed like a retro-orbital tumor or haemorrhage. At autopsy the transplanted bowel showed no gross sign of rejection. This group showed no significant difference with any of the other groups in this experiment.

Pathology specimens

All the pathology specimens were reviewed in a blind fashion by Dr. Elizer Rosenmann from Hadassah Hebrew University Medical Centre. Rejection was rated as grade 0, normal, grade 1, mild, grade 2, moderate, and grade 3 severe, as defined in material and methods. Some specimens could not be analyzed because of autolysis, when the animals were found dead in their cages. No quantitative comparison could be made between groups as the specimens were taken at different days post-transplantation. The histological analysis was used more as a way to confirm our rejection data. Native bowel specimens were used as internal controls. All the native bowel specimens as well as spleen and lymph nodes were read as normal in all experiments, with no sign of rejection. Except for the long term survivors all transplanted bowel were found to have been rejected either acutely or chronically, most of them showing grade 2 and 3 rejection. Two types of rejection patterns seems to arise in the qualitative analysis: one rejection process affecting mostly the mucosa and bowel itself, the other one affecting mainly the mesentery of the transplanted bowel. The

rejection process involving the bowel itself was predominant, and all groups were affected by it in about the same proportion.

Rats in group 1 (control) were all affected by severe rejection at the time of autopsy. Animals in group 2 (systemic DST without cyclosporin) all showed severe rejection on histologic examination. Group 3 (cyclosporin) showed mild rejection on biopsies as early as day 4, but some biopsy specimens showed no signs of rejection at POD 8. After POD 8 all the specimen showed a grade 2 or 3 rejection process. In group 4 (sDST + Cys), two biopsy specimens showed no or mild rejection at POD 8. After POD 8 all specimens showed grade 2 or 3 rejection. There seems to be delayed rejection in group 3 and 4 compared to group 1 and 2, but no real difference between group 3 and 4.

In our second experiment, the long surviving animal in group 6 (XDST + Cys) showed chronic rejection on histological analysis: villi were inexistant, being replaced by a simple flattened cellular epithelium, there was fibrosis seen in the muscular layer, and the vessels showed endothelial cells transformed into foaming cells, narrow lumen, and endotheliitis. All the other specimens in group 6 showed grade 3 rejection except for one specimen which showed mild rejection in the bowel but severe infiltration in the mesentery. Specimens from rats in group 5 (Cys X 28 days) showed the same severe rejection except for one which had the

maximal changes in the mesentery.

Group 7 (Cys 5mg induction) and 8 (XDST + Cys 5mg induction) had similar patterns of rejection, both having two animals showing predominant rejection in the mesentery. One animal in group 7 had only mild rejection at the time of autopsy and showing signs of recovery. One animal in group 7 was a long term survivor, showing no sign of rejection on autopsy but with some area of atrophic mucosa with flattening of the villous pattern. Group 8 also had a long term survivor. This animal showed almost normal bowel on histological examination: There was some slough of necrotic epithelium in the lumen which might indicate a previous process of rejection but there was total regeneration of the mucosa with a normal villous pattern. This necrotic epithelium could also be attributed to a mild perfusion injury with subsequent regeneration.

Group 9 (pDST without Cys) showed severe rejection in all specimens except for one specimen taken at POD 7 which showed no sign of rejection with normal bowel architecture.

In group 10 (pDST + Cys) the severity of rejection was very heterogenous, some animals having only mild grade 1 rejection and some having severe grade 3 rejection. The animal that survived 87 days showed signs of chronic rejection with marked congestion and inflammation of the muscular layer and the replacement of the mucosa with cuboidal epithelium. The two animals that survived beyond 150 days had normal bowel on

histologic examination, with no signs of rejection.The lymph nodes of these two specimens showed complete depletion of regional lymph node with an intact sinusoidal pattern but with complete disappearance of B and T lymphocytes.

Group 11 (pDST + XDST + Cys) showed a surprising pattern on histological examination: even though most of these animals were sacrificed early becau of the appearance of an abdominal mass, only grade 1 and 2 rejection was found on histology. There was no severe rejection noted in this group.

Group 12 (ALS + Cys) also showed an heterogenous pattern of rejection, ranging from grade 1 to grade 3. In the last three groups (13,14,15, which all received ALS, DST, and Cys), there was discrepancies in the level of rejection between animals but most of the donor mesenteric lymph nodes showed lymphoid depletion accompanied by fibroblastic proliferation and fibrosis replacing most of the nodes. The native nodes were intact. These findings resemble somewhat the vascular proliferation or Kaposi sarcoma seen in immunosuppressed patients and AIDS patients.

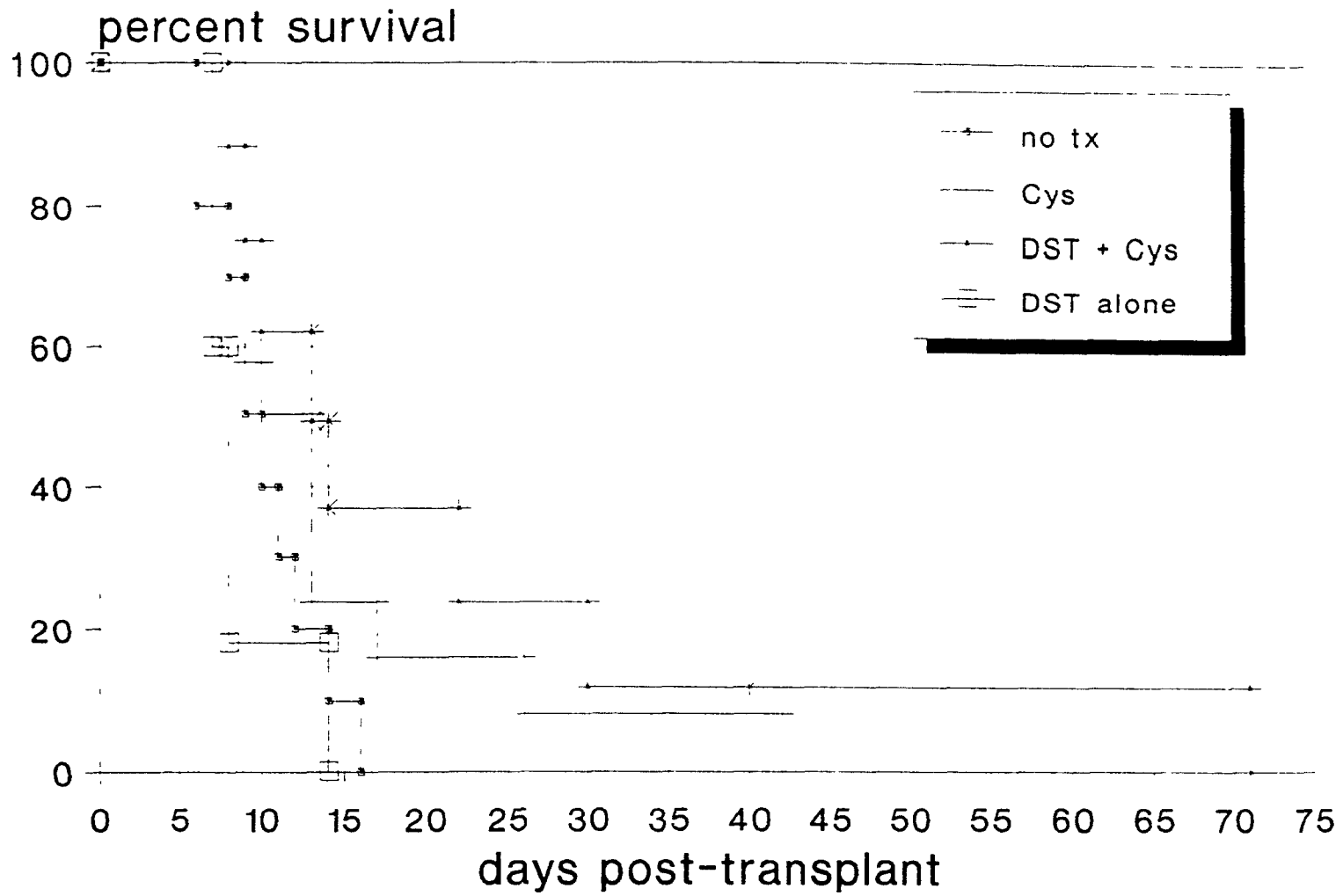
GVHD

Only two animals in this entire project suffered from GVHD. In the fully allogenic model in the rat, rejection predominates over GVHD[17]. GVHD is not usually observed in this model.

The first animal to suffer from GVHD was in Group 8 (multiple systemic DSTs, induction with 5 mg/kg of cyclosporin which was continued for 28 days). It was observed to have what could be classified as a grade 1 GVH at day 8 and 9: mild redness of ears snout and paws, without hair loss or diarrhea. The animal recovered fully after two days. Its graft was found to be rejected at 14 days. The second animal, from Group 12 (ALS plus cyclosporin) had a very similar course. It also had grade 1 GVH at post-operative day 9 and 10, after which it fully recovered. This animal's graft was found to be rejected at 16 days. Both animals were found to have large spleen at autopsy.

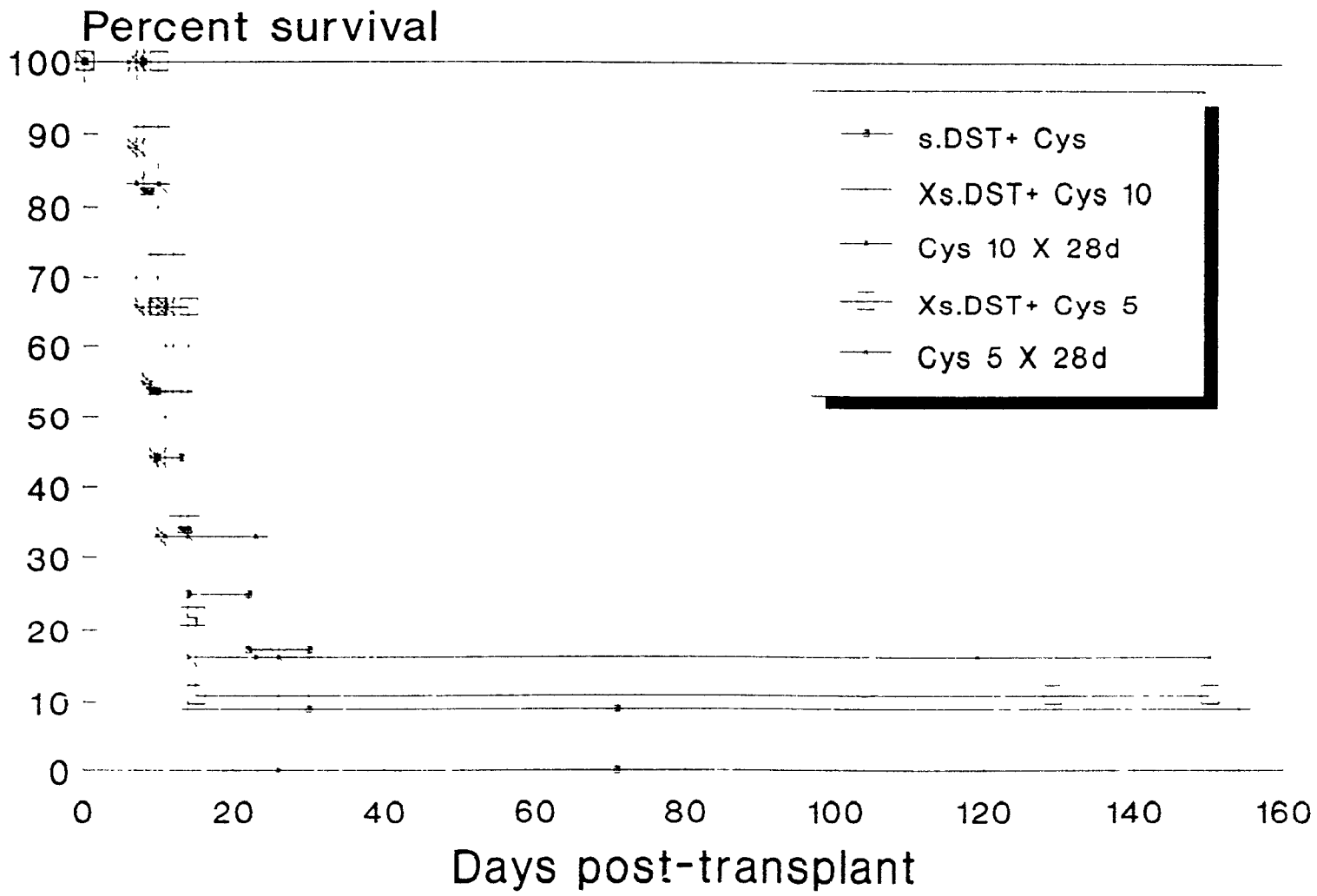
It could be said that both protocols modulated the immune system in such a way as to unbalance the usual rejection-GVHD equation in favour of GVHD. It is surprising that this unbalance did not prolong these animals' graft survival. They both rejected at the mean survival time for their group.

Experiment 1



graphic 1

Experiment 2



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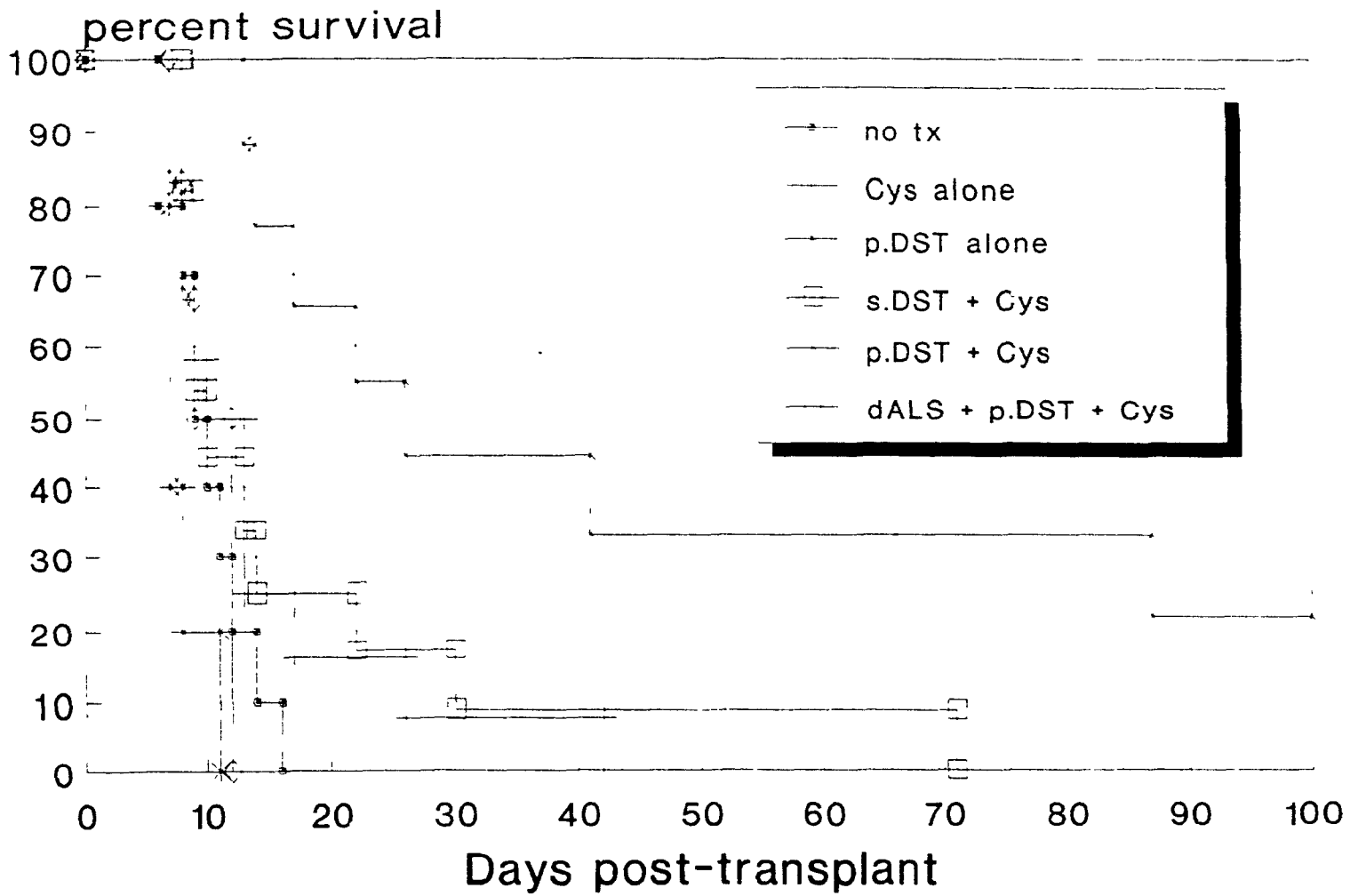
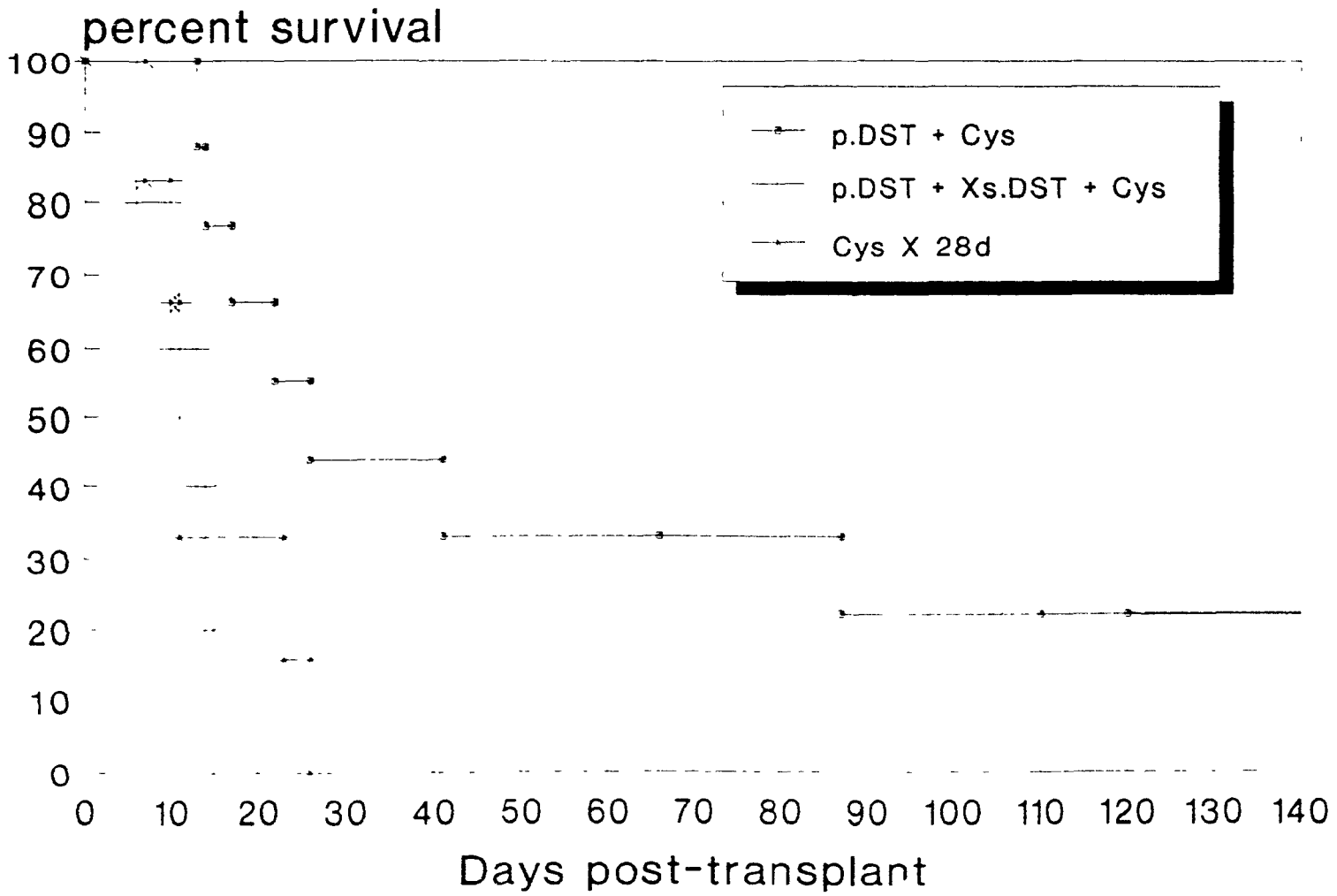
Experiment 3



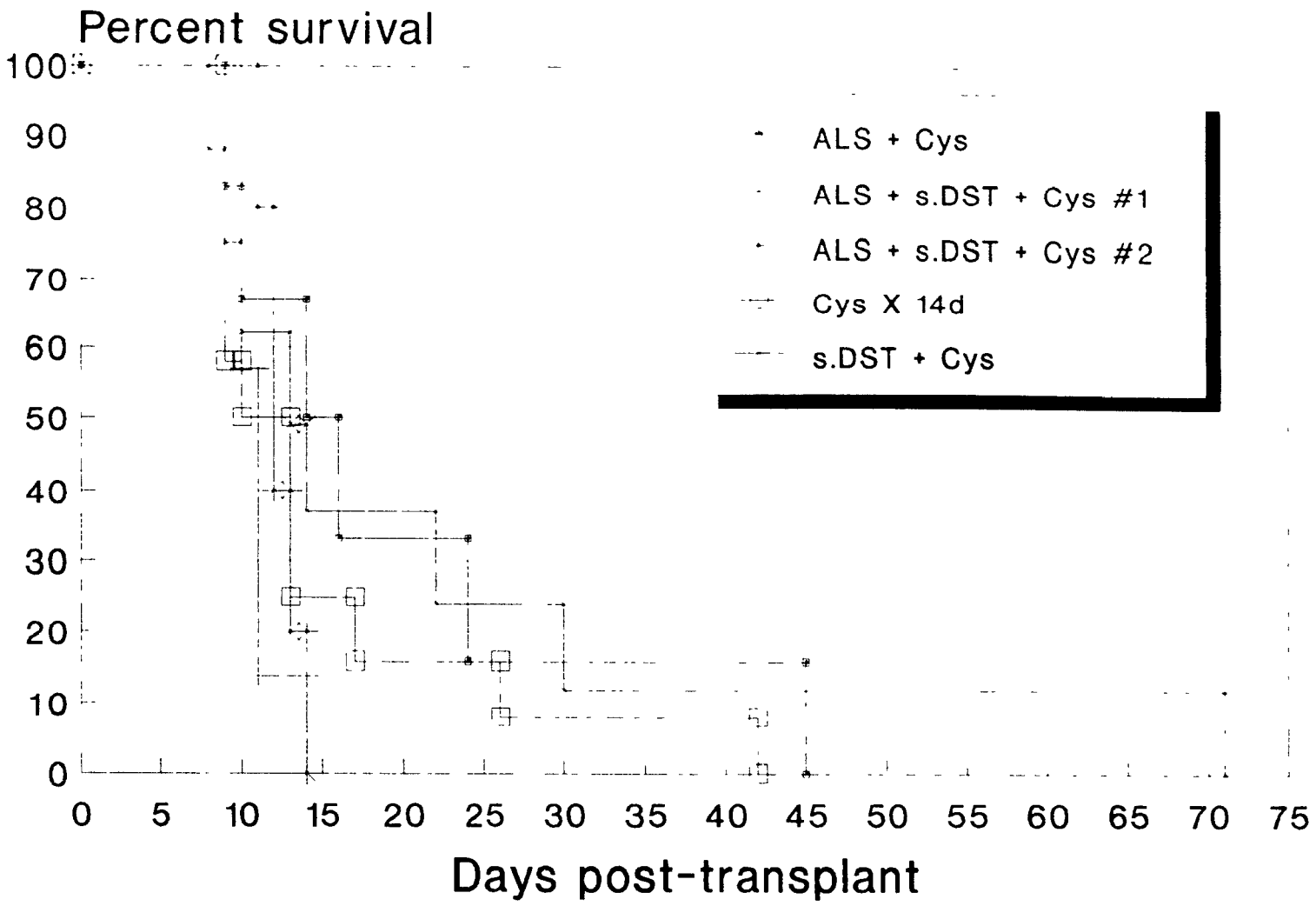
graphic 3

Experiment 4



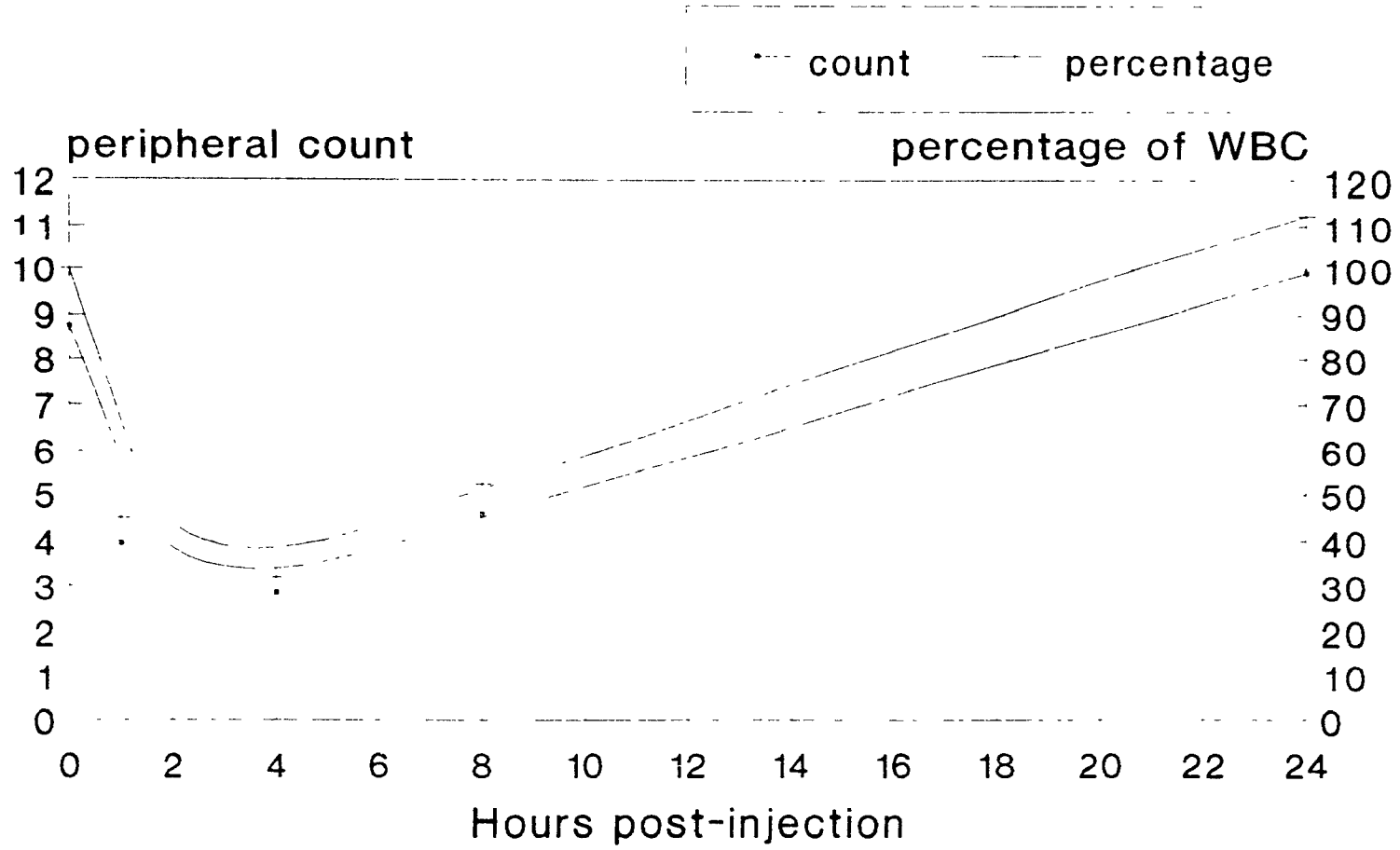
graphic 4

Experiment 5



graphic 5

Peripheral lymphocyte count after ALS injection 1:20 versus time



graphic 6

DISCUSSION

Experimental models of small bowel transplantation

Because the total number of reported human small bowel transplantation (SBT) in the world literature is small, most of our knowledge comes from animal experimentation. In reviewing these studies of bowel transplantation, it is important to bear in mind some of the variables of the experimental models used. There is a marked variation in the immune response elicited by small bowel transplantation in different animal models. Inbred strains of rats have a limited rejection response while outbred strains of a higher order of animals such as pigs and dogs demonstrate much more vigorous reactions.

Bowel transplantation was first attempted by Carrel in 1901[3], who transplanted portions of small intestine into the neck of dogs. But small bowel transplantation was really proven technically feasible in dogs by Richard C. Lillehei in 1959[4]. Lillehei and his colleagues demonstrated that the intestine could survive complete vascular occlusion and autotransplantation, and function adequately post-operatively. The procedure described consisted of transplantation of the entire small bowel with the vascular anastomosis performed between the superior mesenteric artery and vein of the graft

and the respective vessels of the recipient. Dogs receiving autotransplanted bowel survived indefinitely and allograft recipients died of rejection.

The rat model, the most used model even though technically difficult, offers many advantages to the study of small bowel transplantation. The rat heterotopic model was first described by Monchick and Kussell[17] who transplanted the entire small bowel as an accessory graft (Thierry-Vella loop). With the use of inbred parental strains (Lewis and Brown-Norway) and F1 hybrids (Lewis X Brown-Norway), this group demonstrated that the process of rejection could be dissected and studied separately from GVHD. These hybrid rats possess major histocompatibility antigens of both parents and therefore cannot recognise Lewis or Brown-Norway parenteral tissues as foreign. Thus when F1 were used as donors for either Lewis or Brown-Norway recipients, graft rejection occurs without GVHD. Similarly when Lewis or Brown-Norway intestine is transplanted in an F1 recipient, GVHD develops but not rejection[14]. When a fully allogenic model is used, i.e. Brown-Norway to Lewis, a two-way reaction is possible however, rejection predominates over GVHD.The rat model has now become a standard tool for the investigation of small bowel transplantation immunology.

Kort et al.[29] reported a method for orthotopic total small bowel transplantation with vascular anastomosis between the mesenteric artery of the graft and the recipient's aorta

and between the portal vein of the graft to that of the recipient.

Both models, heterotopic or orthotopic small bowel transplantation have advantages and disadvantages. The heterotopic model allows repeat histologic sampling but the animal's survival cannot be equated to the graft survival as the animal is not dependant on the transplanted graft for its nutrition. The orthotopic small bowel transplantation model, while having a higher operative mortality rate, is more pertinent to the clinical situation as the recipient's survival and well being depends directly on a normally functioning intestinal graft.

After the introduction of cyclosporin in the late 1970's, the orthotopic model of bowel transplantation in the dog was first used to assess its effects on small bowel transplantation[30,31]. Even though Reznick et al.[30] demonstrated significant prolongation in graft survival, their overall rate of success was low. The variability in the immunological differences between animals makes the interpretation of survival data with immunosuppression difficult[30,32,33].

The pig model mimics more closely human physiology and has a more defined genetic background than the dog. Ricour and his group were the first to achieve a successful small bowel transplant in the pig and achieve allograft survival[34]. The technical failure rate in this model is relatively high, the

most common problems being intussusception of the transplanted loop, arterial thrombosis, and late sepsis[35]. Both heterotopic and orthotopic models have been described using either portal or systemic drainage[34,35].

History of clinical small bowel transplantation

Clinically, intestinal allotransplantation was first attempted by Lillehei in 1967[36] in the case of a 46 year old white woman with infarction of her entire intestine. The bowel of a cadaveric donor was anastomosed by an end to side superior mesenteric artery and vein to the iliac artery and vein. The patient died of shock two hours after surgery. Okumara and associates[36] reported the second case of SBT also in a patient suffering from superior mesenteric artery thrombosis. The patient died on post-operative day number 6 after the graft had become necrotic. In 1968, Oliver and al.[36] performed an orthotopic intestinal transplantation in a 35 year old patient suffering from polyposis and mesenteric fibromas whose bowel was resected from jejunum to the transverse colon. The patient was treated with AZA,corticosteroids and equine ALG. Rejection was recognized two weeks after the surgery and six-mecarptopurine was then given instead of the AZA but on the twenty-sixth day post-operatively the patient died of septic shock with a necrotic bowel.

The first paediatric SBT was attempted by Alican and Hardy[36] in 1970 in a 10 year old white boy who had strangulated his entire small bowel. After a TPN treatment of four months, the child's mother donated one meter of ileum which was anastomosed to the aorta and the left renal vein. The patient was put on a regimen of AZA, prednisone, and ALG. The graft had to be removed on the seventh post-operative day and the patient was restarted on TPN. He died three weeks later of continued abdominal sepsis and gastrointestinal bleeding.

The fifth reported case was performed by Fortner et al. in 1970[37]. The patient was a 37 year old woman who had undergone massive resection for multiple intestinal polyposis. The patient's HLA-identical sister donated 1.5 meter of lower jejunum and upper ileum. Parenteral AZA and prednisone were used as immunosuppressants. The patient tolerated normal low fat diet two months after the surgery. The patient died on the seventy-ninth post-operative day after suffering from E.Coli septicemia.

Fifteen years elapsed before SBT was attempted again. The advent of cyclosporin rekindled the interest for SBT. The first trial took place in Toronto by Cohen and his group[38] in a twenty-six year old female patient who had undergone resection of her entire small bowel because of a large desmoid tumor secondary to Gardner's syndrome. The patient died at ten days from probable cyclosporin toxicity after having suffered from haemolytic anemia and the beginning of a rejection

episode. Ricour et al. reported having kept several patients alive for more than two years on enteral feeds but with ongoing problems of rejection eventually. Grant and al.[39,40] transplanted a small bowel in a eight year old girl with short gut syndrome who had developed life-threatening problems with limited venous access and early cirrhosis while on TPN. The donor was pre-treated with the monoclonal antibody OKT3. The patient was maintained on continuous high dose intravenous cyclosporin, with cyclosporin levels maintained in the therapeutic range. She also received ALG and AZA until the sixth post-operative day. Tube feeding were started on post-operative day number three but on the sixteenth post-operative day the graft had to be removed because of peritonitis secondary to rejection. The child recovered.

Two recent reports from Williams et al[41] and Starzl et al.[42], described four cases of multiple organs transplantation combining liver, intestine, pancreas and stomach. Williams attempted his first splanchnic transplantation in a seventeen month old male who lost his entire small bowel from the duodenum to the splenic flexure as a complication of gastroschisis. The child was diagnosed with severe cholestasis and cirrhosis from TPN. The stomach, pancreas, the entire small bowel and liver of a six month old donor were transplanted after being irradiated with 10 Gy. Implantation of the composite graft was performed with the intestine being placed in parallel with the remaining

recipient's bowel so that it could be removed and the liver retained if rejection became uncontrollable. The patient died on the forth post-operative day from severe hypotension. At laparotomy a two millimetre defect was found at the aorto-aortic anastomosis.

The second recipient of a splanchnic transplant was a ninth month old boy who had suffered from mid-gut volvulus and infarction of his entire jejunum,ileum, and ascending colon shortly after birth. The donor was pretreated with OKT3. The implantation was performed in a similar manner. On the twelfth post-operative day the patient arrested from acute pericardial tamponade but was successfully resuscitated. He also underwent two subsequent laparotomies for peritonitis secondary to perforation which were closed. Enteric feedings were begun on the fortieth post-operative day and were at full strength three weeks later. Throughout this time cyclosporin was given intravenously with methylprednisone. On the seventy-third post-operative day the diagnosis of a lymphoproliferative disease confined to the liver was made. In spite of cessation of immunosuppressive therapy, the child died of sepsis on the one hundred and ninth post-operative day.

Starzl's experience was similar[42]. His first patient, a six year old girl, died shortly after surgery from hypotension. An exsanguinating haemorrhage had been continuous throughout her surgery. His second patient, a three and a half year old black girl, had suffered perinatal volvulus,

requiring massive resection and had developed liver failure secondary to TPN. She underwent transplantation of the distal stomach, small bowel, liver, and pancreas. The donor had received OKT3 prior to harvesting. Enteral feeding were started two weeks after transplantation and progressively increased. Cyclosporin was given intravenously or enterally and blood levels were maintained in the therapeutic range. The patient was also given a one week course of OKT3 and a prophylactic course of 4.5 Gy of total irradiation. The patient lived one hundred and ninety-two days. She was also diagnosed with a lymphoproliferative disease on postoperative day ninety-one. When immunosuppression was stopped the lesions underwent total necrosis but by day one hundred and sixty-five a new hilar mass had appeared causing obstruction. The obstruction was partly relieved with catheter drainage but sepsis, cardiovascular collapse, and multiple organ failure eventually followed.

More encouraging results were reported at the last International Symposium on Small Bowel Transplantation that was held in London,Ontario in October 1991. Tzakis from the Pittsburg group[43] reported 5 successful SBT, one isolated graft and the others in continuity with a liver. Three were children and two were adults. All five patients were alive on complete enteral alimentation with a median follow-up of 301 days. Several episodes of fungal and/or bacterial translocation were documented and successfully treated in 3 of

those patients.

The London, Ontario group reported 3 out of 6 successful SBT. Two of these were liver and intestinal transplant and the third one was a cluster (stomach, liver, duodenum, pancreas, jejunum, and ileum) transplant[44].

Goulet and Revillion[45] reported the case of a baby girl who suffered total volvulus at birth and was transplanted at the age of five months with the bowel of a iso-blood group O, HLA-mismatch anencephalic neonate. On an initial regimen of quadruple immunosuppressive agents (prednisone, ALG, cyclosporin, and azathioprin), she was maintained on cyclosporin and prednisone. She suffered two episodes of rejection which were successfully treated with OKT3 or ALG. At two years post-transplant, the little girl is totally enterally fed. This group (Ricour-Paris-Hôpital des Enfants Malades) has the largest experience in children, having carried out 11 SBT in 9 children. Deltz et al. have one adult now 3 years post transplantation of isolated small bowel graft doing well. At the recent ACS meeting (October 1992), Tzakis reported that the Pittsburg experience is now up to 29 intestines or liver-intestine allografts.

Small bowel transplant immunology

The small bowel allograft is unique among vascularized

grafts, being a solid organ carrying its own immune system. Similarity can be observed between small bowel transplantation and bone marrow transplantation as both can induce rejection and GVHD.

Investigation of small bowel immunity has revealed an abundance of lymphoid tissues called gut associated lymphatic tissue (GALT)[6].As the largest accumulation of lymphoid tissue in the body, GALT may explain why small bowel transplant is still at the experimental stages. GALT comprises mesenteric lymph nodes, Peyers patches, lymphoid nodules in the lamina propria, and scattered lymphocytes in the lamina propria and epithelium.

The lymphocytes in the epithelium are predominantly T suppressor (Ts) lymphocytes, while those in the lamina propria are predominantly of the T helper (Th) category. Small intestinal epithelial cells express Class II MHC antigens which represent a restriction element in the T cell dependent immune responses. This renders the small intestine more immunogenic compared to other graft as the epithelial cells can act as antigen presenting cells [6].

Peyers patches are macroscopic clusters of lymphoid cells, usually 12-15 in number, found in the serosa of distal small bowel. Peyers patches contain a higher proportion of B cells than peripheral lymph nodes,predominantly lgM bearing cells. Peyers patches are also greatly enriched in Th cells but also contain Ts and Tcs (countrasupressor) which appear to

potentiate the immune response to orally presented antigens. Macrophages and dendritic cells are present in the dome region of the patches and are competent at presenting antigens. Each patch is covered by a matrix of cells including cuboidal epithelial cells, which also express Class II MHC antigens, and microfold cells (M cells) which pinocytose and phagocytose antigens and transport them to the underlying lymphoreticular structures [6]. There the antigens are presented to B cells, macrophages, Th cells and to a lesser extent to Ts and Tcs cells. Some B cells can mature into plasma cells which synthesise a specialized immunoglobulin unique to seromucous secretions (saliva, tracheobronchial secretions, genitourinary secretions,colustrum, and milk) called secretory IgA. Following antigen exposure, Th and B cells which are committed to IgA synthesis are generated in the Peyers patches. They travel to the thoracic duct, entering the circulation before preferentially homing to mucosal surfaces. A feedback control is also exercised by the relative proliferation of Ts compared to Th cells, inducing tolerance. This can be abolished by the induction of Tcs cells.

The lamina propria also contains cells whose functions are less well defined but seem involved in cell mediated cytotoxicity unrestricted by MHC. Natural killer cells, which represent 2-3% of dispersed lamina propria cells, have limited cytotoxic activity but may mediate antibody-dependent cytotoxicity. Mucosal lymphokine activated killer (LAK) cells

are highly cytotoxic cells activated by the exposure to the cytokine IL-2 [6].

Small bowel transplantation interferes with the immune system in an intricate way. First, the immune tissues of the graft are affected by the manipulations involved with the surgery: removal, preservation, and reimplantation of the graft. The resection of the graft from the donor involves the destruction of the proximal lymphatics of the bowel and cisternae chyli. The graft is left with no lymphatic drainage until there is a regeneration and the continuity is re-established with the recipient's lymphatic system. Kocandrle and colleges have used lymphography to demonstrate that lymphatic regeneration requires 21-28 days following intestinal transplantation[46]. However in the long run they remain relatively attenuated and contract less compared with native lymphatics[47]. The immune sequelae of intestinal ischemia, preservation damage, and splanchnic denervation remained to be studied.

Rejection

Rejection predominates in most species in small bowel transplantation, being stronger and occurring earlier than GVHD. One can observe two forms, acute and chronic rejection. Following allogenic transplantation in an untreated host, acute rejection seems to be directed at the vascular

endothelium. Clinically this manifests as bloody diarrhea, progressive inanition and cachexia which begins at about post-operative day 7-8 in the rat and dog models. This course is accompanied by progressive weight loss and culminates in the recipient's death. The immunopathologic and microscopic features have been well described by several authors[17,48,49,50] and may be divided in three phases. Early changes may be confused with those of ischemia secondary to transplantation and handling, but these are corrected by day three.

Phase I begins with rapid graft infiltration by host lymphocytes within 24 hours of transplantation as shown by Lear and colleges with strain specific monoclonal antibodies staining using an indirect immunoperoxidase technique[51]. Endothelial and crypt cell damage begin at day 3 as confirmed by ultrastructural and electrophysiologic techniques[48]. Endothelial cells are enlarged and are associated with an increased number of intravascular lymphoid cells. There is a vascular lesion evident affecting the arterioles and venules at the junction of the mucosa and submucosa. An initial pericapillary aggregation of neutrophils is followed by a build up in lymphocytes in the lamina propria on the sixth day[49,50]. At day 6, an histopathologic evaluation also shows some villus shortening but the villus epithelial cells are morphologically normal with prominent brush border; the crypts are prolonged with extensive cell damage[47].

Phase II begins around day 8 with intensification and

extension of the infiltrate into the submucosal and muscular layer. Villi are blunted and epithelial cells have lost their brush border and begin to slough. Vascular lumens become occluded[52].

Phase III starting about day 10 marks the end of the graft survival with complete mucosal sloughing, heavy transmural lymphocyte and neutrophil infiltration, and serosal inflammation consistent with peritonitis. These changes, ending in mural fibrosis occur similarly in the jejunum and ileum. The native gut is spared.

Lear and his group[51] demonstrated that there was a simultaneous two-way migration of host and donor lymphocytes in the early post-transplant period. Emigration of graft cells were from and almost exclusively within T cell zones of the spleen, Peyers patches, and mesenteric lymph nodes of the host. In non-immunosuppressed animals, the number of graft-derived lymphocytes in host tissue increased daily until day 4, but then decreased rapidly from destruction of lymphoid tissue of the graft.

Multiple factors have been shown to influence the rejection process in small bowel transplantation. Studies have been done to evaluate the effects of length and site of origin of small bowel grafts on the immunologic response[53,54,55]. Kimura showed, in an heterotopic model, a direct relationship between the severity of rejection, but within the same time

frame, and the length of small intestine transplanted. The same was shown to be true for GVHD[53]. Starzl on the other hand, using an orthotopic model, could not demonstrate that the size and origin of the graft (ileal or jejunal) influenced the severity or rapidity of rejection. This study showed that the decreased amount of lymphoid tissue in jejunum segmental grafts did not diminish the effect on survival or rejection in comparison with the effect of grafts having a higher lymphocyte content[55].

Venous drainage of the graft through the portal vein in a physiologic manner was thought by some to delay rejection due to an hepatic filtration or alteration of antigens originating from the graft[56,57]. Schraut concluded from his heterotopic experiments that portal drainage, when associated with splenectomy, decreased the capacity of the host to reject the allograft[56]. Eventually all recipients suffered from chronic rejection. Shaffer et al. reported no significant survival difference between the two drainage techniques, using both an orthotopic and heterotopic model, nor any difference in growth and metabolism in the orthotopic model[58]. They also argued that even though portal drainage would restore normal anatomy, it may be technically more difficult in patients who have undergone multiple abdominal surgery. Schraut later changed his opinion on the subject, agreeing with Shaffer that portal drainage does not confer an immunological benefit.

Chronic rejection occurs after variable periods of

immunosuppression and there is a debate as to whether it is ever fully eliminated in higher order animals. Some would say it is an ongoing subclinical reaction which is suppressed but not eliminated by cyclosporin [59,60,61,62]. In chronic rejection the primary target appears to be the vascular structures[60]. Light microscopy examination reveals a patchy perineural infiltration of lymphocytes and plasma cells[59,60,61]. The mucosa remains normal until the endstage. Jejunum and ileum are affected equally.

Graft versus host disease

Graft versus host disease (GVHD), mediated by passenger T lymphocytes in the graft[63,64], is unique to small bowel among solid organ transplants, although it is also seen in bone marrow transplantation. Small bowel allografts fulfil the three requirements for the appearance of GVHD: (1) the graft is comprised of lymphoid tissues capable of engaging in an immune response; (2) the host possess antigens different from donor tissues; (3) the host is unable to reject the donor[65]. Proven to occur in rats[17] and alleged to occur in dogs[4,66], GVHD has been very hard to demonstrate in clinical experiences. Rejection and GVHD counterbalance each other and it may therefore be the strength of rejection which limits GVHD in higher order models, rather than an absence of GVHD.

GVHD induced by small bowel allograft follows a predictable course. It involves primarily the skin, the host

lymphatic tissues and the host own intestine. In an unidirectional heterotopic GVH rat model (Lew → LBNF1) the first manifestations of the disease appear at 9 to 11 days post-operatively with redness and swelling of ears, snout, skin around eyes, and paws. The skin becomes dry and scaly and is accompanied by hair loss. The animal suffers from diarrhea. The terminal stage, from the 11^{th} to the 16^{th} post-operative day, sees the animal emaciated, cold, listless, sitting hunched on their hind legs. The weight loss becomes precipitous in the last few days. In an orthotopic model, the animal follows the same course of events. At autopsy, enlargement of the spleen and lymph nodes of the host, with thinness and hyperaemia of the host's bowel wall, contrasts markedly with the normal looking allograft bowel. All the animals show sign of peritonitis and generally had perforated their cecum or small bowel[67].

Histologically, at the ninth to the twelfth day,the host bowel reveals a picture very similar to early necrotizing enterocolitis with decrease villus height, sloughing of the villi tips and marked polymorphonuclear infiltration of the mucosa and submucosa. At the fourteenth day there is fulminant necrotizing enteritis[67].

High GVH reactions can be observed in all lymphatic compartments except in the Peyer's patches of the graft and the recipient's bowel. There is a strong expression of GVH reaction within the mesenteric lymph nodes of the semi-

allogenic graft. Immunoblast and epitheloid cells in the paracortical area proliferate[68]. At post-operative day 5 the Peyer's patches and mesenteric lymph nodes undergo a progressive lymphoid depletion, giving rise to a progressive lymphopenia with disappearance of germinal centres and loss of distinction between the cortex and the medulla. The lymphatic tissues of the host undergo a similar course of progressive lymphoid depletion and loss of normal follicular architecture[69]. In the spleen, the loss of lymphoid cells was parallel to the appearance of reticuloendothelial cells that differentiate into histiocytoid cells.

In addition to the small bowel, the skin of the recipient is the main target of anti-host reactions. Microscopic examination of the skin in the unidirectional model shows dyskeratosis, vacuolization of basal cells, and necrosis of keratinocytes. GVHD is immunosuppressive. Thymus and spleen show loss of normal architecture with blurring of the corticomedullary zone and follicular loss respectively. Concomitant suppression of the host humoral and cell-mediated immune response closely correlate with these changes in architecture[69]. Lymphoid organs, skin, liver, colon, and salivary glands, but not kidney and pancreas, are infiltrated with immunoblasts[70].

Studies to determine the mechanism of GHVD have focused on T lymphocytes. Kirkman[71] in 1984 showed that GVHD requires competent donor T cells: a T-cell depleted donor rat could not

induce GVH unless reconstituted with T cells prior to small bowel transplantation. Wallender[64] supported Kirkman's observations, being unable to demonstrate any GVHD in immunocompetent rats transplanted nu/nu rat intestine (phenotypically T cell deficient). Deltz found that the mortality with clinical appearance of a wasting disease was dependent on the quantity of grafted lymphoid tissue, shorter graft eliciting less GVH reactions than longer ones[70].

Other studies of GVHD in the bone marrow model emphasize the importance of effector lymphocytes of donor origin, which are large and granular[72], and natural killer cells of recipient origin, which are active in areas of tissue damage[73]. These studies await corroboration in small bowel transplantation models.

GVHD has been shown to occur in long term survivors of fully allogenic small bowel transplantation. Diflo hypothesised that, because rejection is a much stronger reaction in the fully allogenic rat model, it could mask signs of GVHD. Effective prevention of rejection with cyclosporin A in recipients of fully allogenic small bowel graft would permit the development of a sublethal form of GVHD. The rats developed diarrhea, dermatitis, and weight loss four to six weeks post-transplantation but recovered[74,75].

Donor specific transfusions

Pre-transplant donor-specific transfusions (DST) have had a definite beneficial impact on allograft survival in man. In 1973 Opelz and Terasaki[27] were the first to demonstrate the beneficial effect of third party transfusions in cadaveric renal transplant recipients. A more dramatic effect on allograft survival was obtained with deliberate donor specific blood transfusion in 1-haploidentical living-related renal recipients. In 1980 Salvatierra reported that donor-specific transfusions at 6, 4, and 2 weeks pretransplant in one-haploidentical living related renal recipients,improved the one year allograft survival to 94% compared to 56% in non DST treated recipients[28] . In a latter study, Salvatierra showed that one-haploidentical living related renal recipients that had received 3 DSTs pre-transplant had a comparable 1 and 3 year allograft survival rate and creatinine level to HLA-identical living related renal recipients[76]. However in these studies there was a substantial rate of sensitization to the donor (15%), which precluded the best form of renal transplantation in these patients. The risk of sensitization of prospective transplant recipients by DST has been significantly reduced by the administration of immunosuppressants concomitantly with blood transfusion while maintaining improved renal allograft survival[77]. In fact, studies have demonst rated that DST and cyclosporin are more beneficial together than DST alone[78]. In a more recent

report, Cheigh and his group examined the clinical efficacy of DST and a short course of cyclosporin in recipients of one- and zero-HLA-haplotype-matched renal allografts[79]. They concluded that stored whole blood DST, three times at weekly intervals, with a short course of cyclosporin was minimally sensitizing (4%) but effective in enhancing graft survival even in donor-recipient pairs who did not share a haplotype.

The efficacy of pre-transplant antigen presentation to improve allograft survival has also been investigated in mice and rats; renal, heart, skin, pancreas, and liver allografts. Prolongation of renal allograft survival in rats by pre-transplant antigen presentation has been reported by several investigators. Marquet reported an improved renal graft survival to a mean survival time of 100 days with 0.05 ml of fresh donor blood given from 1 to 2 weeks prior to transplant[80]. When further challenged with a donor-type skin graft, the recipients showed marked prolongation of the skin graft but rejected a third party graft in the normal way. Homan, using splenocytes as the pretransplant antigen presenting agent, showed that he could specifically prolong DA renal grafts in Lew rats after the recipient's pretreatment with 14 days of cyclosporin and two transfusions of 10^8 spleen cells[81].

The effectiveness of DST is most marked in prolonging cardiac allograft survival. Using the same protocol as for the renal allograft , Marquet was able to indefinitely prolong the

survival of cardiac allografts[80]. DST and cyclosporin were shown to be effective in heart transplantation protocols with several changes in timing and dosage[16,82]. The DST effect was also proven to be strain specific in a rat cardiac model: using third party blood (buffalo), no enhancement in allograft survival was demonstrated in an ACI to Lewis model[22].

In skin transplantation, some studies have shown positive results while others have shown no protective effect of pretransplant DST. Marquet showed no effect with a 2 ml pretransplant transfusion 2 weeks to 1 month before transplant[80]. Yamagushi,using splenocytes (3 $X10^6$) given 7 days before transplant, could not improve skin graft survival in a ACI to Lewis combination[83]. In Lehnhard's experiment however, BN or (Lew X BN)F_1 skin grafts survived significantly better in multiple transfused Lew rats than in nontransfused animals[84]. Skin allograft survival was also significantly improved in a strongly incompatible mice combination (DBA/2 to $B6AF_1$) after 4 pre-transplant DSTs combined with anti-lymphocyte serum[85].

Multiple DSTs had no additive effect over cyclosporin in a pancreatic graft survival experiment[86] nor did it show any effect when combined with anti-lymphocyte serum even in weak histocompatibility barrier[87].

The effect of pretransplant antigen presentation was studied in hepatic allograft transplantation with transfusion of splenocytes. Yamaguchi, injecting 3 X 10^6 mitomycin C

treated splenocytes on day -7, was able to prolonged survival of hepatic allografts to >78.9 ± 28.2 days compared to 10.0 ± 4.3 days in control[83]. A group from Changai Hospital was able to induce indefinite survival with the injection of 5 $X10^7$ donor specific splenocytes 7 days before transplantation with the administration of 15mg/kg/day of cyclosporin for 5 days[88].

Only a few investigators have studied the effect of DST in small bowel transplantation, and none have studied the effect of DST at day-1, a protocol clinically relevant to cadaveric transplantation. Four studies have been published using pretransplant antigen presentation as a mode of preventing rejection. Two of these used splenocytes, rather than whole blood. One study from the Netherlands investigated the role of DST as a way to prevent graft versus host disease.

Martinelli and his group first published a study using ACI rats as blood and bowel donor and Lewis rats as recipients[12]. They transplanted 10 cm or 30 cm of bowel orthotopically. The Lewis rats were preconditioned with 1.5 ml of freshly drawn blood 8 days prior to surgery and a concurrent course of intramuscular cyclosporin at a dose of 10 mg/kg/day from day -8 to day -4 and at 2.5 mg/kg/day from the day of transplant to day 30. In the first part of the experiment they compared the effect of donor specific versus nonspecific transfusion on host survival after a 10 cm SBT. Five groups were included in the design of the study: Group 1

had a donor specific transfusion and cyclosporin, Group 2 had third party (Buffalo) transfusion and cyclosporin, Group 3 received only cyclosporin without transfusion, Group 4 were transfused ACI blood but did not receive any cyclosporin, and Group 5 received no treatment at all.

The pretransplant administration of DST alone had no apparent effect on host survival. The administration of cyclosporin alone had a modest but clear effect on survival, increasing survival to 18.3 ± 5.7 days compared to 7.7 ± 1.8 days for untreated controls. The effects of DST in animals treated with cyclosporin were vastly different from the ones observed with nonspecific transfusions. Recipients conditioned with DST and cyclosporin survived an average of 60.3 ± 36.2 days. In contrast the average survival of the animals receiving nonspecific transfusions was only 14.1 ± 5.8 days. The results of the experimental group 1 differed from all controls at the $P<0.001$ level.

In the second part of the experiment, the authors wanted to assess whether the DST-cyclosporin immunosuppressive protocol was effective in prolonging the survival of SB allograft recipients entirely deprived of their native SB and sustained solely on the orthotopic allograft. They transplanted only 30 cm of bowel, as it was found to be the minimum length of SB compatible with the maintenance of acceptable nutritional status. The recipients received the same cyclosporin protocol as in the first experiment. These

recipients of orthotopic SB allograft survived an average of 90 ± 43 days . The untreated controls had a mean survival of 9 ± 3 days. They concluded that DST-cyclosporin conditioning effectively reduces the requirement for intensive immunosuppressive therapy but that the present protocol was feasible only in the event of the availability of living donor. They felt that perioperative conditioning with DST and cyclosporin should be investigated.

De Bruin et al. arrived at the opposite conclusion[13]. They studied the effect of three DST given on days -21, -14, -7 before transplantation in a fully allogenic model of Brown-Norway donors to WAG recipients. An orthotopic transplantation was performed using either 10 cm of proximal jejunum or 10 cm of distal ileum. Cyclosporin was administered intramuscularly at a dosage of 5 mg/kg on day 0, 1, 2, 4, and 6 after grafting. They compared the graft survival with the three DSTs in total small bowel, jejunum, and ileum. None of these groups showed improved survival. In their last two groups, they compared the survival of a total small bowel graft with cyclosporin alone versus the combination DST-cyclosporin. The cyclosporin control group had a mean survival time of >79.6 ± 70.3. The DST-cyclosporin group had a mean survival time of >113.3 ± 95.0, but this did not differ significantly from the cyclosporin only group. They surprisingly observed significantly fewer rats in the DST-pretreated groups showing signs of GVHD as compared to the control groups. This finding,

they advocated, implied that DST may have induced some form of immunosuppression, reflected in the absence of GVHD, but not in the survival time.

Yamaguchi also investigated the effect of an intraperitoneal injection of 3×10^6 mitomycin treated donor-specific splenocytes 7 days prior to transplantation[83]. He performed an heterotopic transplantation from ACI donors to Lewis recipients. This protocol did not increase the small bowel allograft survival significantly when compared to controls (10.3 ± 4.8 vs 8.8 ± 1.8 days). No other immunosuppression was used in combination with the pretransplant antigen presentation.

Wolf and his group from Hadassah University hospital also used splenocytes in their experiment[89] as the antigen presenting agent. They transplanted the small bowel allograft orthotopically, using a semi-allogenic combination of (LEW X BN)F_1 donors to Lewis recipients. They administered 15 mg/kg/d of cyclosporin subcutaneously for 3 days starting on the day of the spleen-cell injection. They compared the effect of timing of the spleen-cell injection (-14 days vs day 0), the route of injection (systemic vs portal), and the number of cells injected (3×10^7 vs 3×10^8). The day 0 injection group had a modest prolongation of survival compared to the -14 day group (26 versus 20 days, $p<0.05$). The largest cell inoculum significantly prolonged survival but it was the portal inoculation that was the major factor in prolongation

of the graft survival ($p<0.01$). They concluded that high-dose donor strain spleen-cell injection via the portal vein carried the potential for inducing hyporesponsiveness.

Only one published paper attempts to ameliorate graft-versus-host disease in small bowel transplant with DST[14]. In this paper an orthotopic total small bowel transplantation was performed in an histoincompatible WAG to Brown-Norway combination. The pretreatment consisted of three recipient-specific (BN) DSTs to the WAG donor on days -21, -14, and -7 days before the operation. No immunosuppression was given to the recipient. This treatment protocol did not prolong the survival time when compared to a no treatment control but surprisingly, the transfusions induced a more severe graft-versus-host reaction. Fifty percent of the animals in the experimental group developed severe GVH when only mild GVH was observed in the control group.

Mechanism of action

The mechanism of action of the pretransplant antigen presentation has not yet been totally elucidated. Several possible mechanisms have been hypothesised to account for the improved graft survival. The bulk of evidence suggests that the primary mechanism whereby DST enhances allograft survival is through the generation of specific suppressor T-lymphocytes. The production of anti-idiotypic antibodies, the

elimination of reactive clone of cells, and the activation of the arachidonic pathway have also been suggested.

Marquet and Heystek demonstrated the presence of suppressor cells in unresponsive recipients of fully allogenic cardiac transplant who had been preconditioned with 1 ml of donor-specific blood one week prior to transplant[90]. It was found that suppressor cells were present in the spleen and thymus but not in the peripheral blood or lymph nodes. Adoptive transfer of 25 $X10^6$ spleen cells from unresponsive recipients led to permanent survival of donor specific grafts in irradiated but otherwise untreated recipients, and transfer of 25 X 10^8 thymocytes always resulted in permanent graft survival. Fractionation of the suppressor spleen cells into T and B cell-enriched population and macrophages revealed that the suppression was mediated by T cells. Those findings were supported by numerous other studies. Shelby[91] confirms the presence of splenic suppressor cells after transfusion and transplantation, as determined by adoptive transfer studies, during the stable maintenance phase of graft survival in transfused recipients with long-term surviving heart allograft. It was also shown that an intact spleen was required to achieve improved allograft survival in mice preconditioned with a DST. Singh, investigating the role of suppressor cells in the blood transfusion phenomenon, showed that transfusions alone were not capable of evoking a detectable number of suppressor cells and that the suppressor

cells are predominantly induced after the transplantation[92]. These suppressor cells appeared to reside in the Ox8-positive T lymphocyte fraction (Tc/s cells). This finding is controversial as some authors[93,94] have found suppressor cells after one and two blood transfusions. Wakely[94], for example was able to demonstrate suppressor cells in both the inductive (after transfusion) and the maintenance (long-term surviving allograft recipients) phases of transfusion-induced suppression. Inductive phase suppressors, however, were less readily detected: the recipients of adoptive transfer had to be sublethally irradiated to provide a more sensitive assay.

Only a few studies evaluated the other possible mechanisms in the transfusion phenomenon. A group from Duke University published two papers on the development of antiidiotypic antibodies following DST[95,96]. In the first paper[95] they demonstrated that DST alone was found to elicit complement-dependent cytotoxic IgM antibody to donor class 1 alloantigens that peaked at 7 days. Following DST alone, antiidiotypic antibodies were detected in the circulation within 7 to 11 days post-DST, with a reduction in circulating alloantibodies. Donor strain kidney transplantation in the presence of those antiidiotypic antibodies resulted in enhanced graft survival, while transplantation prior to the development of detectible antiidiotypic antibodies resulted in rejection. The antiidiotypic antibodies were predominantly IgM, IgG_1, and IgG_2. Their second paper[96] confirmed these

antibody responses. In autologous blood transfused rats, renal allografts elicited high titers of IgM and moderate titers of IgG in the circulation, and high titers of IgM and IgG in the spleens by 5 to 7 days post-transplantation. These titers could not be detected in DST pretreated recipients. This was reflected by a much lower amount of IgM and IgG antibodies eluted from the grafts in DST pre-treated animals. Because IgM fixes complement and IgG triggers antibody-dependant-cellular-cytotoxicity, the reduced deposition of IgM and IgG in the graft may be of particular importance in DST enhancement.

There is some evidences to support the clonal deletion hypothesis. Markmann[97] demonstrated that antigen specific T cell deletion occurs in adult ALS treated male mice, after the intrathymic injection of lymphoid spleen cells. There is little evidence to support the development of chimerism hypothesis to explain the DST effect. Van Twuyver[11] was able to demonstrate a marked reduction in the number of donor-specific cytotoxic T-lymphocyte precursors in the recipient's spleen and in the graft, after the transfusion of donor spleen cells, when compared to a saline transfusion, in a mice skin transplantation study.At the 1991 Symposium on Tolerance induction, K. Wood suggested that part of the DST effect resided in the fact that the DST resulted in a deficit in IL-2 production and a decreased level of expression of IL-2 receptors. Rejecting grafts express low and high affinity IL-2 receptors, while DST conditioned tolerant grafts express only

low affinity IL-2 receptors. This might be a common pathway to both the antiidiotypic antibody and the suppressor cells hypothesis.

Clonal anergy has also been proposed as a mechanism of induction of tolerance. Clonal anergy represents an unresponsive state of antigen-reactive lymphocytes. It was proposed that this unresponsiveness is due to a failure of Th cells to produce the appropriate second humoral activation signal. T helper would recognise the alloantigen, but neither proliferate nor secrete IL-2.[98]

When studied in human, an impaired cell-mediated immunity following blood transfusions has been observed by several groups[99,100]. While a suppressed donor-specific MLC response has been reported, the group from San Francisco also reported non-specific reduction in the MLC response, suggesting that one possible mechanism for the beneficial effect of DST is an early non-specific reduction in immunologic reactivity[101].

The best way to induce immunological unresponsiveness with pretransplant antigen presentation still has not been defined.

Marquet could not detect any influence on the results if he varied the DST dose between 0.05 ml to 2.0 ml[80] while Homan using spleen cells found that the minimum effective number of cells was 10^8 [81].

Shelby also concluded that the amount of blood was a

critical factor in achieving the transfusion effect[91]. On the other hand, she could not find any benefit of multiple transfusions. In contrast, Johnson found that multiple pretransplant DSTs were more effective than a single DST and that the peak effect appeared after six[82]. In data published by Alexander's group in Cincinnati, it was also demonstrated that tolerance induction was doubled when multiple DSTs posttransplant (on days 7, 14, and 21) were given in addition to the pretransplant DST[24]. Timing of the transfusion is thus also a matter of controversy.

While most authors give their transfusions one week prior to transplant, some authors have demonstrated a specific transfusion effect even at 24 hours pretransplant[102,103,16]. Tchervenkov et al. showed that DST 24 hours pretransplant and cyclosporin had a strong synergistic effect on allograft survival, with 10% of the animals achieving permanent tolerance[16]. This conflicts with results from Duke University, where they found that only grafts transplanted during the phase of antiidiotypic antibody production (7-11 days post-transfusion) had enhanced graft survival[95]. The protocols using a long interval between the transfusion and the transplant restrict the transplant surgeon to the availability of a living donor. The perioperative conditioning with DST and cyclosporin extends the pretransplant immunomudulation to the cadaveric organ situation, when blood samples could be obtained from the prospective donor.

Synergism between cyclosporin and DST

Cyclosporin A (Cys), released in 1983 as an immunosuppressive agent, is a fungal metabolite extracted from soil fungi Tolypocladium inflatum. It is insoluble in water but soluble in ethanol and most organic solvents and lipids. Cyclosporin has been used both alone and with other immunosuppressive modalities for experimental transplantation in a broad range of animal species and in many organs. It is the advent of cyclosporin and its superiority as an immunosuppressant drug that renewed the interest for small bowel transplantation in the early 1980s.

Cyclosporin exerts its effect at a very early stage after exposure of the recipient to a tissue allograft. Once the induction of the immune response has taken place, cyclosporin appears to be relatively ineffective. Pre-treatment before transplantation does not influence graft survival suggesting that Cys can only inhibit lymphocytes after an exposure to the stimulating antigen[104].

Recent evidence suggests that the immune activation requires two stimuli: A) the presentation of the antigen by a macrophage and the production of a humoral costimulator, possibly IL-1 :these two factors are essential to the activation of the T helper/inducer lymphocytes(T h/i). B) the T h/i cell provides the second signals including IL-2 for the T cell arm, the B cell growth factor and the B cell

differentiation factor (IL-5, IL-6), the macrophage activating factor, and gamma interferon. Cys inhibits the up-regulation of the immune response by inhibiting lymphokine production, mostly IL-2 and gamma interferon, thus preventing the delivery of the second signal[105]. Cys has little effect on the expression of surface membrane IL-2 receptors[106].

Cys also disrupts the thymic medulla, thereby interfering with the process by which lymphocytes learn to recognize the difference between self versus non-self[107]. In addition, Cys promotes the production by thymic cells of humoral suppressor hormones, which may down-regulate the systemic immune response[108]. There is a gradual increase in suppressor activity post-transplant, possibly promoted in part by an augmented thymic hormone production and by a thymic medullary disequilibrium favouring maturation of thymic-derived lymphocytes toward the suppressor pathway[109]. Cys does not act directly on T suppressor cells , but Hess et al[110] demonstrated that cyclosporin spares a subset of T helper cell that were shown to amplify the T suppressor lymphocyte activity. Cyclosporin preferentially blocks the activity of the cytotoxic inducer T helper lymphocytes while sparing the function of the suppressor inducer T helper lymphocytes.

Most authors will agree that better survival is achieved when the pretransplant antigen presentation is combined with an immunosuppressive agent. Cyclosporin and donor specific transfusion seems to have a strong synergistic effect. The

immunologic mechanisms contributing to this synergism are not fully known. If one retains the hypothesis that the primary effect of DST is through the induction of T suppressor cells, a possible explanation is that cyclosporin acts exclusively on T helper cells and its ability to produce lymphokines such as IL-2. Cyclosporin seems to shift the immune system toward the production of T suppressor lymphocytes and away from rejection. Together DST and cyclosporin may promote the induction of antigen-specific T suppressor lymphocytes that can eventually induce a tolerogenic effect on transplanted organ[16].

Discussion of results

This research was designed to study the effectiveness of donor-specific transfusion in combination with low-dose cyclosporin in small bowel transplantation. The experiments were designed so that all the protocols would be relevant to both cadaveric and living-related donor, i.e. would not require conditionning more than 24 hours before transplantation.

In experiment 1, the hypothesis that, one DST 24 hours pretransplant in combination with a low dose cyclosporin regimen could effectively abrogate the rejection reaction in a fully allogenic rat SBT model, was tested. A similar experiment had been successful in increasing graft survival in

a rat allogenic cardiac model[16]. The graft survival results are found in Table 1.

There is a difference between the results of Tchervenkov et al.'s cardiac allograft experiment[16] and our results. They achieved a 10% permanent allograft survival in their animals. None of our recipients receiving systemic DST day -1 reached permanent graft survival. Perhaps the small bowel allograft is immunologically more difficult to induce recipient allograft unresponsivness, as it carries a more complex amalgam of immunocompetent cells. The heart only carries a small number of passenger leukocytes. Passenger leukocytes may induce antigen presentation and immune reaction as they migrate to the host and home in the endoreticular system of the host. It might be that the immunomosuppressive effect of a DST and low-dose cyclosporin is much more counterbalanced by the rejection reaction in the small bowel transplantation model than in the cardiac transplantation model.

In experiment 2, an attempt was made to push the immune system further towards tolerance by administrating three successive DSTs in the post-transplant period. Group 7 and 8 were designed to test the hypothesis that a smaller induction dose of cyclosporin (day -1) may be more favourable to the induction of suppressor cells or antiidiotypic antibodies by the DST. The induction dose was reduced to 5 mg/kg in both groups : Group 7 received cyclosporin only and Group 8 received both the cyclosporin and the 4 DSTs.

Survival data for this experiment are found in Table 2. A longer regimen of cyclosporin did not ameliorate the graft survival. Adding three post-transplantation DSTs to the pretransplant DST did not significantly change the mean graft survival time. It was speculated that the three DSTs stil did not counterbalance the immune load that the small bowel transplant represented and that the rejection process had already been engaged and could not be reversed. Two animals in that second experiment acheived long term survival. It may be that those two animals responded in a more tolerogenic fashion to the preconditionnig and that the successive DSTs further enhanced that tolerogenic mode. It could also be explained by the fact that those two animals may have received small bowel grafts which were less immunogenic.

Experiment 3 compared the portal route of antigen presentation to the systemic route.It was also determined if intraportal DST can induce long term survival even when given only 24 hours pretransplant. As early as 1967, Cantor and Dumont demonstrated the importance of the antigens being carried to the liver before they reached either the lymphatic system or the general circulation, for the induction of unresponsivness[111]. Oral feeding of antigens suppressed the formation of specific circulating antibodies, a phenomenon that could be abolished by diversion of the portal flow. It was suggested that the mechanism of such unresponsiveness resided in the fact that the immunological conjugates formed

on the absorption of the hapten into the portal blood coursed through the liver sinusoids where the complexes were phagocytized by Kupffer cells. As a result, the "phagocytozed" portions of the complexes, which are usually taken up by immuno-competent macrophages and induce the immunological response, were instead separated and perhaps permanently deposited in the Kupffer cells, which are incapable of inducing antibody production.

Those observations were confirmed by Triger, Cynamon, and Wright in 1973[112]. They showed that repeated injections of small amounts of sheep red blood cells into the portal vein resulted in lower number of circulating antibodies than did injection of equivalent amount of antigens into the inferior vena cava. They also demonstrated a difference in the delayed hypersensitivity reaction, suggesting that the liver may play a role in the mechanism of cell-mediated immunity.

The abrogation of delayed type hypersensitivity reactions by intra-portal inoculation of antigens was further studied by a group at Osaka university[113]. The group showed that such suppression was alloantigen specific and could be rapidly induced, within 11 days, and was long lasting. The hypothesis was made that an antigen-specific tolerogenic factor was released into the circulation after the processing of allogenic cells in the liver.

Work done at the Royal Victoria Hospital[26] showed that dramatic prolongation of rat cardiac allograft survival was

obtained when donor strain mononuclear cells were injected into the mesenteric portal venous system 7 to 10 days prior to transplantation. No difference was demonstrated in the humoral response between rats inoculated systemically versus portally, nor was a diminution in the number of donor specific cytotoxic T cells in the graft demonstrated. With ^{99}mTc-labelled lymph node cells, they showed entrapment of those cells in the liver after portal infusion. They hypothesised that circulating alloreactive cells contacting alloantigens entrapped in the liver became entrapped themselves leading to a functional clonal deletion. In another study[114], this group determined that 7 to 10 days prior to grafting was the optimal time for intraportal inoculation. This is not confirmed by our findings, as we demonstrated that intraportal DST given 24 hours pretransplant was effective a prolonging small bowel allografts.

In an ACI to Lewis combination, Rao et al. were also able to induce prolonged cardiac allograft survival with the injection of 10 X 10^6 donor spleen cells at day -14[115]. In a second experiment, no difference was found when the portal infusion was done day -7, -21, -28, compared to day -14. Those results differ from the previously mentionned results of Lowry et al.

Working on renal allograft, Yoshimura et al.[25] showed prolongation in graft survival with inoculation of intraportal 1X 10^8 lymphocytes at the day of transplant (day 0). They

could not identify suppressor cells in the spleen of tolerant host receiving donor lymphocytes via the portal vein, although suppressor activity was detected in the serum. This would support the hypothesis of the formation of a suppressor factor being produced by the liver, protecting the allograft from rejection.

Besides Wolf's study[89], no other experiment has tested the effect of intraportal DST in small bowel transplantation, and only two studies have tested intraportal DST in the peritransplantation period[89,25].

The graft survival data of experiment 3 can be found in Table 3. With the much improved small bowel allograft survival after portal DST in combination with low dose cyclosporin, it was speculated that the liver plays a central role in processing donor antigens after a small bowel transplantation. It appears that intrahepatic trapping of the donor antigens administered trough the portal vein route may be crucial for inducing the immune system towards suppression and tolerance. Wolf et al.[89] also arrived at the conclusion that portal inoculation was a major factor in prolonging small bowel graft survival.

Kupffer cells, which are characterized as antigen presenting cells, could be responsible for presenting the alloantigens to the host immunocompetent cells in a tolerogenic setting and thus down-regulate the immune system. Schraut hypothesised that antigens may be degraded or altered

during their passage through the liver[56]. This could explain why those alloantigens, if altered by the liver resident macrophages, are not recognized as foreign. One study investigated the role played by the Kupffer cells in the portal DST effect[116]. Blockade of Kupffer cells with the administration of Gadolinium chloride abrogated the prolongation in cardiac allograft survival induced by the infusion of donor-specific spleen cells in the portal circulation. It was suggested that, although Kupffer cells can function as antigen presenting cells, they could also act to down-regulate the immune response and be essential to the development of liver-mediated tolerance.

The adjunct of low-dose cyclosporin to the portal antigen presentation probably plays a major role in setting up a favourable milieu for the induction of this hyporesponsive state.

The deleterious effect of the donor anti-lymphocyte serum on the portal DST protocol probably reflects the fact that a threshold number of donor antigens must be presented to the host to effectively stimulate the suppressive immune response. The dose of ALS might have been to high. In a preliminary study, we tried to determine the killing effect of the systemic inoculation of 1 cc of ALS at a dilution of 1:20 on peripheral blood lymphocytes by white blood cells count, and on lamina propria lymphocytes and other components of the GALT by immunofluorescent stains. Rats were inoculated with 1cc of

ALS 1:20 via the penile vein after a baseline blood sample and an intestinal biopsy were taken. The rats were then periodically bled for determination of white blood cell count at 1 hour, 4 hours, 8 hours, and 24 hours post injection. Intestinal biopsies were taken at 1 hour and 24 hours post-injection.

The serial white blood cell counts revealed a progressive decline in the number of white cells, reaching a minimum at 4 hours with 32% of the original count. The white cell count then progressively came back up with a little overshoot at 24 hours (Table 6).

The pathology specimens revealed that compared to the pretreatment biopsies with a common leukocyte antigen marker, ALS had no effect on the lymphocytic density of Peyer's patches at any of the concentration. There was no evidence of lymphocytic depletion and all the groups were comparable.

In experiment 4 we tested the hypothesis that we could further improve the graft survival of the portal transfusion protocol by giving the recipients successive post-transplant systemic DSTs. We could not transfuse portally as it would have been very difficult technically. Graft survival data can be found in Table 4. To our surprise Group 11, who received a 1cc portal DST day -1, plus three systemic DSTs days 7, 14, 21 with the same cyclosporin regimen as Group 5, fared much worse than Group 10 ($p=0.02$ MW, $p<0.01$ log-rank). We cannot readily explain this result, although the pathological examination of

these allograft only revealed mild rejection. We can only speculate that the systemic injection of antigens in the post-transplant period might have acted as a second signal reversing the tolerogenic effect of the portal DST. The antigens presented by the systemic post-transplant DSTs may have homed to the spleen or thymus and trigger the rejection response.

In our last experiment we tested the hypothesis that we could push the immune response further towards hyporesponsiveness by immunosuppressing the recipients with two drugs, ALS and cyclosporin, instead of only one. Partial depletion of the recipient's lymphocyes with ALS was hypothesized to produce a embryological-like state of immunological immaturity that would allow for an easier induction of tolerance. Graft survival data are found in Table 5. The hypothesis in this experiment proved to be false. While ALS plus cyclosporin was no different than cyclosporin alone, it was significantly different from ALS (at OR) plus cyclosporin, and DST. The ALS is not specific for any type of lymphocytic cells, it will be cytotoxic to both Th and Ts cells. It is speculated that the ALS given at the time of surgery may have actually removed from the circulation Ts induced by the DST given the day before. This can also explain the results of Group 14. In this group the ALS was given 4 hours before the DST, decreasing the total number of lymphocytes to a critical low number before the DST was given.

It might also be that the administered dose of ALS was too high and that a lower dose would have acted as hypothesized.

Conclusions

We have tested the effectiveness of different immunomodulatory protocols using DST 24 hours pre-transplant combined with a short course of low-dose cyclosporin that would be clinically relevant in the context of both cadaveric and related donor small bowel transplantation. Using a fully allogenic (BN to Lew) rat model we demonstrated that one pretransplant DST in combination with a subtherapeutic regimen of cyclosporin improved graft survival time significantly. Adding three post-transplant DSTs to the pretransplant antigen presentation did not have any beneficial effect.

We have found that the portal route of donor antigen presentation with low-dose cyclosporin had the largest immunomodulatory effect with 33% of the grafts surviving more than 85 days. We speculated that the liver played a major role in processing antigen after a small bowel transplantation, and that portal pretransplant antigen presentation in a cyclosporin down-regulated environment effectively pushed the immune system towards an hyporesponsive state. Surprisingly successive systemic post-transplant DSTs were deleterious to the pretransplant portal DST effect.

Using ALS in the pre and post-transfusion period was detrimental to the DST effect. ALS would have adversely affected the induced Ts cells at the same time as the Th cells. The adjunct of ALS before the DST had no effect.

As rejection usually predominates in the allogenic model, grade 1 GVHD was observed in only 1.7 % of the animals and did not alter their graft survival time. Grade 2 and 3 GVHD were not observed.

We suggest that pretransplant portal DST should be considered as an effective mode of immunomodulation in small bowel transplants. This form of pretransplant induction is effective when given only 24 hours pretransplant and could reduce the need for high dose immunosuppressive drugs. Further research should be carried out to investigate the specific mechanisms of action and test this mode of treatment in higher animal species, so that it could eventually be used in the clinical setting.

Bibliography

1. Steigh E,Srp F. Morbidity and mortality related to home TPN in patients with gut failure. American Journal of Surgery 1983;145:102-05.

2. Hodes JE, Grosfeld JL, Weber JB et al. Hepatic failure in infants on total parenteral nutrition: clinical and histopathological observation. Journal of Pediatric Surgery 1982;17:463-8.

3. Carrel A. La technique opératoire des anastomoses vasculaire et la transplantation des viscères. Lyon MEO 1902·98:859-64.

4. Lillehei RC, Goot B, Miller FA. The physiological response of the small bowel of the dog to ischemia including prolonged in vitro preservation of the bowel with successful replacement and survival. Annals of Surgery 1959;150:543-60.

5. Watson AJM, Lear PA. Current status of intestinal transplantation. Gut 1989;30:1771-82.

6. Doe WF. The intestinal immune system. Gut 1989:30:1771-82.

7. Salvatierra O, Vincenti F, Amend W et al. Four-year experience with donor-specific blood transfusions. Transplantation Proceedings 1983;15:924-931.

8. Yasumara T, Kahan BD. Prolongation of rat kidney allografts by pretransplant administration of donor specific antigen extract or whole blood transfusion combined with a short course of cyclosporin. Transplantation 1983;36:603-609.

9. Singh SK, Marquet RL, De Bruin RWF, et al. The role of suppressor cells in the blood transfusion phenomenon. Transplantation Proceedings 1987;19:1442-1444.

10. Downey WE, Baldwin WM, Sanfilippo F. Association of donor-specific enhancement of rat renal allografts with accelerated development of antiidiotypic antibodies and reduced alloantibody responses. Transplantation 1990;49:160-66.

11. Van Twuyer E, Kast WM, Mooijart RJD, et al. Transfusion-induced skin allograft across an H-2 class I mismatch is caused by a clonal deletion of donor-specific cytotoxic T-lymphocyte precursors within the allograft. Transplantation Proceedings 1989;21:1169-70.

12. Martinelli GP, Knight RK, Kaplan S, et al. Small bowel transplantation in the rat: the effect of pretransplant blood transfusions and cyclosporin on host survival. Transplantation 1988;45:1021-1026.

13. De Bruin RWF, Heineman E, Meijssen MAC, et al. Small bowel transplantation in rats: The effect of pretransplant donor-specific blood transfusions on various segments of small bowel grafts. Transplantation 1990;50:928-930.

14. Saat RE, Heineman E, De Bruin RWF, et al. Total orthotopic allogeneic small bowel transplantation in rats: Attempts to ameliorate the graft-versus-host disease by irradiation and transfusions of the donor. Transplantation 1989;47:451-53.

15. Yasumara T., Kahan B.D., Prolongation of rat kidney allografts by pretransplant administration of donor antigen extract or whole blood transfusion combined with a short course of cyclosporin. Transplantation 1983; 36:603-609.

16. Tchervenkov JI, Epstein MD, Alexander JW, Schroeder TJ. The effect of donor-specific blood transfusion, cyclosporin, and dietary prostaglandin precursors on rat cardiac allograft survival: 2. effectiveness of a 24-hour induction period with DST and CsA in inducing long term survival. Transplantation 1989;47:177-181.

17. Monchik GJ, Russell PS. Transplantation of small bowel in the rat: technical and immunological considerations. Surgery 1971;70:693-702.

18. Lee KKW, Hurst R, Schraut WH. Enteral absorption of cyclosporin after small-intestinal transplantation. Current Surgery 1988;205-8.

19. Albrechtsen D, Helgrerud P, Rugstad HE, et al. Very high concentration in intestinal lymph after oral but not intravenous, cyclosporin in rat. Transplantation 1985;40:220-22.

20. Wassef R, Cohen Z, Nordgren S, et al. Cyclosporin absorption in intestinal transplantation. Transplantation 1985;39:496-99.

21. Wassef R, Cohen Z, Langer B. Pharmacokinetic profiles of cyclosporin in rats: influence of route of administration and dosage. Transplantation 1985;40:489-93.

22. Brunson M.E. Tchervenkov J.I., Alexander J.W. Enhancement of allograft survival by donor-specific transfusion one day prior to transplant: Importance of timing and specificity when DST is given with cyclosporin. Transplantation 1991;52:545-549.

23. Fabre J.W., Morris P.J. The effect of donor-specific strain blood pretreatment on renal allograft rejection in rats. Transplantation 1972;2: 608-617.

24. Cotер B.R., Davies C.B., Alexander J.W., Tchervenkov J.I, Fisher R.A. Effects of pre- and postengraftment donor-specific transfusions and cyclosporin on the enhancement of experimental allograft survival. Journal of surgical research 1992;52:663-667.

25. Yoshimura N, Hamashima T, Matsui S, Oka T. Prolongation of rat renal allograft survival by portal venous innoculation with donor lymphocytes. Transplantation proceeedings 1989; 21:3268-3270.

26. Kenick S, Lowry RP, Forbes RDC, Lisbona R. Prolonged cardiac allograft survival following portal venous innoculation of allogenic cells: What is hepatic tolerance? Transplantation Proceedings 1987; 19: 478-480.

27. Opelz G, Sengar DPS, Mickey MR, and Terasaki PI. Effect of blood transfusions on subsequent kidney transplants. Transplantation proceedings 1973;5:253-59.

28. Salvatierra O, Vincent F, Amend W, et al. Deliberate donor-specific transfusions prior to living related renal transplantation. Annals of Surgery 1980;192,543-52.

29. Kort WJ, Westbroek DL, MacDicken I, Lameijer LDF. Orthotopic total small bowel transplantation in the rat. European Surgical Research 1973;5:81-9.

30. Resnick RK, Craddock GN, Langer B, et al. Structure and function of small bowel allograft in the dog: immunosuppression with cyclosporin A. Canadian Journal of Surgery 1982;25:51-5.

31. Craddock GN, Nordgren SR, Resnick RK, et al. Successful small bowel allotransplantation in dog using cyclosporin. Transplantaion 1983;35:284-8.

32. Deliz-Perz HS, McLure J, Bedetti C, et al. Successful small bowel allotransplantation in dogs with cyclosporin and prednisone. Transplantation 1984;37:126-9.

33. Raju S, Didlake RH, Cayirli M, et al. Experimental small bowel transplantation utilizing cyclosporin. Transplantation 1984;38:561-6.

34. Ricour C, Revillon Y, Arnaud-Battandier F, et al. Successful small bowel allografts in piglets using cyclosporin. Transplantation Proceedings 1983;15:3019-26.

35. Prichard TJ, Koltun WA, Madara JL, Kirkman RL. Small bowel tranplantation in the pig. Small-bowel transplantation experimental and clinical fundamentals. Springer-Verlag ed.1986;26-33.

36. Kirkman RL. Small bowel transplantation. Transplantation 1984;37:429-33.

37. Fortner JG, Sichuk G, et al. Immunological response to an intestinal allograft with HLA identical donor-recipient. Transplantation 1972;14:531-35.

38. Cohen Z, Silverman RE, Wassef R, et al. Small intestinal transplantation using cyclosporin. Transplantation 1986;42:613-21.

39. Grant D. Intestinal transplantation: Current status. Transplantation Proceedings 1989;21:2869-71.

40. Grant D, Sommeraues J, Garcia B, et al. Treatment with countinous high-dose intravenous cyclosporin following clinical intestinal transplantation. Transplantation 1989;48:151-2.

41. Williams JW, Slankary HN, Foster PF, et al. Splancnic transplantation. JAMA 1989;261:1458-62.

42. Starzl TE, Rowe MI, Todo S, et al. Transplantation of multiple abdominal visceras. JAMA 1989;261:1449-57.

43. Tzakis A, Todo S, Abu-Elmagd K, et al. Small bowel transplantation in humans. Presented at the International Symposium on small bowel transplantation, London, Ontario, October 1991.

44. McAlister V, Grant D, Wall W, et al. Clincal experience with small intestine transplantation. Presented at the International Symposium on small bowel transplantation, London, Ontario, October 1991.

45. Goulet O, Revillon Y, Brousse N, et al. Small bowel transplantation in a infant: two years follow-up. Presented at the International Symposium on small bowel transplantation, London, Ontario, October 1991.

46. Kocandrle V, Houttuin E, Prohaska JV. Regeneration of the lymphatics after autotransplantation and homotransplantation of the entire small intestine. Surgery Gynecology and Obstetric 1966;122:587-592.

47. Clark E, Ferguson MK, Moynihan HL, Schraut WH. Mesenteric lymphatic function after total small intestine transplantation in the rat. Current Surgery 1989;March-April:115-17.

48. Madara JL, Kirkman RL. Structural and functional evolution of jejunal allograft rejection in rats and the ameliorating effect of CsA treatment. Journal of clinical investigations 1985;35:75-82.

49. Rosemurgy AS, Schraut WH. Small bowel allografts: sequence of histological changes in acute and chronic rejection. American Journal of Surgery 1986;151:470-5.

50. Sonnino RE, Riddle JM, Besser AS. Small bowel transplantation in the rat: Ultrastructural changes during the early phases of rejection. Journal of Investigative research 1988;1:181-91.

51. Lear PA, Cunningham AJ, Crane PW, Wood RMF. Lymphocyte migration patterns in small bowel transplants. Transplantation Proceedings 1989;21:2881-82.

52. Crane PW, Lear PA, Sowter C, et al. Early microvascular changes in small bowel transplantation. Transplantation Proceedings 1989;21:2906.

53. Kimura K, Money SR, Jaffe BM. The effects of size and site of origin of intestinal grafts on small-bowel transplantaion in the rat. Surgery 1987;101:618-22.

54. Kimura K, Money SR, Jaffe BM. The effects of cyclosporin on varying segments of small bowel graft in the rat. Surgery 1988;104:64-69.

55. Starzl MJ, Schraut WH, Moynihan HL, Lee T. Rejection of ileal versus jejunal allografts. Transplantation 1989;47:424-7.

56. Schraut WH, Abraham S, Lee KKW. Portal venous drainage for small bowel allografts. Surgery 1985;98:579-85.

57. Schraut WH, Rosemurgy AS, Riddell PM. Prolongation of intestinal allograft survival without immunosuppressive drug therapy. Journal of Surgical research 1983;34:597-607.

58. Shaffer D, Diflo T, et al. Immunologic and metabolic effects of caval versus portal venous drainage in small-bowel transplantation. Surgery 1988;104:518-24.

59. Diliz-Peres HS, McClure J, Bedetti C, et al. Successful small bowel allotransplantation in dogs with cyclosporin and prednisone. Transplantation 1984;37:126-129.

60. Millard PR, Dennison A, Hughes DA, et al. Morphology of intestinal allograft rejection and the inadequacy of mucosal biopsies in its recognition. British Journal of Experimental pathology 1986;67:687-98.

61. Fujiwara H, Raju S, Grogan JB, et al. Total orthotopic small bowel allotransplantation in the dog: Features of atypical rejection and GvH reaction. Transplantation 1987;44:747-53.

62. Banner B, Dean P, Williams JW. Morphology of rejection in long-surviving canine small bowel transplants. Transplantation 1988;46(5)665-9.

63. Korngold R, Sprent J. Lethal graft-versus host disease after bone marrow transplantation across minor histocompatibility barrier in mice. Prevention by removing mature T cells from marrow. Journal of Experimental Medicine 1978;148:1687-94.

64. Wallander J, Lackgren G, Tufveson G. T-lymphocytes are necessary for fatal graft vs host disease after small bowel transplantation. Transplantation Proceedings 1989;21:2896-7.

65. Elkin WL. Cellular immunology and the pathogenesis of Graft Versus Host Reactions. Progress in Allergy 1977;15:78-187.

66. Cohen Z, Macgregor AB, Moore R, et al. Canine small bowel transplantation: a study of immunological responses. Archives of Surgery 1976;111:248-53.

67. Schraut WH, Lee KKW, Dawson PJ, Hurst RD. Graft-versus-host disease induced by small bowel allografts: clinical course and pathology. Transplantation 1986;41:286-90.

68. Deltz E, Muller-Hermelink HK, Ulrichs K, et al. Development of Graft-Versus-Host reaction in various target organs after small intestine transplantation. Transplantation Proceedings 1981;13:1215-6.

69. Grant D, Zhong R, Gunn H, et al. Graft-versus-host reactions induced by intestinal transplantation in the rat produce host immunosuppression. Transplantation Proceedings 1988;20:1279-80.

70. Garcia B, Grant D, Zhong R, et al. Pathological findings with Graft-Versus-Host reaction induced by intestinal transplantation in the rat. Transplantation Proceedings 1989;21:2890-1.

71. Kirkman RL, Lear PA, Madara JL, Tilney NL. Small intestine transplantation in the rat-Immunology and function. Surgery 1984;96:280-86.

72. Ferrara JML, Guillen FJ, Van Dijken PJ, et al. Evidence that large granular lymphocytes of donor origin mediate acute graft versus host disease. Transplantation 1989;47:50-54.

73. Ghayer T, Seemayer TA, Kongshavn PAL, et al. Graft versus host reactions in the beige mouse: an investigation of the role of host and donor natural killer cells in the pathogenesis of graft versus disease. Transplantation 1987;44:261-267.

74. Diflo T, Maki T, Monaco AP. The existence of graft-versus-host disease in long term surviving fully allogenic rat small bowel transplant recipients. Transplantaion Proceedings 1989;21:2875-6.

75. Diflo T, Maki T, Balough K, Monaco AP. Graft-Versus-Host disease in fully allogenic small bowel transplantation in the rat. Transplantation 1989;47:7-11.

76. Salvatierra O, Vincenti F, Amend W, et al. Four-year experience with donor-specific blood transfusions. Transplantation Proceedings 1983;15:924-931.

77. Anderson CB, Tyler JD, Sicard GA, et al. Pretreatment of renal allograft recipients with immunosuppression and donor-specific blood. Transplantation 1984;38:664-68.

78. Martinelli GP, Racelis D, Giannone G, et al. Interaction of pretransplant blood transfusions and cyclosporin-A in rats. Transplantation Proceedings 1983;15:988-93.

79. Cheigh JS, Suthanthiran M, Fotino M, et al. Minimal sensitization and excellent renal allograft outcome following donor-specific blood transfusion with a short course of cyclosporin. Transplantation 1991;51:378-81.

80. Marquet RL, Heystek GA, Tenbergen WJ. Specific inhibition of organ allograft rejection by donor blood. Transplantation Proceedings 1971;3:708-10.

81. Homan WP, Williams KA, Millard PR, Morris PJ. Prolongation of renal allograft survival in the rat by pretreatment with donor antigen and cyclosporin A. Transplantation 1981;31:423-427.

82. Johnson CP, Munda R, Alexander JW, et al. The effect of donor-specific transfusions on rat heart allograft survival. Transplantation 1984;38:575-78.

83. Yamagushi Y, Harland R, Wyble C, et al. Variable allograft responses to pretreatment with donor splenocytes treated with mitomycin C in the rat. Transplantation 1989;47:360-63.

84. Lehnard V, Renner D, Hansen B, Opelz G. Suppression of antibody response and prolongation of skin graft by multiple blood transfusions in the rat. Transplantation 1985;39:424-29.

85. Okazaki H, Maki T, Wood ML, et al. Prolongation of skin allograft survival in H-2 K and I region -incompatible

mice by pretransplant blood transfusion. Transplantation 1981;32:111-15.

86. Stoffregen C, Schubert G, Kruss D, et al. Effects of multiple blood transfusions and Cyclosporin A treatment on pancreas transplantation in the rat. Transplantation Proceedings 1989;21:1175-76.

87. Perloff LJ, Barker CF. Variable response to donor-specific blood transfusion in the rat. Transplantation 1984;38:178-82.

88. Sun J, WU M, Hou T, et al. Induction of unresponsiveness to rat hepatic allografts by pretreatment with donor splenocytes and peritransplant cyclosporin. Transplantation 1990;50:1070-72.

89. Wolf YG, Dunaway DJ, Harmel RP. Small-bowel transplantation following portal venous injection of donor strain spleen cells. Transplantation Proceedings 1990;22:2493-94.

90. Marquet RL, Heystek GA, Induction of suppressor cells by donor-specific blood transfusions and heart transplantation in rats. Transplantation 1987;31:272-274.

91. Shelby J, Wakely E, Corry RJ. Suppressor cell induction in donor-specific transfused mouse heart recipients. Surgery 1984;96:296-301.

92. Singh SK, Marquet RL, De Bruin RWF, et al. The role of suppressor cells in the blood transfusion phenomenon. Transplantation Proceedings 1987;14:1442-44.

93. Hutchison IV. Suppressor T cells in allogenic models. Transplantation 1986;41:547-55.

94. Wakely E, Amran CJ, Chapman FA, Corry RJ. Prolonged mouse heart allograft survival following adoptive transfer of transplantation-induced suppressors. Transplantation Proceedings 1987;14:1445-47.

95. Downey WE, Baldwin WM, Sanfilippo F. Association of donor-specific blood transfusion enhancement of rat allografts with accelerated development of antiidiotypic antibodies and reduced alloantibody responses. Transplantation 1990;49:160-166.

96. Baldwin WM, Rhoton K, Sanfilippo F. IgM and IgG alloantibody production by splenocytes and deposition in rat renal allografts are decreased by donor-specific blood transfusion. Transplantation 1991;51:481-85.

97. Markmann JF, Odrico JS, Bassiri H, et al. Clonal Deletion in the adult after intrathymic inoculation with lymphoid cells. Presented at the Annual scientific meeting American Society of Transplant Surgeons, Chicago, 1992

98. Bretscher P, Cohn M. A theory of self-nonself discriminastion. Science 1970;169:1042-49.

99. Fisher E.,Lenhard V.,Seifert P. Blood transfusion-induced suppression of cellular immunity in man. Human Immunology 1980;1:187-194.

100. Fehrman I, Riggnden O, Moller E, et al. Is cell-mediated immunity in the uremic patient affected by blood transfusion? Transplantation Proceedings 1981;13:164-166.

101. Lockard-Marduel A, Gumbert M, Tomanovich S, et al. Immunologic alterations induced by donor-specific transfusion. Transplantation Proceedings 1989;21:1171-72.

102. Tsuda T, Kahan BD. A potential synergistic effect of donor antigenic extract and cyclosporin on the prolongation of rat renal allografts. Transplantation 1983;35:483-89.

103. Yasumura T,Kahan BD. Prolongation of rat kidney allograft by pretransplant administration of donor antigen extract or whole blood transfusion combined with a short course of cyclosporin. Transplantation 1983;36:603-609.

104. Morris PJ. Cyclosporin A. Transplantation 1981;32:349-54.

105. Kahan BD. Cyclosporin pharmacology: Pharmacokinetics and pharmacodynamics of cyclosporin. Transplantation proceedings 1989;21(1):9-15.

106. Miyawaki T, Yachie A, Ohzeki S, et al. Cyclosporin A does not prevent expression of Tac antigen, a probable TCFG receptor molecule, on mitogen-stimulated human T cells. Journal of Immunology 1983;130:2737-42.

107. Hess AD, Horwitz L, Beschorner WE, et al. Development of graft-vs-host disease-like syndrome in cyclosporin-treated rats after syngeneic bone marrow transplantation. Journal of experimental medicine 1985;161:718-730.

108. Dardenne M, Savino W, Feutren G, et al. Stimulatory effects of cyclosporin A on human and mouse thymic epithelial cells. European Journal of immunology 1987;17:275-279.

109. Whisler RL, Lindsey JA, Proctor KVW, et al. Characteristics of cyclosporin induction of increased prostaglandin levels from human peripheral blood monocytes. Transplantation 1984;38:377-81.

110. Hess AD, Donnenberg AD, Tutschaka PJ, et al. Effect of cyclosporin A on human lymphocyte responses in vitro: V. Analysis of responding T-lymphocyte subpopulation in primary MLR with monoclonal antibodies. Journal of Immunology 1983;130:717-721.

111. Cantor HM, Dumont AE. Hepatic suppression of sensitization to antigens absorbed into the portal system. Nature 1967;215:744-45.

112. Triger DR, Cynamon MH, Wright R. Studies on hepatic uptake of antigen.1. Comparison of inferior vena cava and portal vein routes of immunization. Immunology 1973;25:941-949.

113. Qian JH, Hashimoto T, Fujiwara H, Hamaoka T. Studies on the induction of tolerance to alloantigens 1. The abrogation of potentials for delayed-type-hypersensitivity responses to alloantigens by portal venous inoculation with allogenic cells. Journal of Immunology 1985;134:3656-3661.

114. Lowry RP, Kenick S, Lisbona R. Speculation on the pathogenesis of prolonged cardiac allograft survival following portal venous inoculation of allogenic cells. Transplantation proceedings 1987;19:3451-

115. Rao VK, Burris DE, Gruel SM, et al. Evidence that donor spleen cells administered through the portal vein prolong the survival of cardiac allografts in rats. Transplantation 1988;45:1145-1146.

116. Kamel T, Callery MP, Flye MW. Kupffer cell blokade prevents induction of portal tolerance in rat cardiac allograft transplantation. Journal of Surgical Research 1990;48:393-396.