University of Alberta

The Feasibility of Segmental Intestinal Transplantation Using FK506

by

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled *The Feasibility of Segmental Intestinal Transplantation Using FK506* submitted by David Charles Williams in partial fulfillment of the requirements for the degree of Master of Science in Experimental Surgery.

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to my mother and father, for their unfailing support and encouragement

Abstract

Successful segmental small intestinal transplantation (SIT) has been achieved in the large animal model, encouraging development of living-related segmental SIT in humans. However, with cyclosporine based immunosuppression, rejection is a problem, and interest has turned to the more potent immunosuppressive FK506. This study examines the feasibility of segmental SIT in the pig model using FK506. We wish to test the hypotheses that 1) FK506 does not affect growth, nutrient absorption or intestinal adaptation in the pig, and that 2) segmental small intestinal transplantation is possible with the graft's vascular pedicle based on the ileocolic artery and vein using FK506 based immunosuppression.

Three groups of animals were studied. The resection group (n=5) served as controls and underwent a small bowel resection of all but the distal 150 cm of ileum. The resection + FK506 group (n=7) underwent an identical operation and received FK506 (mean level 13.2 ± 2.7 ng/ml) and corticosteroids. The transplant + FK506 group (n=14) underwent a two-stage segmental SIT of 150 cm of ileum and received FK506 (mean level 16.1 ± 9.4 ng/ml) and corticosteroids. Animal well-being and weight gain were followed for four weeks. *In vivo* and *in vitro* permeability studies, reflecting nutrient absorption, along with histologic studies of small bowel specimens were performed.

There was a high rate of technical complications seen in the transplant + FK506 group with six animals surviving the initial transplantation procedure. Rejection continued to be a problem despite therapeutic FK506 levels. Animals receiving FK506 based immunosuppression showed significant weight loss and altered intestinal

adaptation. Permeability studies suggest that there is an overall increase in nutrient absorption.

We conclude that segmental SIT in the pig is possible with the intestinal graft's vascular supply based on the ileocolic pedicle, but there is a high rate of associated technical complications. FK506 alters the normal process of intestinal adaptation and adversely affects weight gain. We have shown that this effect is not due to a decrease in nutrient uptake at the intestinal level, but may be due to a metabolic effect of FK506 which has been shown in other models to impair ATP production at the enterocyte level and decrease the ability of the animal to use glucose efficiently.

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List of Abbreviations

ACTH	Adrenocoticicotropic Hormone
ALG	Anti-Lymphocyte Globulin
ANOVA	Analysis of Variance
Amp	Ampere
BID	*Bis in die*
CD	Cluster Differentiation
cm	Centimeter
CPM	Counts per Million
C:V	Crypt to Villus Ratio
DPM	Disintegrations per Minute
EGF	Epidermal Growth Factor
G	Gap Period of Interphase
GVHD	Graft-versus-Host Disease
Hr	Hour
IL-2	Interleukin-2
Isc	Intestinal Short-Circuit Current (μAmp/cm^2/hr)
IM	Intramuscular
IV	Intravenous
IVC	Inferior Vena Cava
J_{sm}	Serosal to Mucosal Isotope Flux (nmol/cm^2/hr)
kcal	Kilocalorie

kg	Kilogram
Km	Michaelis Constant
KS	Kansas
L	Litre
M	Mitosis
mg	Milligram
MHC	Major Histocompatability Complex
MLC	Mixed Lymphocyte Globulin
mM	Millimole
mRNA	Messenger RNA
MW	Molecular Weight
NF-AT	Nuclear factor of Activated T-lymphocytes
ng	Nannogram
nmol	Nannomole
N/I	Not-Interpretable
OR	Operating Room
PGE_2	Prostaglandin-E_2
RNA	Ribose Nucleic Acid
SI	Stimulation Index
SIT	Small Intestinal Transplantation
TPN	Total Parenteral Nutrition
VIP	Vasoactive Intestinal Peptide

^{99}Tc-DTPA	^{99}Technitium-Diethylenetriamine Pentacetic Acid
μmol	Micromole

Chapter I

Research in the area of transplantation has allowed the transplantation of organs and tissues that was once thought impossible. Heart and kidney transplantation is now viewed by many as being routine, with good control of rejection and improvements in techniques of organ harvesting and preservation responsible for long-term graft survival. However, the transplantation of small bowel is still seen by many as an experimental procedure, with problems ranging from rejection to a shortage of available allografts, slowing the progress of this potential therapy for short bowel syndrome.[1,2]

From the very first attempts at small bowel transplantation in the laboratory during the early part of this century,[2] to the first clinical attempts at small bowel transplantation in the 1950's and 1960's, it was realized that transplantation of small intestine was destined to failure unless rejection could be controlled.[3] Corticosteroids, azathioprine, and cyclosporine were drugs which could certainly prolong intestinal graft survival, but long-term survival was limited.[4,5] Then, in 1984, the discovery of FK506, an immunosuppressive drug with a similar mechanism of action to cyclosporine but much more potent, by the Japanese drug company Fujisawa again stirred up interest in small bowel transplantation.[6] Clinical success has been realized in several patients.[7] The Pittsburgh group has carried out the largest number of transplants, with generally good results.[8] This improvement is likely due to the better

control of rejection under FK506 based immunosuppression as well as a better understanding of the transplantation process as a whole.[9]

The shortage of small bowel allografts available for transplantation has further hindered the development of clinical small bowel transplantation. The shortage is likely secondary to the commonly held attitudes of organ procurement agencies and medical professionals alike that small bowel transplantation is still experimental. In an attempt to overcome this shortage, investigators have turned to segmental intestinal allografts, in hope that one cadaveric donor might provide several allografts, or that living-related small bowel donation might be considered between family members.[10,11,12,13]

The work presented in this thesis looks at the feasibility of segmental small intestinal transplantation in the pig model using a 150 cm small intestinal allograft with its vascular supply based on the ileocolic artery and vein. We have elected to use FK506 based immunosuppression as it is currently the standard in the clinical situation since cyclosporine has not provided adequate immunosuppression.[14,15,16]

This thesis starts with a review of short bowel syndrome in Chapter II. The etiology of the syndrome is discussed along with the various treatment options available today, both medical and surgical. The study itself is presented in a paper format in Chapter III. A somewhat abbreviated version of this chapter will be submitted for publication. The thesis finishes with a discussion of the future of small intestinal transplantation presented in Chapter IV, suggesting the need for further

research in both the laboratory and clinical settings before small bowel transplantation becomes a common and accepted clinical reality.

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Chapter II

Short bowel syndrome is a disease entity with often devastating effects for the patient. There are many etiologies of the disease, both in the pediatric and adult patient. The body attempts to adapt to its new state of shortened bowel, but for an extended period of time, and occasionally indefinitely, the patient is dependent on medical therapies, often with life-threatening side effects. Adjuvant surgical treatment has been attempted over the years with varying degrees of success. Small bowel transplantation has been thought of for a long time as the potential cure for short bowel syndrome, but there are several problems which yet have to be overcome; rejection is the most troublesome. There is a great deal of research being carried out in the field of small bowel transplantation, and transplantation in general, and all the acquired knowledge will hopefully allow transplantation of small bowel to become a successful clinical reality in the near future.

Short Bowel Syndrome

Short bowel syndrome is a condition resulting from the massive loss of small bowel. In the pediatric age group, by far the most common cause is necrotizing enterocolitis. Other causes include midgut volvulus, atresia, gastroschisis, and congenital short bowel. In the adult patient, etiologies include mesenteric infarction, Crohn's disease, radiation enteritis, tumors, and trauma.[1,2] There is some debate as to the length of small bowel required to fulfill the definition of short bowel syndrome.[3] Initially it was believed that the problems with massive small bowel resections were

proportional to the total percentage of small bowel resected.[4] However, it is likely that the absolute length of bowel remaining is the important factor. In 1967, Benson suggested that 25-30 cm of small bowel is necessary along with the ileocecal valve to permit survival.[5] In 1977, Wilmore presented a case series of 50 infants and concluded that survival was dependent on the length of small bowel remaining together with the absence or presence of an ileocecal valve.[6] It was unlikely for a child to survive with less than 40 cm of small bowel and the absence of an ileocecal valve, or with less than 25 cm of small bowel in the presence of an ileocecal valve. Despite the many attempts at creating a precise definition of short bowel syndrome, it is probably not necessary to do so. The disease state resulting from an inadequate length of small bowel, whatever the etiology, resulting in inadequate calorie and nutrient uptake, can be accepted as short bowel syndrome.

Short bowel syndrome has been traditionally divided into three phases, each phase describing a distinct clinical condition of the patient.[1,7] The progression from one phase to the next is possible through the process of adaptation, which will be discussed in detail later. Therapy through each of these stages is primarily medical, but there has been interest in the past, and there continues to be, in surgical therapy.

Stage I is a time during which the patient suffers from massive diarrhea, the amount often exceeding two liters per day. Fluid and electrolyte monitoring with appropriate replacement is of utmost importance during this stage. Total parenteral nutrition (TPN) should be started early in the course and diarrhea can be decreased

with oral codeine. Gastric acid hypersecretion can be a problem during this phase: pancreatic lipase is inacticated, resulting in steatorrhea, and there is an increase in osmotic load, accentuating diarrhea. Gastric acid hypersecretion should be treated with H_2- antagonists, resulting in improved nutrient absorption.[8,9,10,11] Stage I lasts from one to three months.[1]

Stage II is a period of gradual adaptation of the remaining small bowel. Oral intake is gradually increased and slowly TPN can be decreased if the patient is receiving adequate oral nutrient intake. Diarrhea is usually limited to less that two litres per day. There is some debate as to what diet should be given to these patients, but a regular diet is probably best.[12] This stage lasts from three months to one year.[1]

Stage III is a time when maximum adaptation is achieved. If a patient can maintain adequate nutrition by mouth, TPN may be discontinued. Some patients never achieve this goal, but the advent of home TPN programs has improved the outlook and quality of life for these patients.

Intestinal Adaptation

The progression from one stage to the next is possible through the process of adaptation. Like the heart and other organs, the intestine has a reserve capacity to function which is seldom required.[13] The coefficient of variation for post-natal small bowel length was found to be 24%, six times that for body length. This variation in length suggests that, to some degree, there is a surplus in length that is immediately available to respond to disease states such as local intestinal disease or resection.[14]

However, when the length of bowel remaining is insufficient for adequate nutrient and calorie absorption, the bowel can change its functional capacity through adaptation.

Epithelial cell kinetics were first described by Leblond in 1956 through techniques of mitotic counting.[15] Subsequent studies using autoradiography techniques have confirmed his data.[16,17] These latter studies use tritiated thymidine to label cells undergoing mitoses. Labelled cells can be observed and cell cycle phases can be identified along with the rate of cell migration along the intestinal villus. The original work was carried out in rats, but the technique has been modified so that mucosal biopsies from humans can be examined.[18,19] Cell division in the small intestine takes place in the base of the crypts of Lieberkuhn. Rates of nucleic acid synthesis and activity of thymidine kinase are highest in this area.[20] Enzymes involved in the metabolism of nucleic acid precursors in epithelial cells at varying degrees of differentiation has allowed examination of the cell proliferation rate. Cell division in the small intestine takes place in the base of the crypts of Lieberkuhn. Thymidine kinase is found in young proliferating cells located in the crypts, and it is here where rates of nucleic acid synthesis are highest. Other enzymes, including thymidilate phosphatase and adenylate deaminase, are found uniformly from the crypts to the villus tips.[20]

The complete cell cycle includes the period of cell division, or mitosis, and the period between cell divisions, or interphase. The replication of DNA occurs only during the synthesis (S) phase of interphase. The S phase is preceded and followed by

two gap periods of interphase, G_1 and G_2, respectively, in which no net synthesis of DNA takes place. Cells that are not growing and dividing are halted in the diploid part of interphase (G_1), termed G_0.[21]

In intestinal mucosa in man, the total cell cycle time lasts at least 24 hours.[22] The M (mitosis) phase lasts only one hour, while the S phase (DNA synthesis) lasts from six to 11 hours. Proteins are produced throughout the S phase as well as during the pre-mitotic (G_2) and post-mitotic (G_1) gaps.[23] As cell turnover is so fast, cells do not usually enter G_0.[23] The cell cycle is rapid enough that the entire small bowel epithelium is completely replaced within three to six days.[24] The uptake of thymidine is gradually lost after two cell divisions, and the cells migrate onto the villus as mature columnar cells with their associated enzymatic machinery.[23] Cells continue to migrate toward the villus tip until they are extruded into the intestinal lumen.

All four cell types found in the small intestinal mucosa (columnar, mucous, Paneth, enteroendocrine) are thought to arise from a single undifferentiated cell at the crypt base, but the latter two do not undergo mitoses. Enteroendocrine cells migrate slowly along the villus, but Paneth cells remain in the crypt where they will eventually degenerate and become phagocytosed by an adjacent crypt-base cell.[18,25] Columnar cells constitute the majority of the epithelial cells in the small intestine, accounting for approximately 90% of the cell population.[26] Surrounding structural cells also undergo division and replication.[27] Zajicek has proposed the idea of an "intestinal proliferon",

made up of the epithelial, neural, vascular, and connective tissue elements, which autoregulates the migration of the various cell elements.[18,28]

The proliferation rate of cells in the small intestine is not constant.[18] Factors such as circadian rhythms,[29] governed by oral intake of food[30] or the autonomic nervous system,[31] along with age,[32] have been shown to affect crypt cell proliferation.[18] The largest body of data looking at altered cell renewal in the small bowel came from looking at partial intestinal resection.[18]

Structural changes have been studied using the rat model in which the animals were subjected to extensive resection of the small intestine.[33] Results show that compensatory growth of the small bowel involves full thickness bowel, but the most striking feature is villus hyperplasia.[33] This effect is most pronounced following proximal versus distal resection, and is maximal near the anastamotic site.[34] The degree of intestinal response increases in a stepwise fashion as the amount of tissue removed is increased.[35] The compensatory growth of the small intestine involves all layers of the small bowel, and includes dilatation and lengthening of the intestinal remnant and an increase in villus height and crypt depth due to hyperplasia rather than hypertrophy, as shown by the unchanged number of cells per unit length of villus[35] and unaltered RNA/DNA ratios.[36] Studies examining mucosal wet and dry weights, and the contents of DNA, RNA and proteins, have confirmed an increase in mucosal mass.[36]

The timing of these structural changes have been looked at experimentally. Using measurements of DNA, RNA, and tritiated thymidine uptake, ileal mucosal hyperplasia has been identified within 48 hours of jejunectomy.[37,38,39,40] It then peaks at seven to 12 days and can continue for months.[41]

Kinetic studies have shown a slight decrease in the overall duration of the cell cycle. The S or G_1 phase are affected.[42] The accelerated cell production is reflected in an increase in pyrimidine synthesis and greater activity in the pentose phosphate shunt.[42,27] Cell turnover, the time from cell differentiation in the crypt to extrusion into the bowel lumen may be increased early on post-resection[43]; however, as the crypt villus is elongated, and the migration rate is higher, the overall cell turnover rate may be unchanged.[18,44]

Functional changes are also seen in the small bowel following resection. With the morphological and kinetic changes discussed above, one would expect a state of altered nutrient absorption. In general, there is an increase in the absorptive capacity of the remaining bowel. This was first shown in the human in a study carried out in 1966 which showed increased absorption of glucose after intestinal resection.[45] Another study showed that both glucose and disaccharide absorption progressively increases with time after resection with a much greater increase in absorptive capacity in ileal remnants than in jejunal remnants.[46] However, this study found a poor correlation between enzyme activity, hydrolysis of disaccharides, and absorption of disaccharides. This suggests that intestinal compensation after resection results from an increased

number of absorbing cells rather than an increase in individual cell function.[46,33] A later study revealed contradictory results in that ileal enterocytes adapt to proximal bowel resection by selective increases in enzyme expression.[47] Whether there is an increase or decrease in enzyme expression, changes in bowel morphology, that is, an increase in villus height and crypt depth, resulting in an overall increased bowel surface area, allow for a state of increased nutrient absorption.[33]

The signals necessary for adaptation of the remaining small bowel following resection have been examined in great detail in the past, but the exact mechanism responsible still remains a mystery. A consistent finding has been that any procedure exposing the distal bowel to a greater nutrient load produces hyperplasia in that segment.[13] Normally most exogenous carbohydrates, fats, or proteins are almost completely absorbed in the proximal jejunum, and the ileum is exposed to only very small amounts of these substances. This rich supply of chyme might directly stimulate mucosal hyperplasia. This is the theory of topical nutrition resulting in mucosal adaptation proposed by Dowling.[33] Villus enlargement results from experimental hyperphagia and epithelial cells lining the intestine can use glucose and amino acids as a nutrient supply.[48] Different sugars, including glucose, fructose, and mannose, have been shown to stimulate mucosal growth.[47] Fat is the most powerful stimulus for mucosal growth, with bolus enteral dosing showing a greater effect that continuous dosing.[49] The enhanced enterotrophic effects of bolus dosing could be mediated by release of a distally located gut peptide, perhaps enteroglucagon.[49] Studies using

complete unhydrolyzed proteins, such a casein, show that the mucosal growth is stimulated to a greater extent when exposed to complete protein versus its hydrosylates.[50] The importance of luminal nutrient exposure is supported by the absence of intestinal mucosal adaptation in animals and humans receiving TPN. Following small bowel resection in the dog, the villus height was reduced in those animals receiving TPN when compared to those animals fed orally.[48] Villus atrophy has been observed in humans after three weeks of TPN and no oral intake.[47] This study also showed a decrease in enterocyte enzyme activity in the patients receiving TPN. The theory of topical nutrition and its effects on intestinal adaptation is simple, and therefore attractive, but there still remain a few unanswered questions. How does this theory explain small bowel adaptation following sub-total colectomy.[51] Clearly, there are other, likely systemic, factors which must come into play.

Pancreaticobiliary secretions will increase as the oral intake of food increases. These secretions have been shown to exert a strong trophic effect on small bowel mucosal villi.[52] Experiments have shown that when the panceaticobiliary secretions are diverted to a more distal portion of the bowel, then that portion shows prompt hyperplasia.[53] The effect is even greater following proximal jejunal resection.[53,54] Pancreatic juice does not produce its hyperplastic effects through the cleavage of proteins, which would produce amino acids for luminal nutrition.[55] When pancreatic juice is infused into a portion of small bowel, the resulting hyperplasia is greater than an infusion of amino acids.[52] When the effects of bile alone are examined, it is found

that high luminal concentrations of bile causes transient cell proliferation in ileal mucosa. However, the additional presence of pancreatic juice prolongs this adaptive response.[56] How large a part pancreaticobiliary secretions play in intestinal adaptation is not completely known. They may act in concert with intraluminal nutrients since the presence of food in the intestinal canal is necessary for the secretions to exist in high enough concentrations to exert their trophic effect.

There is evidence in the literature for a trophic effect of various gastrointestinal hormones. Hormones might conceivably enhance cell proliferation and mucosal mass (trophic action) or inhibit them (antitrophic action).[51] The gut can be thought of as an enormous endocrine organ which is capable of producing many different hormones, any of which may have either a positive or negative trophic effect.[57,58] Gastrin and its effects on intestinal cell growth have been studied extensively in the past, but there is no conclusive evidence that gastrin has any effect.[55] There is no doubt that gastrin has a role in maintaining mucosal integrity in the gastric fundus and proximal duodenum.[58] Hypogastinemic states result in mucosal atrophy as shown by gastric mucosal hypoplasia following antrectomy and gastroduodenostomy.[59] One study looking at small bowel adaptation shows a role for gastrin in the proliferative response of small intestinal mucosa after resection, but the relationship between serum gastrin levels and adaptive changes in the bowel are inconstant.[60,71] In general, gastrin does not appear to have a major role in the adaptive process beyond the stomach and duodenum [13]

Cholecystokinin is another intestinal hormone which may have a positive effect on intestinal adaptation, but again, the evidence is inconclusive. When infused in dogs receiving TPN, villus hypoplasia is prevented.[13] However, when rat gut mucosa is exposed to cholecystokinin, no direct trophic effect is seen.[61] If there is a trophic effect, it may be secondary to the stimulation of pancreaticobiliary secretions.13,61

Enteroglucagon is a gastrointestinal hormone with perhaps the greatest trophic effect on the small intestine. Cells producing enteroglucagon are found throughout the intestine and especially in the small intestine.[58,63] Polyamines are known to influence tissue growth with an increase in RNA and DNA synthesis, resulting in cellular hyperplasia.[13,58,61,63] Polyamines are produced via the ornithine decarboxylase enzyme system.[61] Enteroglucagon stimulates ornithine decarboxylase, raising polyamine production, resulting in cellular hyperplasia and intestinal adaptation.[61] Dowling *et al* have suggested that enteroglucagon is released from enterocytes following exposure to carbohydrates and triglycerides. The induction of ornithine decarboxylase and the resultant increase in polyamine biosynthesis are critical for the normal growth and especially for adaptive hyperplasia of the intestinal mucosa.[62] Enteroglucagon blood levels have been found to be raised in a variety of clinical situations, the common denominator being the ileum's exposure to an abnormally rich chyme.[53] Levels were significantly elevated in patients with proximal small bowel resections.[64] Patients with previous vagotomies and drainage procedures who suffered from the dumping syndrome had significantly raised enteroglucagon levels.[65] Jejuno-ileal bypass

procedures also increased enteroglucagon levels.[67] This evidence supports enteroglucagon as playing an important role in intestinal adaptation.

Somatostatin is unlike enteroglucagon in that it has inhibitory effects on intestinal adaptation.[68,69] It has been found to decrease enteroglucagon levels; whether somatostatin directly alters crypt cell proliferation or acts through its depression of enteroglucagon is not understood.[68,69]

Other hormones produced in the gastrointestinal tract include serotonin, histamine, secretin, and vasoactive intestinal peptide. Serotonin and histamine have dose dependent effects: both hormones, when injected in small doses cause an increased cell proliferation rate. However, when injected in larger doses, they have an inhibitory effect on crypt cell mitosis.[70] Secretin causes a decreased mitotic index in intestinal epithelium and therefore has an inhibitory effect on cell proliferation.[15,71] Vasoactive intestinal peptide (VIP) levels were measured in patients with short bowel syndrome and were found to be significantly elevated above those of healthy control patients.[72] It has been suggested that the elevated VIP levels may account for the diarrhea seen in these patients.[72] This has remained unproven. It seems that VIP has no direct affect on gut cell proliferation.[13]

Epidermal growth factor (EGF) is a growth factor that is produced in the submandibular salivary gland, Brunner's glands in the duodenum, and small intestinal Paneth cells.[13,74] Experiments using fetal human jejunum in tissue culture show clearly that EGF, in varying concentrations, decreases the epithelial cell labelling index. In a

dose dependent manner, the addition of EGF inhibits the tissue accumulation of sucrase, trehalase, and glucoamylase activities without affecting alkaline phosphatase. Lactase activity is increased. This work shows that although the cell proliferation rate is decreased, EGF has, for the most part, a positive effect on brush border enzymes.[73] Another study looked at intravenous administration of EGF in rats in which intestinal epithelial cell proliferation had been reduced by maintaining the rat on TPN. Intravenous EGF restored cell proliferation in the hypoproliferatine intestine.[74] These studies suggest that intravenous but not topical EGF is trophic to the intestine.

Prostaglandin E_2 is another substance with a trophic effect. It causes mucosal hyperplasia in the proximal small bowel of normal rats.[75] Using a PGE_2 analogue, 16-16-dimethyl-prostaglandin E_2,administered orally in rats subjected to a jejunoileal resection, an increase in mucosal mass proximal to the anastamosis was seen. There was a slight, but not significant, increase in mucosal mass in the remaining distal small intestine. The authors concluded that prostaglandins augment mucosal adaption following massive small bowel resection.[75]

Glutamine is a non-essential amino acid which acts as an important nitrogen carrier from the peripheral tissues to the splanchnic area.[13] Nucleic acid syntheses, and thus cell proliferation, requires the presence of purines and pyrimidines. Glutamine plays the important role as primary nitrogen donor for purine and pyrimidine synthesis. A study looked at the use of glutamine in total parenteral nutrition using a rat model. Results showed that glutamine stimulated sucrase, lactase, and maltase activity in the

small intestinal brush border. However, there was no change in the small bowel villous height. Of note, this study showed that the addition of glutamine to TPN solutions protected the liver from fatty infiltration when compared to standard TPN solutions.[76]

Glucagon treatment has been shown to alter the electrophysiological characteristics of rat jejunum. Glucagon caused a marked increase in the potential difference across the brush border membrane along with a significant increase in glucose and galactose absorption. The authors conclude that glucagon increases nutrient uptake across the small bowel, but there is no evidence to suggest that the hormone causes structural adaptive changes.[77]

Short chain hydrocarbons are produced when the colon's bacterial population ferments carbohydrates and fiber to form acetic acid, proprionic acid, and butyric acid.[78] Studies have looked at the addition of short chain hydrocarbons to TPN solutions and have found a reduction in mucosal atrophy seen with TPN use following small bowel resection. This suggests that short chain fatty acids may help in the small intestinal adaptive response following intestinal resection.[79]

The pituitary axis has been examined for playing a possible role in intestinal adaptation. Experiments have been done where the pituitary was removed in rats following small bowel resection. Both the control animals and the hypophysectomized animals showed a reduction in small bowel mucosal mass, disaccharidase activity, villus height, crypt depth, and galactose absorption. However, the magnitude of these adaptive changes was significantly less following hypophysectomy. This study

concluded that the pituitary does influence intestinal adaptation after resection since the effects of hypophysectomy on mucosal regeneration cannot be explained by the observed decreased food intake alone.[80] Whether it is an effect of the pituitary alone, or an effect through one of its target organs has been examined. Hormones present further down the axis have been shown to play a part in the adaptive response. Mineralocorticoids have been shown to cause structural changes in small intestinal mucosa similar to an adaptive response.[81] Studies looking at glucocorticoids show that withdrawal of glucocorticoid hormones by adrenalectomy causes atrophy of the intestinal mucosa and an decrease in jejunal crypt depth. With stimulation of glucocorticoid production by injection of ACTH, the mitotic index of rat jejunum is increased, associated with an increased villus height and cell migration rate.[70] Thyroid hormone has been shown to stimulate the crypt cell mitotic index and to cause an increase in the crypt cell population.[70] Testosterone also stimulates epithelial cell proliferation demonstrated by crypt cell kinetics of testosterone treated mice. The intestinal cell labelling and mitotic indices were significantly raised, suggesting an increase in growth fraction.[82] The loss of the adaptive response with the removal of the pituitary gland can therefore be explained by the pituitary's target organs, (the adrenals, gonads, and thyroid) losing their stimulus to secrete with a resulting loss of the intestinal adaptive response.

Medical Treatment of Short Bowel Syndrome

Although the process of intestinal adaptation allows improved nutrient absorption in the patient with short bowel syndrome, the patient rarely, if ever, returns to normal following massive small bowel loss. Over the years, there have been a variety of treatments devised, both medical and surgical, in an attempt to allow short bowel patients to lead a normal life.

The mainstay of medical therapy over the years has been total parenteral nutrition (TPN). This treatment has been life-saving, but it is not without its problems. TPN was first introduced into clinical practice during the late 1960's and has been used since to support patients with a variety of malabsorption and malnutrition syndromes. Certain situations require only a short period of TPN, whereas the massive loss of intestine as seen in the short bowel syndrome requires the patient to rely on TPN entirely and indefinitely.[83] TPN has allowed the survival of many patients who would otherwise not have been able to receive adequate calories or nutrients via the enteral route. Through the widespread use of TPN, however, many potential risks of the therapy have become apparent. Hepatobiliary complications are probably the most life-threatening, but TPN is also associated with economic, catheter-related, psychological and metabolic problems.

Hepatobiliary problems may develop in patients on TPN. Most are seen in those receiving the therapy for an extended period of time. Problems range from steatosis, to elevated liver enzymes, to cholestasis, to frank liver failure. Raised liver

enzymes are seen in virtually all patients on TPN.[84] Histological examination of these patients revealed that the most frequently observed alteration is steatosis and this is seen relatively soon after TPN is instituted.[83] The pathogenesis of steatosis is not exactly known, but it is likely multifactorial. Proposed mechanisms include decreased hepatic triglyceride secretion,[85] intestinal overgrowth with anaerobic flora inducing toxic steatosis,[110] excessive carbohydrate calories,[86] insulin/glucagon imbalance,[87] and toxic breakdown products of amino acids present in the solution.[90] Steatosis correlates with the clinical situation of elevated liver enzymes in which elevated transaminases and alkaline phosphatase are seen along with elevated bilirubin.[88] These changes are reversible when TPN is discontinued.[89] It has been suggested that steatosis is an early and often transient effect of TPN with cholestasis supervening later and usually persisting as long as the TPN is continued.[84,90]

Cholestasis is seen in patients receiving TPN for a longer period of time.[91] Again, the etiology is likely multifactorial. An interesting finding shows the risk of chronic cholestasis was significantly higher in resected short bowel patients versus unresected patients. This suggests that altered metabolism of unabsorbed bile acids may play a prominent role in the etiology of cholestasis.[92] The lipid make-up of the TPN solution may have an important role in the development of cholestasis.[89]

Continued administration of TPN can result in chronic and sometimes irreversible liver disease. The pathology ranges from periportal fibrosis to cirrhosis.[84] The incidence of irreversible liver disease tends to differ among age groups receiving

long-term TPN. A series of adult patients reported a 10% incidence of severe liver disease.[94] The etiology in this age group is again multifactorial, but a consistent finding is that those patients with a massive loss of small bowel have a higher risk of developing severe liver disease.[95] The incidence in infants and children ranges from 30 to 40%.[91,96] This population has multiple contributing factors. Depending on the reason for instituting the TPN, the young patient may have to contend with sepsis, dehydration, prematurity, gastrointestinal obstruction, and prolonged fasting, all of which may contribute to severe and progressive liver damage.[84,96] It should be noted that short bowel syndrome itself impairs liver function and causes autoimmune-like hepatitis. The precise mechanism remains unclear. It is suggested that activation of self-reactive lymphocytes may take place in the liver after intestinal resection.[97]

Long-term TPN has also been shown to increase the risk for gallbladder disease. A series looked at patients 15 years or older who had received a minimum of three months of TPN.[98] Patients with ileal disease (i.e. Crohn's disease, ileal resection, or both) were considered separately as they have a higher propensity to develop gallbladder disease secondary to the interruption of the enterohepatic circulation.[97] There was a significantly higher incidence of gallbladder disease (acalculous cholecystitis and cholelithiasis) in both groups compared to similar groups not receiving TPN. A similar study examined patients less than 15 years of age and their risk of developing gallbladder disease.[99] 21 children receiving long-term TPN were prospectively evaluated for the presence of gallstones and nine (43%) were found to

have cholelithiasas. In those children with ileal disease, the prevalence of stones was 64%, twice that for a similarly defined population not receiving TPN. This study suggests that the use of TPN in patients with ileal disease or resection significantly increases their risk for cholelithiasis.

In order to infuse TPN in adequate doses required for complete nutrition, a central venous catheter must be used. Broviac first described an indwelling silastic atrial catheter in 1973, and its development has allowed widespread use of TPN.[100] A paper from 1985 prospectively followed 270 infants and children with 335 Broviac catheters placed.[101] Sepsis was diagnosed in 91% of the patients, the most consistent sign being a temperature elevation. Although sepsis is the most frequently encountered complication of long-term indwelling catheters, the benefits of TPN outweigh the disadvantages, and septic episodes can be successfully treated in the vast majority of patients.[101] Another study reviewed 107 infants with Broviac central venous catheters inserted for TPN administration and reviewed the complications. The most common complications were thrombosis and infection. The authors concluded that the risk of complications increases with prematurity, and that in their study population, there was no increase in mortality associated with the presence of a complication.[102]

When TPN first became a clinical reality, formulations were crude. As a result, fluid and electrolyte problems were seen along with micronutrient and vitamin deficiencies. However, the establishment of specialized TPN services within hospitals and close patient monitoring, together with new and improved TPN formulations, has

resulted in fewer metabolic problems in TPN patients. Severe deficiencies in Vitamins D and B_{12} along with zinc, iron, and essential fatty acids can develop and patients must be closely monitored. The problem of metabolic bone disease still remains. The etiology is likely multifactorial and altered metabolites of calcium, phosphate, vitamin D and aluminum have been implicated.[103] The disease process presents as back pain, periarticular bone pain, and fractures in the adult, and as osteopenia, fractures, and rickets in children.[103] The end result can be debilitating.

The underlying disease together with the chronic use of TPN can have adverse effects on the patient's well-being.[104] A study looked at intellectual and perceptual-motor performance of children receiving prolonged TPN.[105] All children functioned within the normal range of intelligence, but the majority of children showed some deficits in perceptual-motor performance. Patients' lifestyles are changed when they are receiving TPN, both as a consequence of the TPN and its complications as well as the underlying chronic disease. The economic aspect cannot be overlooked. Prior to the advent of home TPN programs, the patient and/or the government would be faced with the enormous hospital costs. Since home TPN programs have become popular, the cost per patient has dramatically dropped. However, the treatment is still very expensive. In 1980, the cost for home TPN for one year was $21 465.[106] Today, home TPN casts on average $280/day or $102 200/year.[107] Added to these basic costs are repeated hospitalizations for TPN related complications.[108]

Enteral feeding is another very important aspect of medical therapy for short bowel syndrome. Unfortunately, enteral feeding is not an option when the patient has suffered total small bowel loss. These patients are entirely dependent an parenteral nutrition until small bowel transplantation becomes a safe alternative.[109] As mentioned, enteral feeding may be an important mechanism for small bowel adaptation with a resultant increase in the absorption of water, electrolytes, and nutrients. For this reason, if there is a length of small bowel remaining, enteral feeding should be started early and the amount increased until the patient's tolerance is reached. Agents which slow down intestinal transit such as loperamide may be tried. Loperamide has the added advantage of having an antisecretory action. Antibiotics may also be useful when there is an overgrowth of bacteria in the remaining small intestine.[110] Gastric acid hypersecretion is commonly seen following small bowel loss.[8] This results from an increased level of circulating gastrin, leading to gastric acid hypersecretion.[111] Surgical therapy was initially used to treat hypersecretion, but now medical therapy, through the use of H_2-antagonists, is generally employed.[9]

Enteral feeding, if possible, should be instituted as early as possible to facilitate the process of adaptation. Unfortunately some patients will be dependent on TPN indefinitely as they do not have the small bowel to satisfy their nutrient and calorie intake. Nevertheless, with the advent of new TPN solutions, and meticulous techniques used for central venous catheterization, these patients have a good chance of fulfilling a normal life.

Surgical Management of Short Bowel Syndrome

There has been a lot of interest over the years in the field of surgical management of short bowel syndrome. A principle which should be kept in mind is that at the initial procedure which could possibly result in short bowel syndrome, the maximum amount of bowel possible should be removed. At the time of initial operation, no adjunctive procedures should be performed.[13,112] The remaining bowel should then be allowed to adapt and the patient watched and supported with medical therapy. There is controversy about when, or even if, surgical treatment should be attempted. However, if life-threatening complications of TPN start to develop, surgical options need to be considered. The common goal of surgical therapy is to increase the absorptive surface of the remaining small intestine. There are several different techniques tried over the years, each with varying degrees of success.[113,114]

Intestinal loop lengthening was described in 1980 by Bianci.[115] Unlike other procedures which will be described later, the intestinal loop lengthening technique does not rely on procedures which depend on mechanical intestinal obstruction for their beneficial effects. In this procedure, the dilated loop of small bowel is transected longitudinally after division of the mesenteric leaves and directing alternate vessels to either half of the divided bowel. The result is a loop of twice the length and half the circumference. The halves are then anastomosed end to end. The technique was initially performed in pigs and has been successful in the clinical situation with approximately 50% of the patients weaned off TPN.[13] This technique does not create

new mucosa but simply increases the efficiency of already present mucosa by decreasing intestinal transit time.[13]

A technique which incorporates the Bianchi procedure was described in 1994 by Georgeson.[117] This technique has been used when the remaining segment of small bowel is not sufficiently dilated to carry out a Bianci procedure. A nipple valve was positioned in the distal portion of the small bowel to create a partial mechanical bowel obstruction. This, in tun, would cause the proximal segment to become dilated. Once dilated, the loop could be divided longitudinally as in the Bianci procedure. This technique has yet to be proven and has not gained clinical acceptance.

A further modification was described by Kimura.[118] A myoenteropexy is carried out so the loops of remaining small bowel receive some of its blood supply from the anterior abdominal wall. This allows longitudinal division of the bowel once blood supply is established.

Antiperistaltic segments of small bowel have been used in an attempt to slow intestinal transit. There have been varying degrees of success with the operation, likely because there is no standard length of reversed segment which is used. The ideal length of the reversed segment appears to be 10 cm in adults and as short as three cm in the infant.[113] Approximately 30 patients have had this procedure and some studies have questioned the long-term results.[13,113]

The colonic interposition was attempted as a means of slowing the delivery of chyme to the distal bowel. A portion of colon is interposed between small bowel

segments and it effectively prolongs intestinal transit without causing obstruction.[119,120] Some case reports of this operation suggest a good outcome.[121] One study suggests that although the delivery of chyme is slowed, the degree of absorption is not altered.[120] A variation of this operation has been attempted with a portion of colon interposed distally, but in an antiperistaltic direction. Experiments suggest an increase in absorption but at the cost of a higher morbidity rate.[122]

There have been many attempts in the past at the creation of valves distally to produce a proximal partial bowel obstruction and subsequent dilatation. Creation of a nipple valve,[123] an artificial sphincter,[124] submucosal tunneling of the small bowel,[125] and eversion of distal small bowel and telescoping it into the proximal bowel[13,126] have all been attempted. They have all been shown to create some dilatation, but the clinical results are limited.

The creation of recirculating loops of small bowel have been attempted in an effort to allow repeated exposure of luminal contents to absorptive surface area.[127] There is a high morbidity and mortality associated with this procedure with no objective increase in nutrient absorption.[127]

In an attempt to slow the transit time of chyme through the small bowel, retrograde electrical pacing has been tried in the dog model.[128] The results show a selective increase in absorption, but there are no reports of this procedure in humans.

In general, the adjuvant surgical procedures for short bowel syndrome have not been a great success. High morbidity associated with most of the procedures have

made medical therapy the prime option for treating patients with short bowel syndrome. However, medical therapy, too, has an associated high morbidity rate. A successful treatment should allow the patient to receive most, if not all, of his calories via the enteral route with a minimal associated complication rate. Small bowel transplantation has been investigated over the past 35 years with the aim of providing such a treatment. There have been problems with this surgical approach to short bowel syndrome; however, with improvements in organ harvesting and preservation, together with improved immunosuppressant drugs, small bowel transplantation may soon be the treatment of choice.

Small Bowel Transplantation

Small bowel transplantation has a long history. It was in 1902 when Alexis Carrel first reported the technique of transplantation of intestinal segments into the neck of dogs. He predicted that for small bowel transplantation to be successful, the formidable barrier of graft rejection had to be overcome.[129] Very little work was carried out in this field until the late 1950's, when, in 1959, Lillehei published a paper describing the transplantation of autografts in dogs following four hours of cold ischemia.[130] These animals were followed for time periods ranging from weeks to several years. Lillehei showed small vessel anastomoses (2-4 mm) can be successfully performed and remain patent indefinitely, and that the transplanted bowel could survive and maintain the nutrition of the dog without requiring neural connections with the central nervous system. He documented normal motility of the transplanted bowel, suggesting that the

myenteric plexus functioned without central nervous system control, and demonstrated normal mucosal architecture by microscopic examination.[131]

The same group, some 10 years later, examined small bowel allografts placed heterotopically in the neck of dogs.[132,133] Problems of rejection arose with the limited immunosuppression of the day which included azathioprine and steroids.[134]

At that time, research into small bowel transplantation essentially halted. Without the drugs necessary to prevent rejection, it was thought, albeit correctly, that the operation would never be a complete clinical success. However, in the late 1970's, the advent of cyclosporine rekindled interest in small bowel transplantation.[135] In 1982, a study published by a Canadian group from Toronto (Reznick *et al)* demonstrated that small bowel allotransplantation was possible in the dog using cyclosporine. The animals received a small bowel allograft from the duodenojejunal flexure to the ileocecal valve on the superior mesenteric artery and vein. The graft was flushed with heparinized Ringer's lactate solution. The vascular anastomoses were performed between the superior mesenteric artery and vein and the abdominal aorta and inferior vena cava, respectively. Intestinal anastomoses were performed end-to-end. Ten control dogs received no immunosuppression and ten transplanted dogs received cyclosporine (25mg/kg/day), IM for 30 days, then changed to PO. The ten control dogs survived a mean of 12.5 days and the treated animals survived a mean of 90.6 days.[136] A study published in 1983 by a Toronto group (Craddock *et al*) also showed that small intestinal transplantation was possible with an extended survival when the

cyclosporine was used.[137] The donor and recipient dogs had their entire small intestine removed on its vascular pedicle, from the duodenojejunal flexure to the ileocecal valve. The graft was flushed with heparinized saline, and the donor superior mesenteric artery was anastomosed to the recipient aorta, and the superior mesenteric vein to the recipient inferior vena cava. End to end intestinal anastomoses were carried out so the graft lay in an orthotopic position. 34 dogs were used, divided into three groups. Group I received no immunosuppression. Group II received cyclosporine in one dose IM (25 mg/kg) per day starting one day prior to transplantation, and continuing for one month, after which the same dose was given orally. Group III received cyclosporine orally only, starting one day after transplantation. The IM treated dogs survived for a significantly longer period (mean of 103.8 days) than the orally treated (mean of 30.4 days) or control dogs (12.5 days). These two papers concluded that small bowel allotransplantation is possible in the large animal using cyclosporine, and that cyclosporine is effective in prolonging survival after total small intestinal allotransplantation in the dog.

These studies were the first to show successful long-term survival of small intestinal allografts in the large animal model using cyclosporine. The study was carried out using the dog model. However, the physiology of the domestic swine is closer to that of the human;[138] therefore, the next logical step in large animal intestinal transplant research was to see if small bowel allografts could be successfully transplanted using the pig model and cyclosporine. This was done in 1983 by

Ricour.[139] This study was able to achieve long-term pig survival, up to 200 days. Ricour's paper concluded that small intestinal transplantation was possible in the pig using cyclosporine. In the lomgest surviving animals, cyclosporine was initially given intravenously (8mg/kg/day) and then switched to orally (25 mg/kg/day) when intestinal transport normalized. The cyclosporine levels required for long-term survival were up to 2000 ng/ml measured by radioimmunoassay. A 1988 paper by Grant again used the pig model and used cyclosporine as the immunosuppressant.[140] Again, cyclosporine was administered intravenously (15mg/kg/day) for seven to ten days, followed by oral cyclosporine (30mg/kg/day) in tapering doses. He found that cyclosporine trough levels in the extended survival group were greater than 4 000 ng/ml measured by radioimmunoassay. These levels would be too high in the human population with resultant complications. So it was shown that small intestinal transplantation is possible in the large animal model. However, the levels of cyclosporine required to prevent rejection would not be tolerated in the human recipient. Interest then turned to newer immunosuppressants.

FK506 was first isolated in 1984 from fermentation broths of *Streptomyces tsukubaiensis*.[141] Its properties and mode of action are very similar to cyclosporine, as both have phosphatase calcineurin as the common target.[142] Large animal studies have been carried out and the results suggest that FK506 is therapeutically superior to cyclosporine in preventing graft rejection.[143]

It was not only large animal work which allowed the ground-work to be laid for successful clinical small bowel transplantation; small animals also have a role to play. In 1971, Monckik and Russell proposed a model for small bowel transplantation in the rat.[144] The bowel was placed heterotopically and was a technically demanding procedure; however, the initial heterotopic placement of the graft improved survival.[132] This model allowed for the standardization of the technique of small bowel transplantation in the rat, and with the knowledge of the genetic background of the rat, this allowed work to centre around the immunological events in small bowel transplantation.[132] Other rat work included small bowel transplantation orthotopically. This was described in 1973 by Kent and it has been suggested that the more normal environment for mucosal enterocytes is a benefit and facilitates physiological studies following small bowel transplantation.[129] The mouse has also been used as a model for small bowel transplants,[144] but of the small animals, the rat is studied more extensively.

There was initial encouragement from the success of the first animal transplants. However, as previously mentioned, the control of rejection in animal models was a formidable barrier. A similar problem was seen in the first clinical small bowel transplants in the 1960's and 1970's.[146] A total of seven patients underwent intestinal transplants and received conventional immunosuppression of the day -- azathioprine, steroids, and antilymphocyte globulin. There were no survivors with five technical complications and two rejection complications.[147] Five of the cases will be described in more detail below.

In 1967, a 46 year old lady suffered diffuse mesenteric thrombosis and was treated with resection of the intestine from the ligament of Treitz to distal rectum. The patient was transplanted using donor bowel from the ligament of Treitz to the mid-transverse colon on the superior mesenteric artery and vein. Arterial and venous anastomoses were performed to the iliac artery and vein. Soon after the operation, the jejunostomy and colostomy darkened and the patient went into shock and died.[131]

In 1968, 1.7 meters of small bowel was transplanted heterotopically in a patient with total resection of the small bowel secondary to thrombosis of the superior mesenteric artery. Again, the mesenteric vessels were anastomosed to the iliac vessels. Both distal and proximal ends were brought out through the abdominal wall as enterostomies. The allograft became necrotic on the sixth postoperative day and was removed. However, the patient did not survive.[131]

In 1969, an orthotopic intestinal allotransplant was performed in a 35 year old male who suffered colonic polyposis and mesenteric fibromas. The recipient had his jejunum, ileum, and right and transverse colon removed. He was transplanted with an entire small intestine along with the right and transverse colon. The graft's mesenteric vessels were anastomosed to the host mesenteric vessels, the donor jejunum to the host jejunum, and the distal end of the graft was brought out as a colostomy. Immunosuppression consisted of azathioprine, corticosteroids, and ALG. He experienced a rejection episode and was given 6-mercaptopurine. However, the allograft became necrotic and the patient died of septic shock.[131]

In 1969, a young 10 year old male was diagnosed with total small bowel strangulation secondary to a mesenteric band. He did well on TPN but suffered from numerous infections of indwelling catheters. He was transplanted with a one meter graft of ileum taken from the child's mother. The graft's mesenteric vein was anastomosed to the left renal vein and mesenteric artery to the aorta. Both ends of the graft were brought out as proximal and distal stomas. The patient was treated with azathioprine, prednisone, and ALG. The graft did not do well and was removed on the seventh day post-op. The patient continued to have problems with sepsis and gastrointestinal bleeding and died one month following transplantation.[131,148]

In 1970, a 37 year old woman underwent a massive intestinal resection for multiple intestinal polyposis. The entire small bowel and right colon were removed. One and a half meters of lower jejunum and upper ileum were removed from the patient's sister and transplanted. The graft was placed orthotopically in continuity. Intravenous azathioprine and prednisone were used for immunosuppression. She initially did well, but developed GI bleeding and multiple pulmonary emboli 75 days post transplant. She died 79 days post-op.[131]

These results were less than encouraging, and by the mid-1970's, enthusiasm for intestinal transplantation was lacking.[146] The advent of cyclosporine and the success in the animal model reaffirmed the feasibility of small intestinal transplantation.[137,146,147] Again, attempts at clinical transplantation were seen.

The first clinical transplant using cyclosporine was carried out in Toronto in 1986. The recipient was a 26 year old female with Gardner's syndrome and a large abdominal mass diagnosed as a desmoid tumor. The tumor, which encased the entire superior mesenteric artery, was removed after attempts at conservative treatment. The patient was resistant to home TPN and small bowel transplanation was carried out. The donor's entire small bowel was transplanted with the superior mesenteric artery on its aortic cuff anastomosed to the recipient aorta and the superior mesenteric vein anastomosed to the recipient inferior vena cava. Both ends of the intestine were exterorized as stomas. The patient received cyclosporine to maintain trough levels between 200-300μg/ml . She also received solumedrol in tapering doses. Unfortunately the patient developed neurological complications and became comatose. It was thought that this may have been due to severe GVHD and the graft was removed. The graft looked good with patent vascular anastomoses at removal. She never recovered and became inotrope dependent. She died on the tenth post-op day. It is suggested that her neurological complications were a result of cyclosporine-induced central nervous system toxicity.[149]

The next series of transplants using cyclosporine took place in 1987 and 1988 and are reviewed in Grant's paper "Intestinal Transplantation: Current Status".[146] Groups in Chicago (graft removed ten days post-op due to rejection), Kiel, Germany (graft removed 12 days post-op due to rejection), and Paris (two grafts removed one and 206 days post-op due to enterocolitis and sepsis, respectively, with the latter

patient dying) all attempted small bowel transplantation in 1987. In 1988, the London, Ontario group started their transplant program. The first patient was an eight year old girl with severe short gut syndrome secondary to the removal of the entire small intestine for congenital neuromyopathy. She was initially treated medically, but she developed severe liver disease. She was transplanted with the entire jejunum and ileum from a six year old donor with a proximal anastomosis and a distal stoma. The venous drainage was via the portal system, and the arterial supply via an aortic conduit. She was immunosuppressed with cyclosporine (levels between 400-500 ng/ml), steroids, azathioprine, and ALG. She developed pulmonary sepsis, and with a decrease in immunosuppression, she developed graft rejection. The graft was removed on day 15 post-op.[150] A second recipient was a 41 year old woman who had thrombosed her superior mesenteric artery after myocardial infarction, necessitating removal of her entire small intestine and right colon. She was treated medically initially with TPN, but due to an antithrombin III deficiency, she had many problems with thrombosis of venous access sites as well as recurrent infections. Due to the antithrombin deficiency, a combined liver and small intestinal transplant was carried out.[150,151] She developed transient, mild GVHD and an episode of rejection, both of which were easily managed. She was discharged from hospital eight months post-op and her graft remains normal.

In 1990, the Paris group reported their results of small bowel transplantation in children using cyclosporine.[152] Six transplants in five children were carried out. The first patient's graft was marked by early necrosis just after unclamping and the graft

was removed. The patient was discharged home on TPN and was retransplanted two years later. There were three cases of early graft rejection managed with ALG or OKT3. One patient had his graft removed because of early acute graft rejection. Five episodes of delayed acute rejection were seen in four patients. One patient developed severe infection and graft rejection with subsequent removal of the graft. Another patient developed visceral failure at six months and her graft was removed. She died one week later. This left two patients with grafts. One of these two developed chronic rejection manifested by abdominal pain and vomiting. His graft was removed 17 months after transplantation.[152]

The last patient was written up in 1992, two and one half years post transplantation.[153] She is fed entirely enterally. She has developed three rejection episodes despite heavy immunosuppression therapy including cyclosporine (10 mg/kg/day po), methylprednisolone (0.5 mg/kg/48 hours) and azathioprine. The authors discuss the absence of GVHD in this patient, and how this may be attributable to the neonatal origin of the graft. The patient is free of parenteral support six years after transplantation.[154]

In 1993, the Pittsburgh group published their experience of small bowel transplants peformed from May 1990 through November 1992.[155] In all, 30 patients underwent transplantation. 15 were children and 15 were adults. FK506 was used in all cases for immunosuppression as well as steroids and prostaglandin E_1. The transplants were done either as isolated small bowel grafts (nine patients), or combined

liver and small bowel grafts (17 patients), or abdominal multivisceral grafts (four patients). Of the patients receiving isolated small bowel grafts, eight are alive with one patient's graft removed due to rejection. The remaining seven patients with grafts are maintaining their nutritional requirements without TPN support at time of the paper's publication. Of the patients receiving combined liver and small bowel grafts, 13 are alive. Four patients died from sepsis or post-operative lymphoprolifertaive disease. All of the surviving patients, except for one, are completely free from TPN at time of the paper'spublication. Of the patients receiving abdominal multivisceral transplants, all four are alive with functioning grafts at the time of the paper's publication. The authors conclude from their series that intestinal transplantation is becoming a practical clinical reality.

At the Fourth International Symposium on Small Bowel Transplantation held in Pittsburgh in October 1995, there were 15 individual centre reports on clinical small bowel transplantation experience.[154] The transplants consisted of isolated small bowel grafts, combined small bowel and liver grafts, and multivisceral grafts. In total, 116 new clinical transplants have taken place around the world since 1992. Both dedicated transplant centres and smaller centres are now carrying out these technically demanding procedures. In general, the results are good. Many authors attribute the success of small bowel transplantation on new immunosuppressive agents such as FK506. Most believe that small bowel transplantation can be an effective treatment for short bowel syndrome, but there is room for improvement in all aspects of this

procedure to allow intestinal transplantation to be applied routinely in the treatment of short bowel syndrome.

Conclusion

In conclusion, short bowel syndrome is a disease with varying etiologies in both the adult and pediatric population. The patient's response to the disorder, through the process of adaptation, can help in allowing the patient to gain his nutrition and caloric needs through enteral feeding. Unfortunately, adaptation is not always sufficient, and the patient must depend on medical treatment. The use of TPN is not without its complications and can have a high associated morbidity. Several surgical approaches have been attempted over the years in an effort to allow the patient to remain on enteral nutrition. Small bowel transplantation has been investigated since 1959, and, with the advent of new immunosuppressive agents such as FK506, is quickly becoming a routine clinical reality. With a small bowel transplant, the patient with short bowel syndrome will be closer to carrying out a full and normal life without the psychological effects and associated physical morbidity of TPN.

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Chapter III

Introduction

Small bowel syndrome results from a massive loss of small bowel. There are many etiologies to this disorder, and they vary depending on the age group in question. In the pediatric population, by far the most common cause is necrotizing enterocolitis. Other causes include midgut volvulus, atresia, gastroschisis, and congenital short bowel. In adults, etiologies include mesenteric ischemia, Crohn's disease, radiation enteritis, tumors, and trauma.[1,2] Although the process of intestinal adaptation allows improved nutrient absorption in the patient with short bowel syndrome, the patient rarely, if ever, returns to a normal physiological state following massive small bowel loss.[3,4]

Various adjunctive surgical procedures have been attempted in order to improve the patient's nutritional status, but most have met with limited success.[5,6] Medical therapy in the form of total parenteral nutrition (TPN), has served as the basis for treating these patients. However, TPN is not without its complications,[7,8,9,10,11] perhaps the worst of which isTPN induced liver failure, which has an attendant mortality rate greater than 60% when bilirubin levels rise over 150-200 μmol/l.[12] Thus, although medical therapy is the best option at present, it is not without a significant morbidity and mortality. It is this poor outlook which has led to a crisis in therapies, and is the push behind our efforts to perform intestinal transplantation. An adequate

treatment for short bowel syndrome should provide a means of sufficient caloric intake with minimal morbidity and mortality to the patient. Neither medical nor traditional surgical therapies have been successful in fulfilling these provisions. If clinical small bowel transplantation could be performed with an acceptable morbidity and mortality, it would offer a more physiologic approach to the problem of short bowel syndrome. It is this goal to which we are striving.

Early attempts at small bowel transplantation ended in failure.[13,14] Without an adequate means of preventing rejection, it was believed that the treatment would never be a success. It was not until the late 1970s, with the advent of the immunosuppressant cyclosporine that small bowel transplantation began to seem a real possibility.[15] Both Reznick and Craddock showed that extended survival was possible after small intestinal transplantation in the canine model.[16,17] Successful transplants in the pig were carried out by Ricour in 1983[18] and Grant in 1988[19]. Grant required high doses of cyclosporine to maintain levels greater than 4 000 ng/ml in order to achieve long-term survival. These levels would not be tolerated in the human, with the resulting complications and some doubted at this time that small bowel transplantation would ever be clinically applicable. Nevertheless, these studies created some enthusiasm.

Unfortunately, early clinical transplant attempts ended in failure,[14,20] due to both technical problems and rejection, even though the prognosis has improved with the advent of cyclosporine.[20,21] In the 1980s, groups in Toronto, Chicago, Kiel, and Paris all attempted clinical transplantation with cyclosporine immunosuppression with

varying degrees of succeess.[20,22] In 1990, the Paris group carried out six transplants using cyclosporine. There were problems ranging from early graft necrosis to chronic rejection. One patient, six years after transplantation, is free of parenteral support.[23,24]

The discovery of FK506 was a great advance in the field of clinical small bowel transplantation. FK506 was first discovered in the early 1980s in Japan.[43] The drug is 50 to 100 times more potent than cyclosporine and has a better therapeutic index.[25] FK506 works primarily through its inhibition of the signal transduction pathway following T-lymphocyte activation.[26] The drug binds to its intracellular receptor, an immunophilin, known as FK-binding proteins (FKBP).[27] The drug-receptor complex has calcineurin as its target, a calcium and calmodulin dependent phosphatase which, in its unbound state, dephosphorylates the nuclear factor of activated T-lymphocytes (NF-AT).[28] This nuclear factor, when dephosphorylated, binds to DNA and allows transcription of several genes coding for early cytokines such as IL-2.[29] Thus, when calcineurin is bound, cytokine production is inhibited. It is here where FK506 has its mechanism of action. Its introduction spurred hope that the problem of rejection, which has plagued small bowel transplantation could be overcome. It is important to note, however, that there have been no clinical trials looking at the efficacy of FK506 in small bowel transplantation and the animal literature is sparse.

The Pittsburgh group published their experience in 1993 of small bowel transplants performed from May 1990 through November 1992 using FK506 based immunosuppression. A total of 30 patients received a combination of isolated small

bowel grafts, combined liver and small bowel grafts, or abdominal multivisceral grafts with a generally good success rate.[30]

At the Fourth International Symposium on Small Bowel Transplantation held in Pittsburgh in October 1995, 15 centres reported on clinical small bowel transplantation.[23] 116 clinical small bowel transplants have taken place around the world since 1992 with improving results. There has been a significant reduction in morbidity and mortality over earlier transplants. Many have attributed the improved success of small bowel transplantation to the new immunosuppressive FK506, which has proven successful not only in clinical small bowel transplants, but also in heart, liver, and kidney transplantation.[31]

Most successful small intestinal transplants have used total length allografts.[32,33,34] However, a few recent reports have discussed segmental small intestinal transplants which used grafts from living-related donors.[35,36] Living-related small bowel would solve the current shortage of small bowel grafts. Presently, many patients die waiting for transplants due to the lack of available allografts. Living-related segmental intestinal transplantation would help alleviate this problem as a portion of small bowel can be removed from a living donor and transplanted into the recipient, much the same as living-related kidney transplantation which has proven itself as a viable option over the years. A further benefit of segmental transplantation is immunological. Not only can closer HLA matching be achieved with presumably improved rejection control, but the reduction in the amount of lymphoid tissue in the

intestinal graft lessens the severity of GVHD (graft versus host disease).[37] For these reasons, the model of living-related segmental intestinal transplantation deserves further investigation. Our model uses a small bowel allograft based on the ileocolic artery and vein which are appropriate end vessels on which a sufficient length of small bowel can be harvested for transplantation with minimal morbidity to the donor.

Using a pig model, our study examined intestinal segment transplantation with FK506-based immunosuppression. Our study used the pig model as a large animal model, as previous studies have shown it to be immunologically, physiologically, and anatomically similar to the human.[93,94] We tested the hypotheses that 1) segmental transplantation in which the intestinal graft's vascular supply is based on the ileocolic pedicle is possible with FK506 based immunosuppression, and that 2) FK506 treatment would allow pigs to grow at a normal rate, suffering no nutrient absorption or intestinal adaptation problems.

Materials and Methods

Study Design

The study design was based on previous work where transplantation of an intestinal segment based on the superior mesenteric artery and vein was possible with cyclosporine-based immunosuppression.[38] In this study, we looked at three groups of outbred female Yorkshire Landrace pigs. The first group (resection) served as controls and underwent a small bowel resection of all but the terminal 150 cm of small bowel as shown in Figure I. This group tests whether the animals are capable of

growing on a 150 cm intestinal segment, and will generate information on normal intestinal adaptation following massive small bowel resection. The second group (resection + FK506) underwent an identical operative procedure but received an immunosuppressive regimen of FK506 and steroids. This group, when compared to the resection, will show any effects of the immunosuppressive regimen on animal growth and intestinal adaptation. The third group (transplant + FK506) was transplanted with a 150 cm segment of distal small bowel in a two-stage operation. The graft's vascular supply was based on the ileocolic artery and vein (ileocolic pedicle) as shown in Figure II. At Stage I, the graft was initially placed in a heterotopic position (Figure III), and the recipient animal retained all its native small bowel. At Stage II, in a second operation 14 days later, the native small bowel was resected, and the graft was placed in continuity (Figure IV). These animals received an identical immunosuppressive regimen to that of the resection + FK506 group. Mixed Lymphocye Culture (MLC) assured discordant grafts. This transplant + FK506 group tests whether segmental intestinal transplantation is possible with the graft's vascular pedicle based on the ileocolic artery and vein. This group allows us to examine the effects of transplantation on animal growth and nutrient absorption.

All resection and transplant pigs weighed between 13-17 kg. The donor pigs weighed between 25-30 kg.

The resection and resection + FK506 animals were followed for a four week period following the initial bowel resection. The transplant + FK506 animals were

followed for a four week period after the Stage II hook-up procedure. Animals were weighed twice weekly. Stool records were kept. Animals that lost greater than 20% of their initial post-op body weight were euthanized.

At the end of the four week study period, *in vivo* intestinal permeability was assessed by urinary recovery of ^{99}Tc-DTPA. *In vitro* permeability studies were carried out using Ussing chambers. The animals were anaesthetized and a repeat laparotomy performed. Samples of normal jejunum and ileum were also obtained at the time of the donor procedure. Results from the resection animals were compared to normal ileum, providing information on the normal adaptation response of the small intestine. *In vitro* permeability results from smaples taken from the resection + FK506 and transplant + FK506 animals were compared to those taken from the resection animals to see what effects FK506 and transplantation have on intestinal adaptation. Proximal and distal small bowel, liver, and skin biopsies were taken for histology. Following tissue sampling, the animals were euthanized with sodium pentobarbital (100 mg/kg).

All animals were housed in individual pens which provided a standard 12 hour day/night cycle. Room temperature was maintained at 22 ± 2°c. Animals had free access to feed (Start S200, Sunglo Feeds, Inc., Hesston, KS, providing 56% carbohydrate, 6% fat, and 38% protein) and water except during the immediate post-operative period. The animals were fasted for 12 hours pre-operatively.

Our experimental protocol was approved by the Animal Use and Welfare Committee at the University of Missouri at Kansas City.

Operative Technique

Anaesthesia

Anaesthesia consisted of a ketamine (20 mg/kg IM) and xylazine (2 mg/kg IM) induction, followed by endotracheal intubation (using a #6 cuffed endotracheal tube) and isoflurane inhalation (1-2%) via mechanical ventilation. Tidal volumes were 10 ml/kg. Continuous pulse oximetry was monitored throughout general anaesthesia. Following the operative procedures, isoflurane was discontinued and the animals received buprenorphine 0.1 mg IM once extubated. They were closely monitored until considered stable when they were returned to their pens.

Donor Operation

Following induction of anaesthesia, a vertical midline incision was made and the abdominal cavity entered. The proximal and distal ends of a segment running 150 cm proximal from a point 10 cm proximal to the ileocecal valve were transected using a GIA stapler. A 2 cm length of small bowel at the segment's proximal end, together with a 2 cm segment of jejunum from a site just distal to the ligament of Treitz were taken for Ussing chamber analysis. The ileal segment's vascular supply, based on the ileocolic artery and vein, was isolated. Heparin (50 units/kg IV) and phentolamine (0.5 mg/kg IV) were administered. The graft was removed following proximal vascular clamping, immediately placed on ice, and flushed with one litre of lactated Ringer's + 2.4% Mannitol with 2 000 units of heparin per litre. Iliac vessels were retrieved for use as vascular conduits if necessary. Blood was drawn for MLC studies, and the animal

was euthenized using sodium pentobarbital (100 mg/kg). The recipient animal was then prepared.

Recipient Operation

Stage I

A #7 French double lumen Broviac catheter was introduced via cut-down into the right external jugular vein; ports exited from the back, posterior to the scapula. A vertical midline incision was used to enter the abdominal cavity. The abdominal viscera was packed to the left of midline and the posterior peritoneum was dissected off the inferior vena cava (IVC) and aorta, at a point inferior to the renal artery and vein. The IVC and aorta were then cleared of surrounding connective tissue. We took care not to injure the lymph sac on the anterior aorta. The animal was given heparin (50 units/kg IV) and the graft was brought into the operative field. A side clamp was placed on the IVC, and a IVC was incised. An end-to-side venocaval anastomosis was performed using a running 6-0 prolene suture. The side clamp was removed from the IVC after a bulldog clamp was applied proximal to the anastomosis. A side clamp was placed on the aorta, and the aorta was incised using a #11 blade and a 2.5 mm aortic punch. An end-to-side anastomosis of graft ileocolic artery to recipient aorta was made using a interrupted 6-0 prolene suture. Prior to revascularization of the graft, 10 mg/kg of calcium chloride and 1 mEq/kg of sodium bicarbonate were given intravenously. Clamps were then removed and the graft was reperfused. The distal end was brought out as a stoma on the anterior abdominal wall. The graft was left in a

heterotopic position. The abdomen was closed using a running 0-Vicryl suture, and the skin closed with interrupted simple 4-0 Vicryl suture.

Stage II (Hook-up)

Fourteen days later, a double lumen #7 French Broviac catheter was inserted into the left external jugular vein of the recipient pig. The abdomen was entered through a vertical midline incision. All adhesions were taken down by sharp dissection. The transplanted bowel was examined, and if it appeared viable, the operation proceded. The native small bowel was resected between a point 10 cm distal to the ligament of Treitz and a point 10 cm proximal to the ileocecal valve. The stoma was taken down, and the graft was placed in continuity using end-to-end anastomoses created with a single layer of interrupted 4-0 silk sutures. The abdomen was closed with simple interrupted 0-Vicryl sutures and the skin closed with simple interrupted 4-0 Vicryl sutures.

Resection Operation

The resection and resection + FK506 groups were all treated in exactly the same manner operatively except that all the small bowel, from a point 10 cm distal to the Ligament of Treitz to a point 160 cm from the ileocecal valve, was resected. A jejunoileal anastomosis was performed . A "sham" transection 10 cm proximal to the ileocecal valve was performed, and was followed by re-anastomosis. The anastomoses were single layer using interrupted simple 4-0 silk sutures. The abdomen was closed

with a running 0-Vicryl suture, and the skin approximated using interrupted simple 4-0 Vicryl sutures.

Post-operative Care

All animals were allowed water *ad lib* following the operations. In addition, they received intravenous D5 lactated Ringer's (10-20 ml/kg BID) until oral intake was judged adequate according to urine output. Animals in the resection and resection + FK506 groups, and the transplant + FK506 in Stage I of the procedure were given solid food on post-operative day two. The transplant + FK506 animals in Stage II of the procedure (hook-up) were given water *ad lib*, but solid food was withheld until the animal passed stool. Solid food was supplemented with an enteral formula (Vivonex, Sandoz Nutrition Corp., Minneapolis) which supplied 300 kcal/day. Analgesia in the form of buprenorphine (0.1 mg BID IV) was given for two days post-op. Cefazolin (500 mg bid) and ranitidine (25 mg bid) were administered while the Broviac catheters remained in place. The catheters were removed three to five days post-op unless they were required for prolonged fluid management.

Immunosuppression

FK506 and steroids formed the basis for immunosuppression. The immunosuppressive protocol has as its basis the clinical immunosuppressive protocols used for liver and intestinal transplantation.[39,40,41,42,43] Resection + FK506 and transplant + FK506 groups received identical regimens. FK506 was initially given intravenously (0.1 mg/kg BID) over a three to four hour period beginning four hours

post-op. On post-operative day three, FK506 was given orally, starting at 0.5 mg/kg/day in divided doses. The intravenous and oral doses were adjusted to keep blood levels between 10-20 ng/ml. Levels were routinely drawn on post-operative days two and five and then were taken only as required. Doses were modified according to levels. Levels from whole blood were determined using a microparticle enzyme immunoassay (IMX, Abbott Laboratories, Abbott Park, IL). Methyl-prednisolone was also given following the operative procedure. The dose was 4 mg/kg/day IV for post-operative days one and two and 2mg/kg/day IV for post-operative days three and four. 1 mg/kg/day of oral prednisone was given on post-opertative days five and six, and 0.5 mg/kg/day of prednisone was given for the rest of the experimental period. An equivalent dose of prednisolone was given intravenously to recipient pigs following the hook-up procedure, and oral prednisone was substituted when oral feeding was initiated.

Mixed Lymphocyte Culture (MLC)

Mixed lymphocyte culture assays were performed at the Midwest Organ Bank, Wichita, Kansas. The technique for MLC has been described in detail elsewhere.[47,48] In brief, stimulator cells irradiated with 3000 rads of gamma radiation from recipient animals were mixed with responder cells from donor animals. The cells were incubated for 7 days and then pulsed with ^{3}H-Thymidine. The cells were incubated for a further 18 hours to allow for the radioactive label to be incorporated. The cells were

harvested using a cell harvester and the β-radiation emitted was measured using a scintillation counter.

Histopathology

All histology specimens were fixed in 10% buffered formalin. 4μM sections were prepared, and slides were stained with hematoxylin and eosin. Morphological measurements using an ocular micrometer examined villus length, crypt to villus ratio, and *muscularis propria* thickness. Rejection criteria were based on general architectural changes and the extent of inflammatory cell infiltrate as presented in Table I. Skin and liver were examined for evidence of occult graft versus host disease (GVHD) in the transplant + FK506 group. Photomicrographs were prepared using an Olympus photomicroscope.

Permeability Studies

In Vivo Permeability

Three days prior to euthanization, a ^{99}Tc-DTPA *in vivo* permeability study was carried out. Animals were gavaged using 2 mCi ^{99}Tc-DTPA in 2 ml of distilled water. Animals were then placed in individual metabolic cages and allowed access to water *ad lib*. After seven hours, the total urine volume was measured, and two 1 ml aliquots of urine were analyzed with a gamma counter. Prepared test solutions were also analyzed. The percent recovery of the labelled probe was calculated as follows: % ^{99}Tc-DTPA recovery = (total urine volume x dpm/ml)/(test solution volume x dpm/ml x dilution factor).[49]

In Vitro Permeability

In vitro permeability studies examined the dominant pathway for glucose absorption as described by the sodium-glucose co-transport theory. Glucose enters the enterocyte via facilitated diffusion, driven by a sodium concentration gradient, maintained by a Na^+-K^+ ATPase system. Once in the enterocyte, the glucose diffuses into the interstitial fluid and then into the blood.[95] Measurement of the intestinal short circuit current (Δ-Isc) with the addition of glucose allowed us, through mathematical manipulation of the data, to determine the maximal velocity of glucose transport (V-max), and the carrier affinity (Km).[96]

In brief, segments of bowel resected for *in vitro* permeability studies were flushed with ice-cold Ringer's and preserved in iced Ringer's containing 20 nM fructose. Intestinal segments were split along the mesenteric border and 2 cm sections were stripped of their serosa and *muscularis propria*. The sections were clamped into Ussing chambers and bathed in normal Ringer's solution bubbled with carbogen (95% oxygen, 5% carbon dioxide). The solution had a pH of 7.4 and a temperature of 37°C.

Addition of glucose in 2,4,8,16, and 64 mM increments at 10 minute intervals was used to determine Vmax, Km, and Δ-Isc. Steady state conditions were ensured with a 20 minute equilibration period between glucose addition. Transepithelial electical potential (PD) was recorded electronically as the 100 μA current clamping the tissue was shut off for 3-5 seconds every 10 minutes. Tissue resistance and conductance were calculated using Ohm's law.

The serosal chamber contained 1 mM ^{3}H-inulin and 1 mM ^{14}C-mannitol. Seven 1 ml aliquots were taken from the "cold" mucosal chamber prior to glucose additions, allowing the calculation of the serosal to mucosal (J_{sm}) isotope flux at differing glucose concentrations. Radioactivity was measured using a Packard 1900CA Tri-Carb Liquid Scintillation Analyzer.

Tissue viability was assessed at the termination of the experiment by the addition of 5 mM theophylline to the mucosal chamber. Tissue pairs were discarded if electrical conductivity varied by greater than 15%.[47,48,50,51,52,53]

Statistical Analysis

Statistical analysis was performed using the SPSS statistical package. Data are expressed as the mean ± standard deviation. Means were compared using ANOVA. Multicomparison tests were carried out using the Tukey HSD test. Survival curves were generated according to Kaplan-Meier. The generalized Wilcoxon test was used to compare survival rates. Differences in proportions were compared using the χ^2-test. A probability value of less than 0.05 was considered significant.

Results

Survival and Complications

All animals in the resection and resection + FK506 groups survived the four week study period. There were no complications. These animals were playful and had good appetites.

Animals in the transplant + FK506 group did not fare as well; early deaths resulted from technical, infectious, and rejection complications.

Transplant + FK506 animal survival data is presented in Table II. In the early phase of technical development, five animals died on day zero to one of vascular complications, and we have excluded these animals from the statistical analysis. Two animals died from small bowel obstructions (T8 and T9), one at day three, and one at day 17, three days following the hook-up procedure. This group experienced a technical failure rate of 22%

Rejection was seen in two animals (T12, T11) at days six and 14. Following hook-up, T10 was sacrificed at day 33 because of cachexia, and evidence of rejection. The longest surviving transplant, T5, was sacrificed on day 42; the graft was showing evidence of rejection. Two animals, T7 and T13, died following anastomotic breakdown. We suspected that these breakdowns were secondary to rejection, but the pathological specimens were not interpretable due to post-mortem autolysis. The clinical course of T7 and T13 were identical to those animals showing rejection, and we have thus included these animals into them into the rejection analysis. These animals all showed a similar pattern of rejection -- cachexia, lethargy, weight loss, and prolonged diarrhea. The overall rejection rate was 66%.

T14 also showed a similar clinical course for rejection, but this animal developed a pneumonia, manifested by a cough dyspnea. This animal showed a rapid deterioration, and at sacrifice, examination of pathological specimens showed

complete ischemic necrosis of the intestinal graft and lung consolidation. Pneumonia is a common end-stage problem with debilitated animals, and although rejection might have contributed to this debilitated state, we have included this animal in the infectious complication group. This produces an infectious complication rate of 11%.

Although the technical and infectious complication rates in the transplant + FK506 group are higher than the resection or resection + FK506 groups, the difference does not reach statistical significance.

Survival curves for all three groups are shown in Figure V. Only transplant animals surviving after the Stage II operative procedure (n=6) were included in survival analysis in order that equal four week periods can be studied. The transplant + FK506 animals survived 12 ± 10 days. The resection and resection + FK506 groups both survived 28 ± 0 days. The transplant + FK506 animal survival rate differs significantly from that of the resection and resection + FK506 groups ($p<0.05$).

Weight Gain/Loss

Weight change over the study period is summarized in Table III and Figures VI and VII.

Animals in the resection group all thrived. The mean weight gain was 37% ± 19.

Animals in the resection + FK506 group all had prolonged diarrhea and no animal produced a solid stool. The mean weight loss was 18% ± 8 ($p<0.05$).

All animals in the transplant + FK506 group also experienced prolonged diarrhea. Only animals surviving longer than one week after hook-up were included in weight gain analysis. These three pigs, T5, T10, and T14 averaged a weight loss of -22% ± 2 ($p<0.05$).

Immunosuppression

Animals in the resection + FK506 group had an FK506 level of 13.2 ± 2.7 ng/ml. Animals in the transplant + FK506 group had an FK506 level of 16.1 ± 9.4 ng/ml. These levels are not statistically different.

Mixed Lymphocyte Culture (MLC)

MLC assays between the donors and recipients in the transplant + FK506 group were all reactive. The results are shown in Table IV and are expressed as counts per million (CPM) and stimulation index (SI).

Histopathology

The results from small bowel, liver and skin histology from animals in the transplant + FK506 group is shown in Tables V. Two animals showed severe rejection (T11, T12), one animal showed moderate rejection (T10), and one animal showed mild rejection (T5). Representative photomicrographs of mild, moderate and severe rejection are shown in figures VIII, IX, and X. Skin and liver sections did not show any evidence of GVHD.

Villus height, crypt to villus ratio (C:V), and *muscularis propria* thickness for all the groups are presented in Table VI.

Villus height is greatest in the resection group with a crypt to villus ratio (C:V) of less than one in both the proximal and distal segment (Figure XI). In the resection + FK506 group, there was marked villus blunting with the villus height approaching 50% of control height (Figure XII). The villi were found to be significantly shorter than proximal and distal controls ($p<0.05$). A more active crypt zone was present in the resection + FK506 group, which had a significantly higher C:V proximally and distally than proximal and distal controls ($p<0.05$). The transplant + FK506 group showed no statistical difference when compared to controls, but there was a trend towards a lower villus height and an increased C:V (Figure XIII). There was no significant difference between the resection + FK506 and transplant + FK506 groups.

Within each group, there was no significant difference between villus height and C:V when measured at the proximal and distal ends of the segment.

The thickness of the *muscularis propria* did not differ significantly among the three groups.

Permeability Studies

In Vivo Permeability Studies

In vivo permeability studies are summarized in Table VII. Normal animal data shown in the table is for comparative purposes only, but is not part of the study. When compared to normal animals, the resection group shows a significantly lower permeability reflected in a smaller percentage recovery of ^{99}Tc-DTPA. Of the resection and resection + FK506 groups, the latter showed a significant increase in permeability

to ^{99}Tc-DTPA. There was a decrease in permeability in the transplant + FK506 group, but the decrease was not statistically significant due to the small sample size (one pig).

In Vitro Permeability Studies

Ussing chamber analysis gave data for Δ-Isc, V-max, and Km, along with overall serosal-to-mucosal flux of ^{14}C-Mannitol and ^{3}H-Inulin. Data comparing normal ileum to the proximal and distal segment of the resection group is summarized in Table VIII. A significant decrease in Δ-Isc and V-max was seen in both the proximal and distal portions of segments; numbers tended toward normal jejunal values. Km, the Michaelis constant, was significantly increased in the distal portion of the segment; a proximal increase did not reach significance. The serosal-to-mucosal flux of inulin was significantly decreased in the proximal segment; it was also decreased in a non-significantly manner distally. Again, these changes tended towards normal jejunal values.

When compared to control resections, there was a significantly higher Δ-Isc and V-max in the resection + FK506 group's proximal segment. There was no significant difference in the distal segment. Neither Km nor mannitol and inulin serosal-to-mucosal fluxes show any significant difference.

Only one animal in the transplant + FK506 group had bowel suitable for Ussing chamber analysis. Although our findings are not statistically significant, this sample did show a decrease in Δ-Isc and V-max in both proximal and distal segments with a greater decrease seen proximally when compared to controls. Km was increased at

both ends of the segment and bowel permeability to inulin and mannitol was increased proximally.

Changes in Δ-Isc related to intestinal adaptation, FK506 treatment, and transplantation are further illustrated in Figure XIV. Within the normal group, the ileum had a higher Δ-Isc than did the jejunum. Note that in the resection group, both the proximal and distal segment were ileum. Here, the adaptation response caused a decrease in Δ-Isc both proximally and distally; a larger proximal decrease indicated that this segment was behaving like normal jejunum. The distal segments in the resection and resection + FK506 groups behaved similarly, but glucose absorption was significantly greater in the proximal segments of the resection + FK506 group. This higher transport capacity suggests that FK506 inhibits the normal jejunalization of the proximal segment. The transplant + FK506 group showed a decrease in Δ-Isc both proximally and distally, with a greater decrease occurring proximally. Again, no significant difference was seen because of the small sample size.

Discussion

This study was designed not only to investigate the feasibility of segmental small intestinal transplantation on animal growth and nutrition, but also to examine the effects of FK506 based immunosuppression on animal growth, nutrient absorption, and intestinal adaptation.

The FK506-based immunosuppression protocol used in our study is very similar to that used in clinical transplant medicine.[39,40,41,42] As we will see in the

upcoming pages, the immunosuppressive regimen was found to have many effects on the animals, both at a macroscopic and microscopic level. It is difficult with our model to say that all the effects are due solely to FK506, with corticosteroids playing an important role in the immunosuppressive regimen. However, in the past, several studies have used high dose corticosteroids in the swine model looking at allograft survival.[54,55,56,57,58,59,60,61] Heart, pancreas, and kidney transplantation experiments in pigs have all used corticosteroid immunosuppression, either alone or in combination with other drugs such as cyclosporine or azathioprine. Doses of corticosteroid range from 0.5 mg/kg/day to 1 gm/day with study periods ranging from two weeks to one and a half years. No study reviewed looks at weight gain of the animals as critically as we have done. One study examines the effects of pancreatectomy followed by segmental pancreatic allotransplantation. A control group of non-pancreatectomized pigs transplanted with a segmental pancreatic allograft received high dose corticosteroids (Methylprednisolone 1 gm/day for seven days, followed by 1 mg/kg/day of prednisone) showed a low overall mortality, suggesting that the corticosteroids has no adverse effects on the animals. However, specifics of weight gain or weight loss were not discussed, and the study period was only one month. A study looking at cardiac transplantation using azathioprine and prednisone based immunosuppression showed animals with a survival period of greater than one year. Again, there were no problems of weight loss discussed. A study looking at small intestinal transplantation using FK506 or cyclosporine-based immunosuppression

together with corticosteroids in similar doses to those used in our study showed the animals had problems with weight loss of 200-500 g/day.[60] However, in this study, the control animals not receiving immunosuppression with resection of the large bowel and creation of a distal ileostomy lost a similar amount of weight. From these studies, it is not clear whether corticosteroids have any adverse effects on intestinal function. It is unfortunate that a study has not been caried out examining the effects of corticosteroids on nutrient absorption. To be confident that corticosteroids cause no ill effects on intestinal function in our study, another group of experiments needs to be designed to investigate the independent effects of FK506 and corticosteroids.

Previous transplant studies have shown that 150 cm of small bowel is an adequate length for animal survival.[37,38] We chose to transplant the ileum not simply because of its greater absorptive potential, but also because of its anatomy.[62] A segment can be easily isolated on the ileocolic vascular pedicle, thus providing us with a model for living-related graft donation. Placing transplanted grafts in the heterotopic position initially has proven successful in several animal models. This procedure provides a simple means of allowing the graft to recover following the transplantation period and is analagous to maintaining the patient on TPN in the immediate post-transplant period.[48,63] We elected to use systemic venous drainage because it is technically easier and safer. The immunological and metabolic advantages of portal drainage are controversial.[64,65,66]

The transplant procedure itself had a high rate of technical complications, especially vascular thromboses. All of the thromboses were seen in the early animals in the transplant + FK506 group with no vascular complications seen after the first six animals. Other studies using a segmental intestinal transplant model in the large animal have not shown such a high rate of technical problems, but these pevious studies have not been as technically demanding as ours.[67] No study has yet attempted to transplant a graft with the vascular supply based on the ileocolic artery and vein. These vessels are small, with an internal diameter averaging two to three millimeters. There was a change in surgical technique following this initial high technical failure rate. Initially our arterial anastomoses were performed with running vascular prolene, but as the technique of parachuting down the anastomosis was introduced, the patency rate was increased to 100%. Thus, a higher success rate was achieved.

In our transplant group, there were two cases of suspected GVHD (graft versus host disease) recognized by lethargy of the animal and a transient dermatitis. FK506 levels drawn at the time of recognition were slightly below therapeutic values in both animals, and the GVHD quickly resolved following an increase in the FK506 dose. There was no histologic evidence of GVHD seen in any animal at necropsy. Previous studies have shown a similar mild GVHD picture, except for one where a severe picture was seen.[37,48,69,70] This study used cyclosporine-based immunosuppression, and the graft was placed in the heterotopic position.[48] It has been suggested that heterotopic placement of the intestinal allograft may predispose the

animal to develop GVHD, as the recipient's native intestine increases the potential pool of activation sites for donor lymphocytes.[68,71,72] FK506 has been shown to decrease the severity of GVHD when compared to cyclosporine, and it may be for this reason that our cases were few in number and transient in duration.[73]

Infectious complications were seen only in those animals receiving FK506. This group had a high rate of rejection, and we had expected to see a higher incidence of infectious problems in this group. With rejection comes a higher permeability of the bowel resulting in a greater degree of bacterial translocation.[74] We suspect this is the etiology of the pneumonia seen in the one transplant animal. Other studies have shown high rates of infectious complications.[61,75,78] Had our sample size been larger, we would have likely seen more animals with infectious complications, especially with the less than optimal control of rejection.

As we have noted, rejection was a problem in the transplant + FK506 group despite the use of the potent immunosuppressive FK506 along with corticosteroids. While there is, as yet, no clearly defined clinical target defined target range for FK506, levels in this study were in line with the general consensus that a 12 hour trough level of 10-20 ng/ml should be maintained.[77] The nine animals in this group had a total rejection rate of 66%, a rate of rejection greater than those seen in previous studies on the pig which used either cyclosporine or FK506-based immunosuppression. A recent series of segmental small intestinal transplants using a very similar model to ours examined the effect of MHC matching on animal survival following transplant.[72]

Cyclosporine-based immunosuppression was used. The MHC-mismatched group had a 50% rate of rejection. Although our study did not involve MHC matching, we assured discordent grafts through the use of MLC reactions. All were positive. As FK506 is more potent than cyclosporine, we would have expected to see a lower rate of rejection in our study. This suggests that therapeutic FK506 levels in the pig may not be reflective of therapeutic levels in the human. Greussner *et al*'s large series of small intestinal transplants carried out in pigs using FK506-based reports a total rejection rate of 18%.[60]It is possible that the difference in rejection rate is the result of the fact that our animals received FK506 orally for the majority of the study period, whereas FK506 was given intravenously in Greussner's study. The pahrmacokinetics of FK506 are such that there is a high degree of variability in the bioavailability of drug depending on whether the drug is administered orally or intravenously. [78,79] However, it has been suggested that as long as adequate trough levels are maintained and monitored with drug levels, oral administration of the drug is feasible. While we achieved what are considered adequate trough levels through oral administration, these levels were lower than those found Gruessner's study, where levels were maintained between 20-40 ng/ml. Of note, these high trough levels of FK506 were associated with a high rate of infectious complications. Along with the high rejection rate, this difference in trough level-related response suggests that the pig may require higher levels of FK506 than humans do. The immunological compatibility between pig and man becomes an issue.

Although research has shown many similarities between the human and the pig immunological responses, there still remain some unanswered questions.[80] For instance, in humans, T-lymphocytes are activated when their T-cell receptor interacts with donor alloantigen in conjunction with native MHC class I or II. The donor antigen is first processed by antigen presenting cells (APC's which include B-cells, macrophages, and dendritic cells), then the antigen is expressed on the cell surface of the APC in conjunction with MHC antigen and is presented to the T-lymphocyte. CD4+ T-helper lymphocytes recognize antigens complexed to class II MHC and CD8+cytotoxic T-lymphocytes recognise antigen complexed to class I MHC. T-lymphocytes in man are thus said to be MHC-restricted. Activated CD4+ secrete cytokines, including IL-2, which enhances the cytolytic activity of CD8+ cytotoxic T-lymphocytes.[81,82] This cytolytic activity is perhaps the most important factor in acute graft rejection. In the pig, however, there is evidence of a combined CD4+ and CD8+ subpopulation of T-lymphocytes making up 25% of the pig's lymphocyte population.[83] This unique population of cells unique to the pig model could confer a "resistance" to FK506 if the cells exhibit both helper and cytotoxic properties. Once the lymphocyte was activated, not only would the cell produce cytokines, but the cell might also have a direct cytotoxic action. This mechanism would essentially bypass the immunosuppressive effects of FK506. Another unique characteristic of swine T-lymphocytes involves a reversal of the CD4:CD8 ratio. The pig normally has 25% CD4+ lymphocytes and 40% CD8+ lymphocytes (0.6 CD4:CD8 ratio) while the ratio

seen in humans is 1.5-2.0.[83] The functional importance of these unique features of swine T-lymphocytes is not clear. It may be these differences which necessitate higher FK506 levels in the pig in order to maintain a rejection-free graft.

Renal allograft models using the pig with cyclosporine-based immunosuppression have found that similar levels of cyclosporine are required to prevent rejection in the pig when compared to humans. Cyclosporine levels of 300-400 ng/ml measured by RIA are suggested in the human to prevent rejection.[97] One paper examined renal allograft rejection in the pig. Cyclosporine levels were maintained at 100 ng/ml, levels stated as being slightly sub-therapeutic.[98] Unfortunately, the method of level determination was not discussed. Nevertheless, we can conclude that both human and pigs require roughly similar levels of cyclosporine to prevent renal allograft rejection. Why then is a higher dose of FK506 required in the pig? The small bowel is unique in that a transplanted allograft has altered permeability characteristics when compared to normal small bowel. Presumably the degree of organ injury during harvesting and re-perfusion alters these permeability characteristics. We hypothesize that with an increase in permeability, the antigen load presented to the host is increased with a subsequently stronger rejection response. Allograft injury may have been greater in our model when compared to the human situation. Our model used lactated Ringer's + 2.4% mannitol which potentially did not protect the graft as well as the more expensive University of Wisconsin solution used in human allograft harvesting.[99] This is speculative, but suggests a possible reason for the higher level of

FK506 required in the pig to prevent allograft rejection when compared to the human. A greater degree of injury during harvesting initiates a stronger immune response by the recipient, which, in turn, necessitates a higher level of FK506 to prevent rejection.

However, FK506-based immunosuppression was seen to adversely affect the overall well-being and survival of these animals. Although there was no significant difference between the resection and the resection + FK506 groups in terms of survival, a significant weight loss was seen in those animals receiving FK506. No animals in the resection + FK506 group were sacrificed because of weight loss, but all experienced prolonged diarrhea, and all but one animal came close to our criteria for euthanization. Similarly, all animals in the transplant + FK506 group had prolonged diarrhea. Of the transplanted animals which survived longer than one week following orthotopic placement of the graft, two were euthanized for weight loss secondary to rejection. The surviving animals approached the 20% weight loss criterion but only one lived the entire four week period. This animal showed histologic evidence of rejection. While rejection would explain the rapid and severe weight loss and prolonged diarrhea in the transplant animals, it cannot explain the weight loss and diarrhea in the resection + FK506 group. Perhaps FK506 altered the normal adaptive changes seen in small bowel following resection.

Intestinal adaptation is a normal process following small bowel resection and allows for improvement in the small bowel's nutrient absorptive capacity.[84] In man, the early phase of short bowel syndrome is characterized by massive diarrhea. Over two

weeks to three months, the remaining small bowel adapts, nutrient and fluid absorption improves, and the diarrhea decreases in severity.[84] The time period necessary for this early phase of adaptation seems to be shorter in the pig. The resected pigs in this study produced solid stool in three to four days and ... well, after an initial period of weight loss. Similar results have been seen in ... r study.[85] In our study, the pigs which underwent a small bowel resection and received FK506 did not follow this expected course. Thus, we hypothesize that FK506 slows this normal recovery period by impairing intestinal adaptation.

Work on adaptive morphological changes in the pig model has shown a significant increase in villus height, resulting in an increase in villus surface area and a corresponding increase in absorptive surface.[85] Our resection group showed a similar pattern, displaying a crypt to villus ratio of less than one. The resection + FK506 group had significantly shorter villi with a deeper crypt zone, resulting in a crypt to villus ratio of greater than one. This increase in crypt depth relative to villus height could explain the diarrhea seen in this group of animals as the resulting increase in secretion would cause a secretory diarrhea. The histologic picture is similar to that seen in celiac disease, which is accompanied by a striking blunting of the intestinal villi.[86] It thus appears that FK506 significantly impairs the normal adaptive response. As one animal in the transplant + FK506 group had bowel suitable for histological study, no statistical analysis was possible. However, the villi of this specimen did appear blunted. Unfortunately, evidence of mild-moderate rejection was also seen in

the specimen. As blunting is part of the normal rejection picture, it is difficult to assess the relative importance of FK506 and rejection to the blunting process.

The normal adaptive response involves crypt cell hyperplasia and increased cell turnover. If the process of migration from crypt to villus is slowed, an increase in crypt cell population and a decrease in villus cell population would develop. Cell turnover is an active process and is energy dependent. has been shown to impair normal mitochondrial function at the enterocyte level causing a decrease in ATP production.[87] We therefore hypothesize that the non-adaptive changes seen with FK506 administration may be due to metabolic derangements at the enterocyte level. Alternatively, FK506 may adversely affect the function of hormones on intestinal adaptation. For example, enteroglucagon has been shown to have a strong trophic effect on the small bowel during small intestinal adaptation.[100,101,102] FK506 functions through its inhibition of calcineurin, a calcium and calmodulin-dependent serine phosphatase.[103] Enteroglucagon functions by binding to cell surface receptors, and stimulating cAMP.[104] Downstream steps in enteroglucagon's biochemical pathway also involve calcium and calmodulin-dependent reactions.[105] Thus, if there were a protein which interacts with FK506 in such a way as to prevent the calcium and calmodulin-dependent reactions, enteroglucagon may be prevented from exerting its trophic action, thus inhibiting adaptation. This is speculative, and more research is required to investigate the potential effects of these FK506 binding proteins in enterocytes.

Electrophysiological analysis of nutrient absorption reveals the functional changes associated with a normal adaptive response. The sodium gradient which drives the co-transport mechanism by which glucose is absorbed is maintained by a sodium-potassium ATPase.[88] Ussing chamber measurements of intestinal short circuit current (Δ-Isc) and the derivation of V-max provide a direct picture of transepithelial glucose transport capacity.[49,50,51] The resection group in our study showed a decrease in the transport capacity in both the proximal and distal portion of the ileal segment when compared to normal ileum, with values tending towards those of normal jejunum. A greater decrease is seen proximally. Thus, the proximal segment, that is the portion of the ileal segment which is exposed to the environment normally experienced by the jejunum, behaves like jejunum. Therefore, the normal adaptive response of the ileal segment is jejunalization. The resection + FK506 group showed a different adaptive response. Essentially, FK506 prevented the proximal portion of the ileal segment from becoming jejunalized to its full potential. Samples from this group showed adaptation, but they displayed a significantly higher transport capacity in the proximal ileal segment than did the resection group. Along with higher levels of glucose transport, an overall higher level of *in vivo* intestinal permeability as measured by recovery of ^{99}Tc-DTPA was seen. As FK506 has been shown to affect the mitochondria's ability to produce ATP, and as the uptake of glucose is an active, energy dependent process, this higher glucose uptake is difficult to explain. Perhaps the increase in nutrient absorption compensates for the lack of increase in surface area

which was seen in a previous study.[85] We hypothesize that because the bowel surface area could not increase in response to intestinal resection when treated with FK506, the upregulation of glucose transport may be a secondary adaptive response. Other studies on FK506 have shown a decrease in glucose transport at low doses and an increase in transport (with an increase in *in vivo* permeability) at higher doses.[89] A possible explanation would involve the contribution of solvent drag to the overall absorption process. Pappenheimer has suggested that solvent drag through paracellular pathways (intercellular tight junctions or pores) can account for a large fraction of glucose transport in the epithelia of the small intestine.[90] The presence of glucose in the gut lumen activates contraction of perijunctional actinomysin rings in the apical region of the enterocyte. The primary role of the sodium-coupled active transport mechanism is to provide the osmotic force for convective flow and to trigger contraction of cytoskeletal proteins.[90] With contraction comes the opening of the tight junctions which, in turn, allows transport of nutrients by slovent drag.[90] The morphology of these pores has been shown to be highly dependent on ATP levels within the enterocyte; a dramatic decrease in transepithelial resistance occurs as energy stores are depleted.[91] Accordingly, as ATP levels are decreased by FK506 administration, a conformational change in the tight junction might cause a significant increase in glucose absorption. With this model, although there is a decrease in transepithelial, actively-mediated transport of glucose, there is an increase in paracellular absorption.

In an attempt to quantify the size of the paracellular pores, we studied the flux of ^{3}H-Inulin, a large molecule (MW 5500 d), and ^{14}C-Mannitol, a small molecule (MW182 d) similar in size to glucose. Our results confirm that there is a flux of these non-actively transported molecules, and that the smaller molecule is carried by solvent drag to a greater degree than is the larger molecule. We found a significantly lower flux of ^{3}H-Inulin in the proximal segment of the resection group. This finding is consistent with jejunalization of the ileal segment as a normal adaptive response following small bowel resection. No other changes in the flux of ^{3}H-Inulin or ^{14}C-Mannitol were observed. This change in passive permeability to large molecules can be explained through morphologic alterations seen with adaptation. We hypothesize that hyperplastic morphological changes may alter the characteristics of the epithelial tight junctions, including changes in pore size. If these changes result in smaller channels, then only large molecules would be affected. This theory could explain the relative decrease in ^{3}H-Inulin diffusion in comparison to that of ^{14}C-Mannitol. Electron microscopic examination of the epithelial tight junctions might also reveal differences in morphology which could explain this difference in permeability.

These results confirm the presence of paracellular transport, but they bring up a problem with our explanation of solvent drag's responsibility for the glucose transport increase seen in resection + FK506 animals. As glucose and mannitol are similar in size, an increase in glucose transport via the paracellular route should be reflected by an increase in mannitol transport via a similar route. There has been some

recent reports that there is a significant interspecies variation in the paracellular transport of mannitol. Mannitol transport is low in rats, guinea pigs, and rabbits, and high in cats and humans. This species variation makes it difficult to generalize about paracellular transport in our model using mannitol as a marker.[44]

It is difficult to draw any conclusions from this study about transplantation's effect on the nutrient absorption of the intestinal segment. Although we see a trend toward increased *in vitro* glucose transport in both the proximal and distal portion of the transplant, this animal was undergoing rejection, and the sample size is small.

Other research on sodium-glucose co-transporter mRNA has found a significant increase in mRNA levels as measured by reverse transcriptase-polymerase chain reaction.[92] Functional analysis of nutrient absorption was not carried out in these experiments. Our work did not examine mRNA levels, but the decrease in nutrient absorption experienced by the resected animals as part of intestinal adaptation suggests either a decrease in co-transporter production or an increase in production of a defective enzyme system.

Conclusion

This study using the pig as a large animal model has allowed us to examine several aspects of small intestinal transplantation which may be applicable to the human situation.

Although there was a high incidence of early technical failure in our transplant + FK506 group, the transplantation of a 150 cm segment of intestine based on the

ileocolic . ! vein is a feasible operation. This model provides a reproducible technique with good technical results which will enable us to investigate other aspects of living-related small intestinal transplant.

Our study used FK506-based immunosuppression and we found a significant morbidity with its use. The animals significantly deviated from their normal growth pattern and showed an adversely affected adaptive response at the enterocyte level. In addition, nutrient absorption was significantly altered. These factors, in addition to the fact that rejection was not well controlled, suggests that we need to reassess the widespread use of FK506 as part of the currently used immunosuppressive regimen in human small intestinal transplantation, especially in the pediatric population where normal growth and nutritional status are so crucial.

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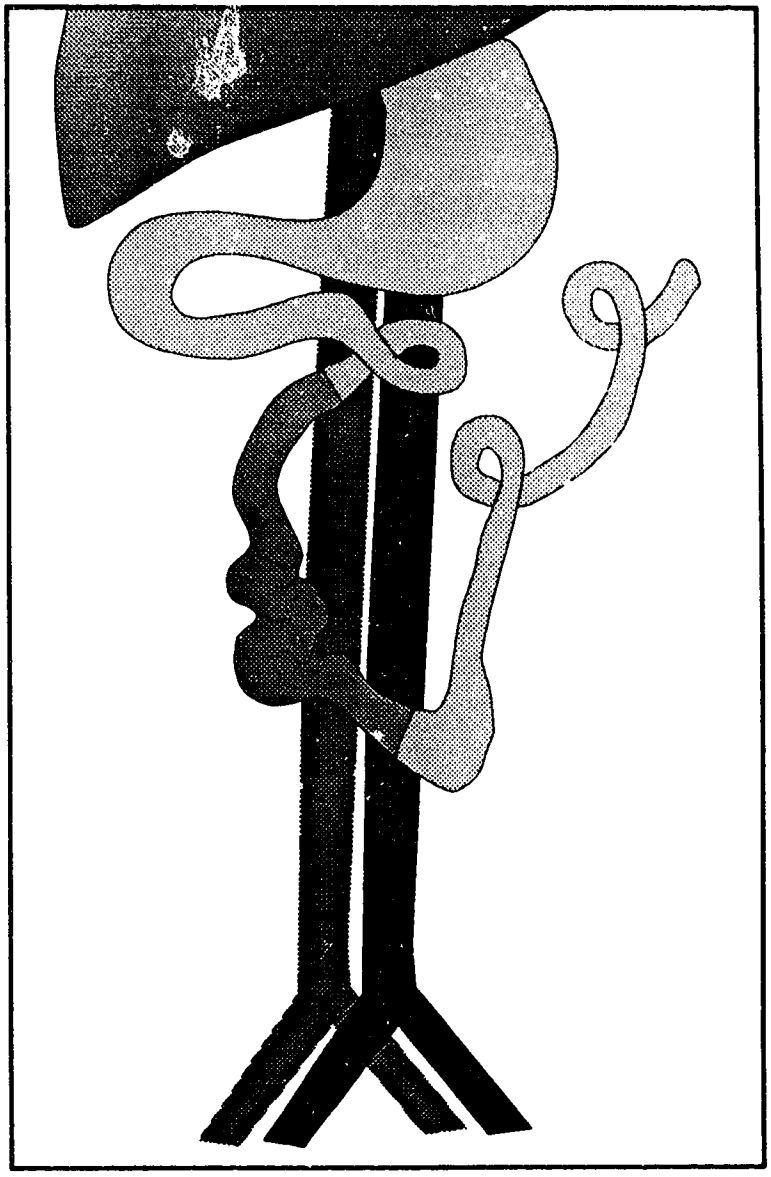
Figure I

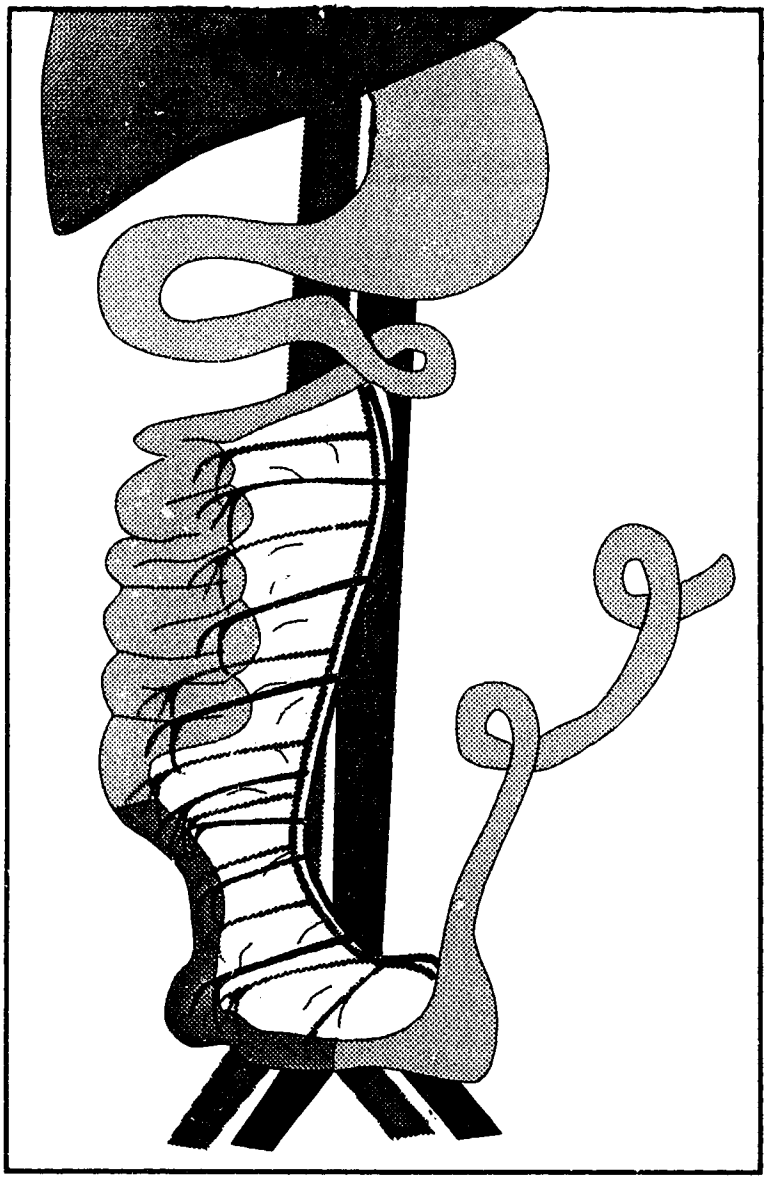
Resection Operation



All but the terminal 150 cm of small bowel is resected with a proximal end-to-end anastomosis followed by a sham distal transection with end-to-end anastomosis.

Figure II

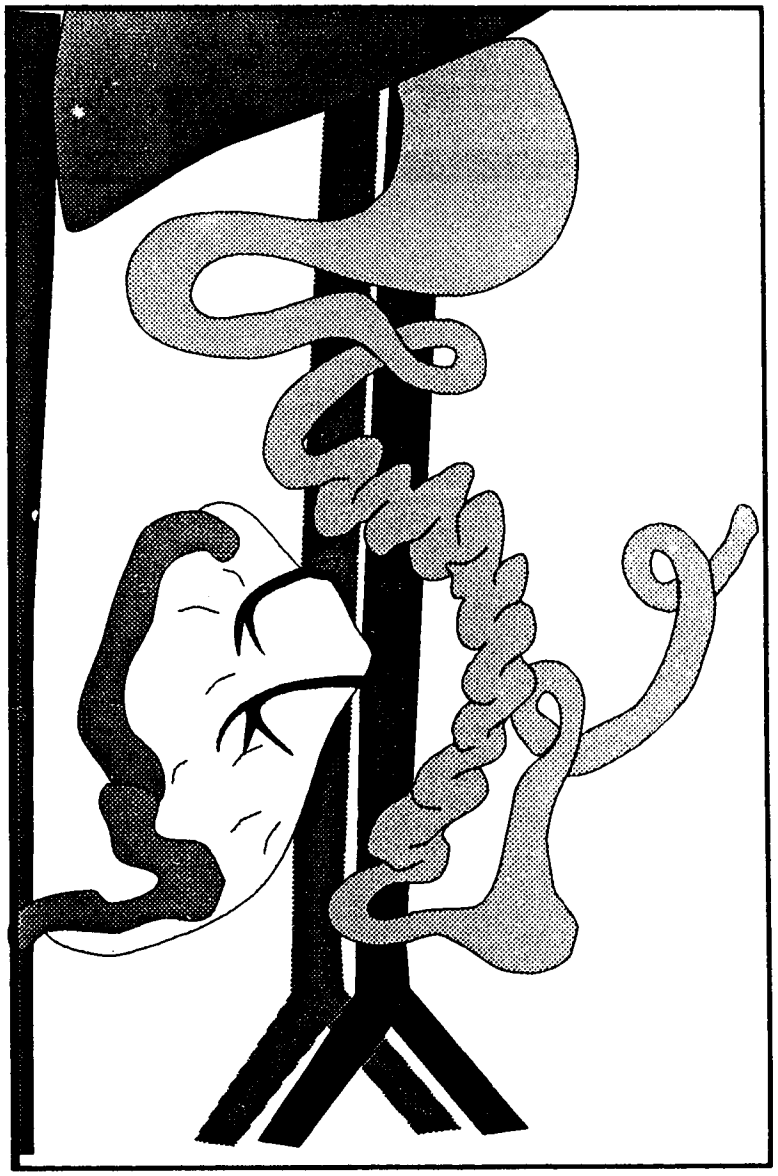
Stage I
Donor Operation



The donor 150 cm intestinal segment is isolated on the ileocolic artery and vein. The graft is removed, flushed, and placed in the recipient.

Figure III

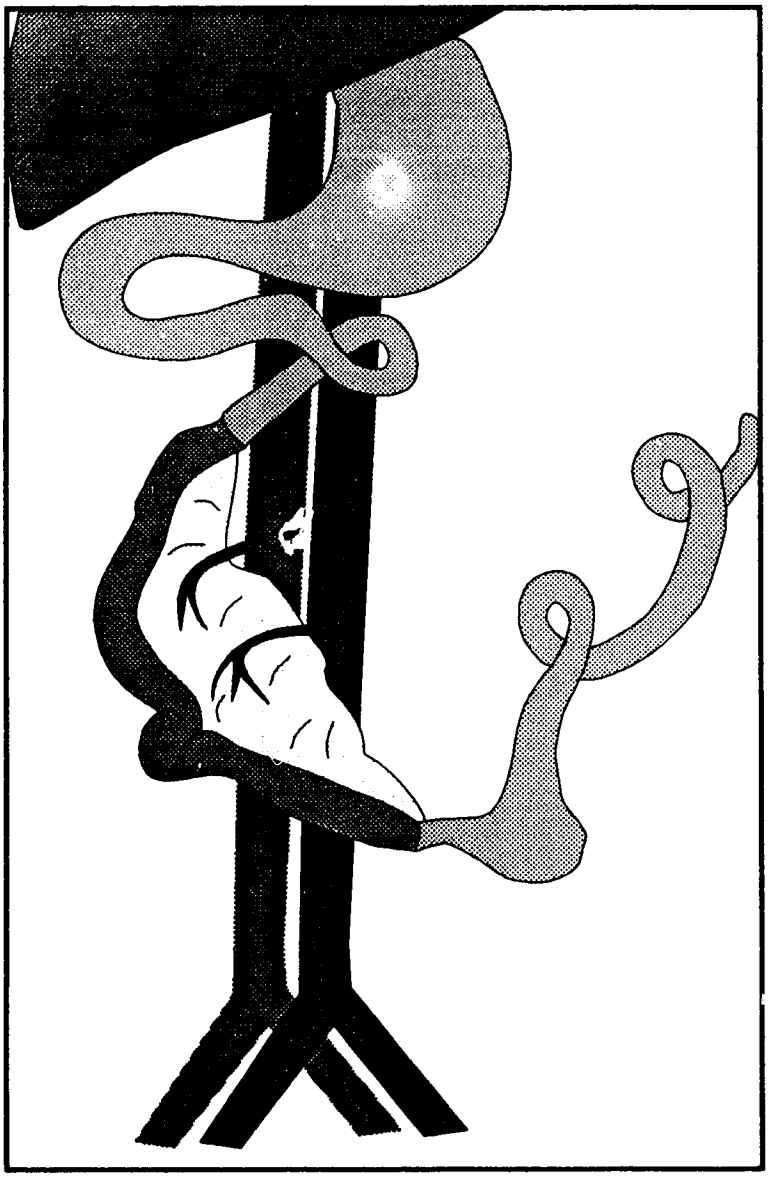
Stage I
Heterotopic Graft Placement



The graft is palced in the heterotopic position with the recipient's native small bowel left in place. The proximal end of the graft is oversewn and the distal end is brought out the abdominal wall as a stoma.

Figure IV

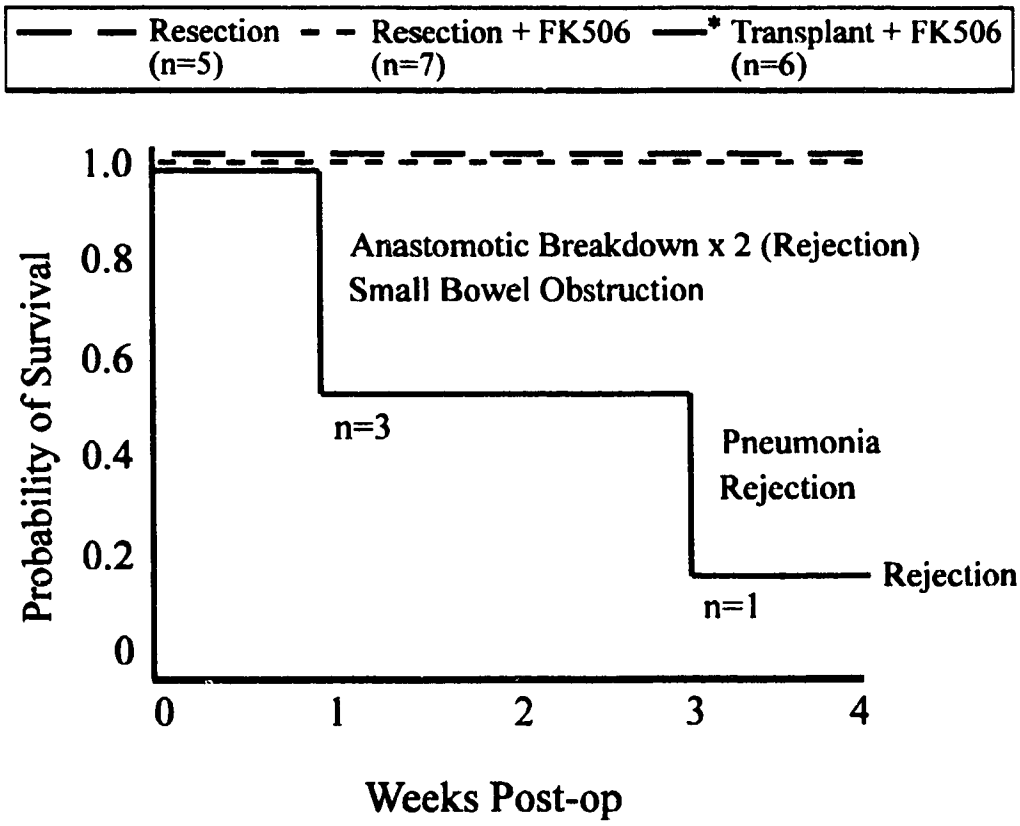
Stage II
Orthotopic Graft Placement



The entire native small bowel is resected and the graft is placed in continuity with proximal and distal anastomoses.

Figure V

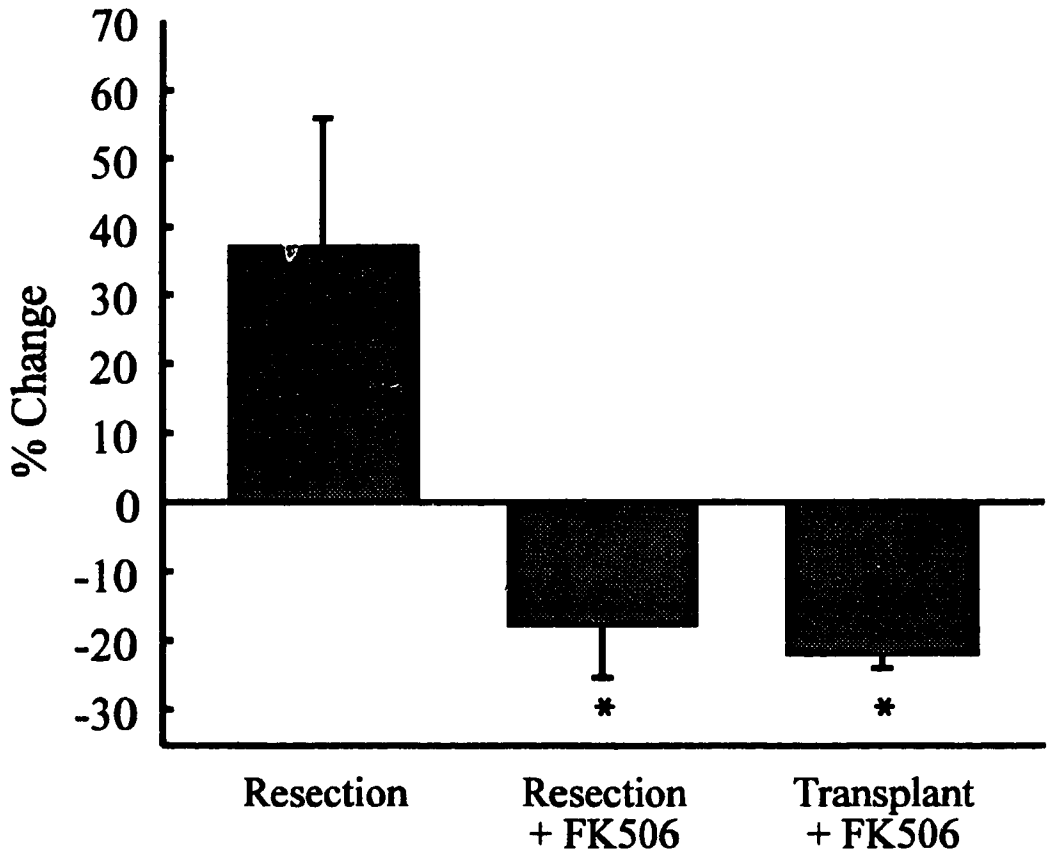
Kaplan-Meier Survival Curve



*Significantly lower survival compared to resection group with generalized Wilcoxon rank test.

Figure VI

Percentage Weight Change from Time Zero to Sacrifice



***Significant difference by ANOVA compared to resection group ($p<0.05$).**

Figure VII

Weight Gain

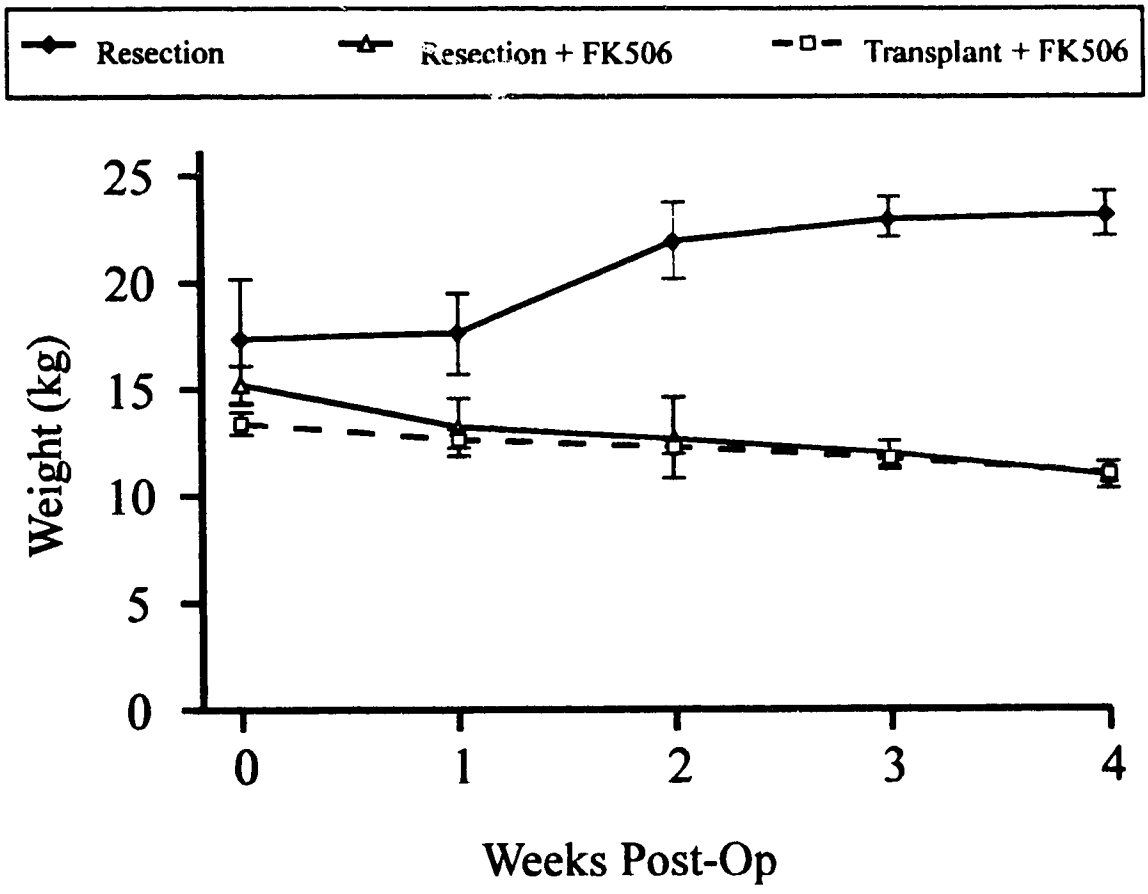


Figure VIII

Mild Rejection

Mild mononuclear cell infiltrate in the *lamina propria.*
Mild villus blunting and edema.

Figure IX

Moderate Rejection

Mixed cellular infiltrate infiltrate in the *lamina propria* and *submucosa*. Minimal epithelial necrosis with moderate villus blunting.

Figure X

Severe Rejection

Inflammatory cell infiltrate in the *lamina propria, submocosa,* and *muscularis propria.*
Diffuse epithelial cell necrosis with areas of complete cell loss.
Severe villus blunting.

Figure XI

Resection Group

Sample of small bowel removed from the resection group showing elongated intestinal villi with a crypt to villus ratio of less than one.

Figure XII

Resection + FK506 Group

Sample of small bowel removed from the resection + FK506 group showing marked blunting of the intestinal villi with a crypt to villus ratio of greater than one.

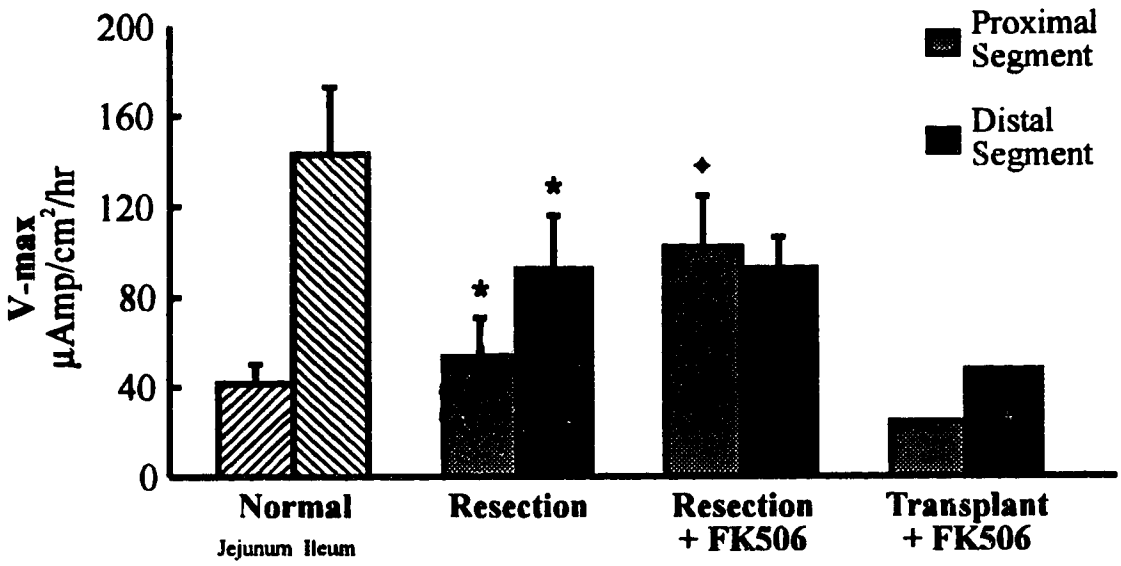
Figure XIII

Transplant + FK506 Group

Sample of small bowel removed from the transplant + FK506 group showing blunting of the villi. There are also signs of rejection present which include villus edema, sloughing of the mucosa, and a mononuclear cell infiltrate.

Figure XIV

Glucose Transport Capacity



Significant difference ($p<0.05$) by ANOVA when compared to
* normal ileum or ◆ resection group.

Table I

Grading System for Small Bowel Rejection*

Rejection Grade	Histologic Changes
None	None
Mild	Mild mononuclear cell infiltrate in the lamina propria, may extend into the submucosa. Mild villus blunting and oedema.
Moderate	Mixed cellular infiltrate in the lamina propria and submucosa, may extend into the muscularis propria. Minimal epithelial cell necrosis. Moderate villus blunting.
Severe	Inflammatory cell infiltrate in the lamina propria, submucosa, and muscularis propria. Diffuse epithelial cell necrosis with areas of complete epithelial cell loss. Severe villus blunting.

*Adapted from Gruessner RWG, Fryer JP, Fasola C, Raouf EN *et al.* A prospective study of FK506 versus CsA and pig ATG in a porcine model of small bowel transplantation. Transplantation 199;59:164-171 and Goulet O, Brousse N, Revillon Y, and Ricour C. Pathology of human intestinal transplantation. In Grant DR, Wood RFM (eds) *Small Bowel Transplantation*. London, Edward Arnold, pp.112-120, 1994.

Table II

Transplant + FK506 Animals Cause and Time of Death

Animal	Cause of Death	Time of Death	
		Days Post Stage I	Days Post Stage II
Stage I Operation			
T2*	Vascular Thrombosis	0	
T4*	Vascular Thrombosis	0	
T6*	Vascular Thrombosis	0	
T1*	Vascular Thrombosis	1	
T3*	Vascular Thrombosis	1	
T8	Small Bowel Obstruction	3	
T12	Rejection	6	
T11	Rejection	14	
Stage II Operation			
T13	Anastomotic Breakdown	15	1
T9	Small Bowel Obstruction	17	3
T7	Anastomotic Breakdown	21	7
T14	Pneumonia	30	16
T10	Rejection	33	19
T5	Rejection	42	28

*Animals excluded from statistical analysis

Table III

Percentage Pig Weight Change from Day Zero to Sacrifice

Group	Resection	Resection + FK506	Transplant + FK506
	65.5	-26	-21.8
	17.3	-21.8	-23.9
	27.9	-24.8	-19.6
	46	-5.8	
	28.5	-8.8	
		-20	
		-16.2	
Average	**37.0**	**-17.6***	**-21.8***
Std. Deviation	**18.9**	**7.8**	**2.2**

*Signifies a significant difference when compared to resection group with ANOVA, $p<0.05$.

Table IV

Mixed Lymphocyte Culture Results

	Test Animals		Media Control	
	CPM	*SI*	*CPM*	*SI*
T5R vs T5D	884	7.8	114	1.0
T7R vs T7D	1683	14.3	118	1.0
T8R vs T8D	789	10.5	7.5	1.0
T9R vs T9D	433	2.1	202	1.0
T10R vs T10D	2276	23.0	99	1.0
T11R vs T11D	520	3.6	146	1.0
T12R vs T12D	1476	7.5	198	1.0
T13R vs T13D	2059	12.6	164	1.0
T14R vs T14D	6539	73.5	89	1.0

R = Recipient

D = Donor

CPM = Counts per Minute

SI = Stimulation Index

Table V

Small Bowel, Skin, and Liver Histology
Transplant + FK506 Group

Animal	Small Bowel Rejection Grade	Skin	Liver
T8	none	normal	normal
T12	severe	normal	normal
T11	severe	normal	normal
T13	N/I (autolysis)	normal	normal
T9	none	normal	normal
T7	N/I (autolysis)	normal	normal
T14	ischemic necrosis	normal	normal
T10	moderate	normal	normal
T5	mild	normal	normal

N/i refers to uninterpretable results.

Table VI

Small Bowel Morphology

	Resection		Resection + FK506		Transplant + FK506	
	Proximal	Distal	Proximal	Distal	Proximal	Distal
Villus Height (μm)	618±37	562±56	338±115*	303±31*	285±21*	268±145*
Crypt:Villus Ratio	0.74±0.08	0.70±0.15	1.64±0.62*	1.59±0.69*	2.07±0.21*	2.31±1.28*
***Muscularis propria* (μm)**	884±174	878±43	1068±199	1004±180	1642±733	1600±269

*Significantly different from resection animals when compared with ANOVA ($P<0.05$).

Table VII

In Vivo Permeability Measurements

Group	^{99}Tc-DTPA % Recovery
Normal	3.8 ± 1.0
Resection	0.9 ± 0.4†
Resection + FK506	1.8 ± 0.8*
Transplant + FK506	0.28

Data expressed as mean ± standard deviation. Significant difference ($p<0.05$) by ANOVA compared to †normal animals and *resection group.

Table VIII

In Vitro Glucose Transport and Permeability Measurements

Group	Δ-Isc		V-max		Km		^{14}C-Mannitol		^{3}H-Inulin	
	Proximal	Distal	Proximal	Distal	Proximal	Distal	Proximal	Distal	Proximal	Distal
Normal	38.3±8.1	132.7±32.0	41.5±8.5	142.6±29.8	5.1±1.6	1.6±0.4	11.5±1.6	12.0±5.2	3.7±1.4	5.5±1.6
Resection	50.0±15.6†	83.9±20.3†	53.3±17.5†	92.2±23.6†	1.8±0.7	2.8±0.4†	9.5±0.6	12.9±0.2	3.7±0.9†	4.8±0.8
Resection + FK506	92.4±21.1*	75.9±16.9	101.8±22.7*	92.4±13.9	2.1±0.6	2.1±0.7	10.0±1.0	11.6±4.0	3.0±1.5	4.6±2.6
Transplant + FK506	22.0	44.8	24.2	47.4	5.4	3.5	16.3	11.1	4.5	3.0

Data expressed as mean ± standard deviation (Δ-Isc, short-circuit current in μAmp/cm^2/hr; ^{14}C-Mannitol, transepithelial flux in nmol/cm^2/hr; ^{3}H-Inulin, transepithelial flux in nmol/cm^2/hr; V-max, maximal transepithelial Na^+/glucose transport in μAmp/cm^2/hr); Km, substrate concentration at which reaction velocity is half maximal *Significant difference ($p<0.05$) by ANOVA compared to †normal ileum (=distal) or *resection group; ¶Permeability studies carried out on one animal.

Chapter IV

The field of clinical small bowel transplantation has seen much progress over the last ten years. Improvements in the technical aspects of organ harvesting and preservation, together with the widespread use of the new potent immunosuppressive FK506 have contributed to this progress.[1] Generally good results have been seen in Pittsburgh, the largest centre performing transplants.[2] However, there are still problems which stand in the way of clinical therapy becoming a standard and routine treatment for short bowel syndrome.

The shortage of small bowel allografts available for transplantation along with the failure to achieve good long-term control of rejection are issues which need to be addressed and overcome if small bowel transplantation is to be a success.

The allograft shortage is likely the result of many health care professionals seeing this therapy as experimental. This suggests a need to educate both medical professionals and the public in general about short bowel syndrome with its often devastating and expensive health effects, and how small bowel transplantation could be a cost-effective and realistic treatment for patients suffering from this disease. Living-related organ donation has proven effective in renal transplantation, and work examining the feasibility of living-related small bowel donation is ongoing.[3,4] Our work has shown that segmental small bowel donation is indeed feasible when the graft is isolated on a distal vascular pedicle. The donor is subjected to minimal morbidity with

the isolation procedure. This idea of living-related segmental intestinal transplantation may be the answer to the shortage of available allografts. Indeed, there have been isolated reports of successful living-related transplants in the human literature.[5,6]

The small bowel is a unique organ in that there are large numbers of immunoreactive cells present in the bowel itself and in the mesenteric lymph nodes. This enormous population of cells provides not only a large site for potential immune attack during the rejection response, but also increases the likelihood that the recipient will become sensitized to the graft. Furthermore, the large pool of transplanted lymphocytes increases the likelihood for a significant graft-versus-host reaction. These factors instinctively suggest that small bowel is difficult to transplant with a good functional result unless there is a good control of the rejection process. A multi-modality therapy strategy in the control of rejection may help to achieve a good end result. Such a strategy might employ a combination of donor selection, host immunomodulation, and anti-rejection pharmacology.

Donor selection with close HLA matching has proven effective in prolonging graft survival in several transplant models of solid organ and tissue transplantation.[7,8,9,10] A close match between donor and recipient in the context of small bowel transplantation should allow for an improved graft survival rate as seen in living-related small bowel transplantation. Unfortunately, there is a shortage of available allografts and consequently a close match is often not possible. Again, educating the general public and health-care professionals alike may help to alleviate

this problem, thus increasing the available donor pool with a greater chance of achieving a close match between donor and recipient

Extensive research is being carried out looking at the feasibility of inducing immune tolerance to the transplanted graft in many different transplant models. Initial work looked at the infusion of donor-specific bone marrow prior to transplantation. Encouraging results have been observed in renal transplant models, but there was a high incidence of GVHD seen in porcine a porcine model of small bowel transplantation with pre-transplant infusion of donor-specific bone marrow.[11,12,13] Other techniques of inducing tolerance may play a role in the future. Injection of donor-specific lymphocytes, soluble MHC complexes, or simply peptides specific to donor MHC into the recipient's thymus may allow the recipient tc become tolerant to the graft.[14,15,16] More work needs to be carried out to gain an understanding of the whole process of tolerance before these techniques are used routinely in small bowel transplant patients.

Much of the progress of small bowel transplantation has been due to the developments in the area of anti-rejection pharmacology. Initial clinical transplant attempts depended on prednisone and azathioprine with poor results.[17] The advent of cyclosporine led to a flurry of activity in both the clinical and laboratory settings.[18] Unfortunately, the results were less that encouraging. The discovery of FK506 in the 1980s was thought to be the answer which would overcome the problem of rejection.[19] The drug is several-fold more potent than cyclosporine and many patients

have been transplanted with small bowel allografts using FK506 with generally good results.[20,21] However, there is still room for improvement in immunosuppressive therapy. FK506 has been shown to have a toxicity profile very similar to that of cyclosporine in that they are both nephrotoxic, neurotoxic, hepatotoxic, and diabetogenic.[20] Our work has found FK506 to also be enterotoxic.

FK506 was shown to adversely affect the weight gain of our experimental animals, a reflection of change in the nutrient absorption and utilization seen with our nutrient absorption studies. At the same time, FK506 adversely affects the normal process of intestinal adaptation on a morphological and functional level. Adaptation is an important reserve on which patients with short bowel syndrome depend,[22] and the effects of FK506 on this reserve can be instinctively carried over to transplanted segments of small bowel. Thus, although FK506 may prevent rejection better than other drugs available today, there are still significant toxicities associated with its use.[23,24,25,26,27,28]

The enterotoxicity observed in our study was attributed to FK506, but as corticosteroids were an important part of the immunosuppressive regimen, mimicking the clinical situation, further studies need to be carried out, examining the independent effects of both FK506 and corticosteroids. Nevertheless, several transplantation studies have been carried out in the past using high dose corticosteroids with minimal adverse effects observed.[29,30,31,32]

Our study did not directly test the ability of FK506 to control rejection, and again, controlled studies comparing FK506 with other immunosuppressive regimens would have to be designed. However, a simple observation can be made: namely, rejection was not well controlled. Whether this is a result of our animal model not reflecting the human situation, with resulting subtherapeutic drug levels, or the result of relying solely on clinical evaluation to diagnose rejection remains debatable.

There are several newer drugs which may play a role in the future of immunosuppression in small bowel transplantation. Mycrophenolate mofetil has proven successful in renal transplantation and may have an important role to play in small bowel transplantation.[33] The drug is more specific for lymphocytes when compared to the closely-related azathioprine. Rapamycin is a drug which has recently enjoyed a resurgence in popularity. It binds to a similar binding-protein as does FK506, but functions through a different mechanism via calcium-independent pathways.[33] Rapamycin may indeed have a big role to play in successful immunosuppression, likely in combination with FK506 or cyclosporine. Other drugs such as bequinar and deoxyspergualin have been investigated in rodent models of small bowel transplantation and the may play an important role in the future. New drugs are continuously being evaluated.[33] These immunosuppressive agents will likely play the most significant role in prolonging graft survival following small intestinal transplantation. New combinations need to be examined so that smaller doses of drugs can be used in order to limit the potential toxicities.

It is clear that a strategy which involves steps in overcoming the rejection process will be necessary if small bowel transplantation is to become a routine and feasible alternative to the current management options for short bowel syndrome. There is still a lot of work which needs to be carried out in both the laboratory and clinical settings. The question remains as to whether or not we should continue to carry out small bowel transplantation in the clinical situation. Small bowel transplantation is not yet a proven treatment option and there is some debate as to whether or not we should be attempting to perfect this treatment on human patients. Unfortunately no animal is as good as the human being when learning about treatments applicable to man. There indeed needs to be continued work in the clinical setting, but this work should be carried out in institutions where the operative procedures are well versed and there is a complement of health-care professionals with a thorough knowledge of the small bowel transplantation process. There is little doubt that with time, small bowel transplantation will become a real alternative to the current medical management of patients with the often devastating short bowel syndrome.

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