FUNCTIONAL AND IMMUNOLOGICAL ASPECTS OF SMALL BOWEL TRANSPLANTATION

an experimental study in dogs

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FUNCTIONELE EN IMMUNOLOGISCHE ASPECTEN VAN DUNNE DARM TRANSPLANTATIE

een experimentele studie bij honden

PROEFSCHRIFT

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Scientific medicine, to be sure, is not always practical,
but it is ever striving to become so,
and practical medicine, though no always scientific,
is constantly leading to that end.
(W.H. Draper, 1886)

What a piece of work is man.
(William Shakespeare, Hamlet, II, 2)

To my parents
To Margaret and Suzanne

CONTENTS

PREFACE

Small bowel transplantation is thought to be the ultimate therapeutic treatment for patients with an irreversible short bowel syndrome and those with intolerance to long-term total parenteral nutrition. Although the small bowel was one of the first organs to be transplanted experimentally, it is the last to be engrafted successfully in humans. The extreme immunogenicity of the small bowel graft and its compromised function after the transplantation procedure still hamper clinical small bowel transplantation as an established therapy.

This thesis tries to answer several questions concerning immunological and functional aspects of intestinal transplantation in a pre-clinical animal model. The experiments were performed at the Laboratory for Experimental Surgery, Erasmus University Rotterdam.

In part A of this thesis an overview of the problems encountered with the short bowel syndrome is given. In addition, the past and present experiences of experimental and clinical small bowel transplantations are extensively described. Part A ends with the scope of this study, formulating questions and objectives of investigation. In part B the general materials and methods used in the experiments are described. Part C is a compilation of adapted versions of original articles, in which the general materials and methods are omitted. These articles are a reflection of the questions raised in chapter 3. The general discussion of our experimental results is given in part D. Finally, several avenues for future experimental and clinical small bowel transplantation are proposed.

PART A

GENERAL INTRODUCTION

CHAPTER 1

THE SHORT BOWEL SYNDROME

1.1 Introduction

The short bowel syndrome (SBS) presents when the length of the small bowel remaining after intestinal resection is insufficient for adequate digestion and absorption of food, water, minerals and vitamins. It is manifested by a picture of diarrhoea, steatorrhoea, malabsorption and weight loss. Although this suggests a well-recognized pattern, we can observe in fact, a wide range of clinical consequences after extensive small bowel resection.

Less than 30 years ago death was inevitable following massive resection of small bowel; surgical morbidity was contributory, but the primary cause was the ensuing protein and caloric malnutrition. The situation is very different now; (neonatal) intensive care has markedly improved and the introduction, development and advances of total parenteral nutrition (TPN) have transformed the outlook for patients with SBS. However, the length and quality of life for patients with intestinal failure have been limited by late sequelae caused by prolonged TPN.

1.2 Causes of the short bowel syndrome

The aetiology of SBS in children can be divided into prenatal and postnatal causes, with the majority of causes occurring in intrauterine life (Table 1.1)(1-3). Prenatal vascular accidents resulting in intestinal atresia are particularly common causes of SBS. Volvulus secondary to malfixation or malrotation also quite often leads to the syndrome and may arise in utero or at any time postnatally. Gastroschisis and idiopathic congenital SBS can also be included as prenatal causes of SBS. Necrotizing enterocolitis and volvulus have been recognized as major postnatal causes of SBS in neonates (1-3). Less common aetiologic postnatal factors include gastroschisis and abdominal trauma. Crohn's disease and radiation enteritis can also cause SBS in children but generally affect an older age group (1,2).

In adults, various causes of SBS are encountered (Table 1.2). The commonest conditions that lead to SBS are intestinal resections following a vascular insult to the small bowel, and Crohn's disease with multiple bowel resections (4). Less common causes include abdominal trauma, primary and secondary neoplasms of the gut, radiation enteritis and jejunoileal bypass for morbid obesity (4).

Table 1.1. Causes of short bowel syndrome in infants and children

Prenatal	Postnatal
Intestinal atresia	Necrotizing enterocolitis
Volvulus	Volvulus
Gastroschisis	Gastroschisis
Idiopathic congenital SBS	Abdominal trauma
	Crohn's disease
	Radiation enteropathy

Table 1.2. Causes of short bowel syndrome in adults

Thrombosis or embolus of superior mesenteric artery
Thrombosis of superior mesenteric vein
Volvulus of small intestine
Strangulated hernia
Inflammatory bowel disease
Abdominal trauma with subsequent bowel resection
Primary and secondary neoplasms of the small intestine
Radiation enteropathy
Jejunoileal bypass for morbid obesity

1.3 Factors influencing the severity of the short bowel syndrome

Several factors, all of which influence nutrient absorption after small bowel resection, can be distinguished: 1. the extent and site of resected small bowel; 2. the presence or absence of an ileocaecal valve; and 3. the degree of adaptation of the remaining intestine and colon.

1.3.1 The extent and site of resected small bowel

The more extensive the resection of intestine, the greater the loss of absorptive surface for nutrients, water, and electrolytes transported by active and passive mechanisms (4-9).

However, the length of small intestine remaining after resection will determine both the area of absorptive surface for luminal contents and the transit time of the nutrients (5-10). Wilmore concluded that among infants less than two months of age at the time of operation, survival was most likely if the remaining segment of intestine exceeded 40 cm in length, but most unlikely if it was less than 15 cm in length (9). In an autopsy study of preterm infants, Touloukian found a doubling of the intestinal length from 142 cm to 304 cm in the third trimester of gestation (11). Therefore, he suggested that the prognosis should not be based on the absolute length of the remaining intestine, but on the percentage of normal for a given gestational age remaining following resection. In adults it appears that approximately 50% of the small bowel may be resected without significant problems in sustaining normal nutritional requirements (4). Adults with more than 75% of their small intestine resected, however, almost invariably have severe malabsorption problems (4).

The site of intestinal resection also plays a pivotal role in determining the metabolic consequences (4,5). Proteins, carbohydrates, fat, most water-soluble vitamins and minerals are absorbed along the length of the small bowel, but particularly in the duodenum and jejunum. A normal ileum will generally take over most of these absorptive functions after loss of the proximal small intestine (12). However, loss of the proximal intestine may result in decreased synthesis of cholecystokinin and secretin, and consequently in diminished release of biliary and exocrine pancreatic secretions, which will further shorten transit time (4).

Resection of ileum is metabolically more important than loss of the proximal intestine, since it is the site for absorption of conjugated bile salts and intrinsic factor-bound vitamin B_{12} (4,12). Loss of less than 100 cm of ileum reduces the amount of bile salts in the enterohepatic cycle and increases the entry of bile salt losses in the colon with subsequent production of bile salt diarrhoea (4). The liver is able to partially compensate for this bile salt loss by increased synthesis, which will counterbalance reduced bile salt micelle formation and decreased absorption of water-insoluble monoglycerides and fatty acids. More extensive loss of ileum will result in severe bile salt loss and hence steatorrhoea. Removal of more than 60 to 100 cm of ileum will lead to malabsorption of intrinsic factor-bound vitamin B_{12}. A deficiency of this vitamin may lead to the development of megaloblastic anaemia, peripheral neuropathy, and ultimately to combined degeneration of the spinal cord (4).

If the colon is resected in conjunction with the small bowel, the clinical consequences are far more pronounced than after small bowel resection alone. After colectomy combined with extensive small bowel resection, dehydration and sodium and potassium depletion are likely to develop (4). Recently, Nightingale evaluated 46 patients with less than 200 cm of jejunum without a colon and 38 patients with similar jejunal lengths in continuity with a functioning colon (13). All patients without a colon and less

than 85 cm of jejunum, and all those with a colon and less than 45 cm jejunum needed long-term TPN. After a median of five years most (25 of 27) of those without a colon who did not need parenteral supplements required oral electrolyte replacement compared with few (4 of 27) with a colon. Conservation of the colon is beneficial, because it absorbs water, sodium, calcium, some nutrients, maintains a normal rate of gastric emptying of liquids, and may stimulate small intestinal hyperplasia (13).

1.3.2 The ileocaecal valve

Of much prognostic significance for patients with SBS is the presence or absence of the ileocaecal valve (7-9). Wilmore suggested that infants whose remaining intestinal segments measured less than 40 cm required an intact ileocaecal valve for survival (9). Galea showed that neonates with a remaining jejunoileal segment of more than 20 cm and an intact ileocaecal valve should be considered salvable, whereas a small bowel segment of more than 30 cm is needed if the ileocaecal valve is absent (7). Resection of the ileocaecal valve results in decreased transit time of the luminal contents within the small bowel (4,9,10). Loss of the ileocaecal valve may result in increased bacterial colonization of the residual small bowel, deconjugation of bile salts, reduced absorption of fat and the fat-soluble vitamins A, D, E and K, as well as increased entry of bile salts in the colon (12). Vitamin B_{12} may also be metabolized by the higher number of bacteria in the small bowel leading to diminished absorption of this vitamin. In addition, the absence of an ileocaecal valve prolongs the length of TPN therapy (8).

1.3.3 The degree of adaptation of the remaining small bowel

In 1912 Flint first demonstrated a significant postresection increase in villus height in man and calculated that the absorptive surface area increased four-fold (14). Since then this phenomenon has been observed in animals and man.

Experimental rat and pig studies showed morphological and functional adaptive changes in the residual small intestine after extensive small bowel resection. In the rat, SBS leads to an increase in the circumference, thickness and height of the villi in the residual ileum (12). Resection of the central three quarters of the small bowel in pigs results in an increase of gross bowel length and diameter, an increase in the size of the villi and increased in vitro, passive ileal uptake of fatty acids, cholesterol and L-glucose (15). Hyperplasia of the residual ileal mucosa after massive small bowel resection occurs from accelerated cellular proliferation and migration, with a reduction of the life span of enterocytes (12). In animal experiments it was demonstrated that enhanced segmental absorption, as a result of adaptive changes, may permit normal growth and development. Increased absorption of glucose, maltose, sucrose, bile salts and vitamin B_{12} after

proximal small bowel resection has been reported (12). This phenomenon appears to be proportional to the number of epithelial cells per unit length or to the mucosal weight, suggesting that these functional, adaptive changes are caused by an increase in the number of absorptive cells, rather than by an enlarged capacity of individual cells (15). Enzymatic and metabolic changes in the hyperplastic mucosa have been shown after proximal small bowel resection in animals; in particular, the activities of Na-K-ATPase, peptide hydrolases, enterokinase and enzymes involved in DNA and pyrimidine synthesis are increased (12).

In humans a gradual improvement in absorption of fat, nitrogen and carbohydrates has been shown after massive bowel resection. Although these functional, adaptive changes may represent a higher number or increased capacity of absorptive cells, the precise mechanisms remain to be elucidated (12). However, lactose absorption is impaired in patients with SBS, probably as a result of secondary lactase deficiency of functionally immature cells (12). In newborn infants who had undergone resection of more than 50% of the small bowel, the time required for intestinal adaptation depended on the residual intestinal length and the presence or absence of an ileocaecal valve. Infants with less than 40 cm of small bowel adapted after a mean of 27.3 months, those with 40 to 80 cm of small bowel after a mean of 14 months (16). In case of preterm infants, Touloukian suggested aggressive management with expectation for survival if the length of remaining intestine exceeds 5% of normal with the ileocaecal valve remaining, or greater than 10% of normal if the ileocaecal valve has been lost (11).

Less is known about the adaptive capacity of the colon after intestinal resection in both animals and man. Preliminary studies have shown that after intestinal resection the colon may increase its absorption of glucose and amino acids (17).

1.3.4 Mechanisms of intestinal adaptation

Several mechanisms that may stimulate intestinal adaptation after extensive small bowel resection have been postulated:

1. The influence of luminal nutrients plays an important role in intestinal adaptation (4). It has been shown that fasting leads to intestinal hypoplasia. Moreover, intestinal hypoplasia also develops in patients receiving TPN only (4). The mechanism involved in this phenomenon remains unclear. In a rat experiment the positive influence of short-chain triglycerides has been shown: when compared with an elemental diet with or without medium-chain triglycerides, an elemental diet supplemented with short-chain triglycerides improved jejunal and colonic adaptive growth, as evidenced by increased mucosal weight, mucosal protein and DNA (18). Another rat study, examining the influence of diets with or without essential fatty acids, showed the dynamic nature of the status of essential fatty acids in tissue and the positive effect of essential fatty acids on

mucosal adaptation after intestinal resection (19). Recently, the importance of the amino acid glutamine as an intestinal stimulant was pointed out (20). Glutamine appears to be an energy substrate for cells with rapid turnover and a precursor for purine biosynthesis; it may become essential at times when maximal cell growth is required. The role of glutamine as an essential fuel for intestinal mucosa regeneration has been studied in a dog model of massive small bowel resection and colectomy (21).

2. Luminal nutrients may stimulate biliary and exocrine pancreatic secretions, which in turn may promote mucosal hyperplasia (4,17). Pancreatic secretions have the most pronounced effect with respect to mucosal hyperplasia and have also been shown to alter the activities of brush-border enzymes in the small bowel (12). Recently, in a rat study it was shown that the somatostatine-analogue octreotide interfered with mucosal hyperplasia of the residual small intestine, probably because of the inhibitory effect of octreotide on exocrine pancreatic secretions (22). Although this effect has to be examined in other animal models, the authors suggest not to use octreotide in the immediate postresection period. This makes the role of octreotide in the treatment of patients with SBS controversial, because others reported a beneficial effect of octreotide - a reduction of intestinal secretions - in six patients with SBS (23).

3. The trophic effects of hormones probably released by luminal nutrients may also lead to mucosal hyperplasia. Administration of secretin and cholecystokinin has been shown to stimulate mucosal hyperplasia, but this effect could be mediated via their action on biliary and exocrine pancreatic secretions, rather than by direct stimulation (5). Enteroglucagon appears to be most important in the induction of intestinal hyperplasia (7). In addition, prostaglandins, epidermal growth factor, and human growth hormone analogues appear to stimulate epithelial cell proliferation after small bowel resection (7). The intestinal enterocyte is the target of these factors, and synthesis of the polyamines putrescine, spermidine and spermine within the cell is the essential step for the development of hyperplasia after resection. The rate-limiting enzyme for polyamine synthesis, ornithine decarboxylase, reacts to trophic stimuli with increased activity. Blockage of ornithine decarboxylase by specific inhibitors is accompanied by the absence of intestinal hyperplasia (7,24,25). In another study inhibition of the putrescine-degrading enzyme, diamine oxidase, enhanced the adaptive response to intestinal resection (26). It remains to be determined whether inhibition of diamine oxidase activity by aminoguanidine will be clinically important for patients with SBS.

4. Other possible mechanisms, such as increased blood flow to the residual bowel, and altered innervation, may play a role in intestinal adaptation (4).

1.4 Clinical characteristics of the short bowel syndrome

In the clinical course of patients with SBS we can distinguish an early, an intermediate, and a late phase. In the early phase diarrhoea is the prominent feature. It is worsened by oral food and fluid intake. Dehydration and depletion of electrolytes may occur. TPN will be required to prevent dehydration and electrolyte and acid-base disturbances. In the intermediate phase malabsorption predominates, characterized by weight loss and nutritional deficiencies. During this phase the process of adaptation takes place. During the late phase body weight stabilizes despite diarrhoea and steatorrhoea. Some patients eventually achieve full adaptation and adequate nutritional status by oral intake. However, even after fully developed adaptation the residual small intestine may be unable to maintain a normal nutritional status without parenteral support.

1.4.1 Diarrhoea

Diarrhoea nearly always occurs after small bowel resection. With extensive reduction of intestinal surface faecal effluents greater than five litre per day are a common feature during the first weeks postresection (12). Hypovolemia, hyponatremia, hypokalemia and with time hypocalcemia and hypomagnesemia will develop if no replacement therapy takes place. Dehydration, weakness, postural hypotension and tetany may occur. Recently, Nightingale examined the relationships between the length of remaining jejunum, the absorptive and secretory response to meals, and the need of parenteral supplements in patients with less than 150 cm of residual jejunum and no colon (27). Long-term parenteral fluid and electrolyte replacements were needed in those patients with less than 100 cm jejunum, demonstrating a secretory response. Jejunal length correlated inversely with fluid, sodium, potassium and energy absorption.

Several factors are involved in the pathogenesis of postresection diarrhoea. First, decreased transit time and motility disturbances due to the bowel resection. Second, malabsorption of carbohydrates may cause osmotic diarrhoea. This may be accentuated by bacterial overgrowth in which disaccharidase activities are decreased. Third, increased secretion of water and electrolytes, especially after ileal resection, secondary to increased colonic entry of bile acids, which stimulate adenylate cyclase activity (28). Fourth, steatorrhoea with increased colonic fatty acids, which stimulate adenylate cyclase and thereby cause secretion of water and electrolytes.

Control of diarrhoea is of utmost importance in the treatment of SBS and may be accomplished by narcotic agents. These drugs and their derivatives act mainly on intestinal smooth muscle to decrease transit time and increase intestinal capacity and have been demonstrated to diminish ileostomy output (29). Intestinal enterocytes contain opiate receptors on their membrane surfaces, which may be involved in ion transport (30). Bile

acid-binding agents such as cholestyramine may be useful to control bile salt diarrhoea (12).

1.4.2 Nutritional deficiencies

Reduced absorption of virtually all nutrients, including fat, carbohydrates and protein, is noted after massive intestinal resection (31). Caloric deprivation will result in weight loss or growth retardation, lassitude, weakness and fatigue. Hypocalcemia may be aggravated by reduced vitamin D absorption secondary to malabsorption of fatty acids (12).

Vitamin deficiency occurs frequently after intestinal resection. Steatorrhoea results in decreased absorption of the fat-soluble vitamins A, D, E and K (31,32). Vitamin A deficiency causing night-blindness, vitamin D deficiency with osteoporosis and osteomalacia, vitamin E deficiency resulting in neurological deficits and vitamin K deficiency with a bleeding tendency have been reported (31,33,34). Although the water-soluble vitamins are generally well absorbed, vitamin B_{12} deficiency and occasionally folate deficiency may result in megaloblastic anaemia (31). After proximal small bowel resection iron deficiency with subsequent anaemia may occur (31).

Trace elements are usually absorbed in adequate amounts like water-soluble vitamins. However, zinc deficiency may develop after massive ileal resection (31).

1.4.3 Gastric hypersecretion

A common complication after small bowel resection is gastric hypersecretion (4,35). This may lead not only to peptic ulcer disease, but also compromises intestinal absorption by inducing mucosal damage, reducing micelle formation and pH-induced inhibiting of exocrine pancreatic secretion. These effects of hypersecretion and the large volumes of gastric juice may cause diarrhoea. The mechanism of gastric hypersecretion after intestinal resection is unclear. Some authors reported elevated serum gastrin levels, suggesting gastric hypersecretion secondary to gastrin stimulation (35). However, other authors found normal gastrin levels and suggested removal of gastrine-competitive hormones as a cause of gastric hypersecretion (12).

After intestinal resection, treatment with H_2-receptor blockers may prevent peptic ulceration, diminish nasogastric tube and ostomy fluid and electrolyte losses, and control metabolic alkalosis (12,36).

1.4.4 Bacterial overgrowth

Patients with SBS are prone to bacterial overgrowth. In those with ileal-colonic resections this phenomenon could be due to the absence of the ileocaecal valve (4). However,

motility disturbances after small bowel resection may be even more important (12). Small bowel bacterial overgrowth in combination with SBS magnifies diarrhoea, malabsorption and nutritional deficiencies.

Detection of bacterial overgrowth in patients with SBS is difficult, because results of noninvasive tests commonly used to diagnose bacterial overgrowth, such as the bile acid breath test, will be abnormal in patients after ileal resections (12). If bacterial overgrowth is suspected treatment, with antibiotics is warranted.

1.4.5 Hyperoxaluria and nephrolithiasis

Hyperoxaluria and renal calcium oxalate concrements occur more frequently in patients after extensive small bowel resection (37). Hyperoxaluria is a result of excess colonic absorption of oxalate. Increased colonic fatty acids bind to calcium, which prevents the formation of insoluble calcium oxalate in the colon. In addition, fatty acids and bile salts may increase colonic mucosal permeability, which results in higher oxalate absorption (37). The pathogenesis of increased renal calcium oxalate concrements remains unclear. Hyperoxaluria, decreased renal calcium-binding anions like phosphate and citrate, and diminished urinary volume may be involved in the development of nephrolithiasis (38). Nightingale found that none of his patients with SBS without a colon developed symptomatic renal stones, in contrast to 24% of patients with a colon (13).

Patients with SBS should limit their intake of high-oxalate containing food, such as chocolate, cola drinks, tea, carrots and nuts, and food containing dietary fat to minimize oxalate absorption. Calcium-containing food may promote intraluminal formation of insoluble calcium oxalate and hence reduce hyperoxaluria.

1.5 Total parenteral nutrition

The mainstay of early and sometimes late management of SBS is TPN (39-42). However, TPN alone reduces the mass of the remaining intestine after small bowel resection (4,43). In a rat study the beneficial effect of short-chain fatty acid supplements to TPN has been demonstrated; after 80% small bowel resection, animals receiving TPN showed significant loss of jejunal mucosal weight, DNA, RNA, and protein, and loss of ileal mucosal weight and DNA, whereas animals receiving short-chain fatty acid-supplemented TPN showed no significant change in jejunal mucosal parameters and a significant increase in ileal mucosal protein content (43). Premature attempts at oral food intake will result in massive diarrhoea, dehydration, and electrolyte and acid-base disturbances (12). However, limited amounts of enteral feeding must be started as soon as possible to stimulate intestinal adaptation, to maintain intestinal integrity, and to minimize bacterial

translocation (44-46).

The period a patient with SBS will require TPN is quite variable and depends on the severity of the metabolic consequences of intestinal resection (8,15,40,42). Intestinal adaptation is indicated by gradual tolerance of increasingly larger amounts of enteral feeding and diminishing support from TPN. Complete adaptation is achieved when all caloric needs are met by enteral feeding and - in infants - a normal pattern of growth and weight gain is established. Full adaptation may take over two years to be achieved (47). Therefore, a patient should not be labelled as having irreversible intestinal failure before two years have passed, unless the residual small bowel is extremely short. Georgeson found that neonates with 40 cm or less of initial small bowel spent a longer time on TPN (24.1 versus 10.6 months) and had a lower weaning rate from it (57 versus 88%) than those with more small bowel (9). Caniano reported a survival rate of 86% in neonates with less than 25% of expected small bowel length for gestational age; 67% of survivors had sufficient intestinal adaptation to permit discontinuation of TPN between 6 and 45 months (40).

Unfortunately, some patients with severe SBS remain permanently dependent on TPN (42,47-49). Gouttebel reported that patients with SBS and no colon required more than 60 cm of small bowel to avoid irreversible intestinal failure (42). To maintain an adequate nutritional status and avoid dehydration and electrolyte disturbances these patients receive home TPN (47).

In the United Kingdom and the United States of America national registers recording details of patients receiving home TPN have been kept since 1977 (50). Not all patients receiving home TPN are included in these registers and not all patients with irreversible intestinal failure receive TPN (50). In particular it is not always considered ethically appropriate to commence TPN in neonates with extremely SBS (48). Lennard-Jones found that approximately two adults per million per year commence home TPN in the United Kingdom and half of these have irreversible intestinal failure (47).

Complications of total parenteral nutrition

Several complications have been recognized in patients maintained on prolonged TPN. They are most often related to the presence of the venous catheter or to metabolic imbalances arising from long-term intravenous feeding.

Catheter-related problems are pneumothorax, hematothorax and, most commonly, infection and sepsis (51). Staphylococcus species and fungi are the organisms most frequently cultured from catheters. Gram-negative bacterial catheter infections are observed in patients with ostomies whose skin is colonized with these organisms. With aseptic techniques, however, the frequency of episodes of catheter-related sepsis can be minimized to one in 905 days (52).

Vascular thrombosis with vena subclavia occlusion and superior vena cava syndrome may occur (51,53). With repeated episodes of venous occlusion, vascular access may become limited and a thoracotomy may be required to implant a catheter in the right atrium (53).

Metabolic complications of intravenous feeding such as hyperglycemia, electrolyte disorders, and hypertriglyceridemia, can occur in patients on home TPN. In addition, several vitamin and trace element deficiency syndromes have been recognized (12). Metabolic bone disease has also been noted (54).

Hepatobiliary abnormalities ranging from asymptomatic cholelithiasis and steatosis to liver insufficiency have been attributed to prolonged TPN, and they are responsible for substantial morbidity and mortality (51,55-59). Infants tend to develop a cholestatic illness, whereas adults predominantly exhibit hepatocellular disease (51). Several mechanisms for TPN-related hepatic disorders have been proposed such as increased production of toxic secondary bile acids (lithocholic acid) due to lack of oral nutrition (51), decreased hepatic triglyceride secretion (56), intestinal overgrowth and translocation of intestinal bacteria causing cholestatic jaundice (57), excess of carbohydrate calories (58), excess of amino acids contributing to cholestasis (51), and insulin/glucagon imbalance (59). End-stage liver disease associated with TPN accounts for almost all deaths in recently reported studies of infants with SBS (60). Excessive dextrose administration should be avoided; glucose utilization is maximal between 5 to 6 mg dextrose $kg^{-1}min^{-1}$, and additional energy needs should be supplied using lipid, although excess Intralipid must also be avoided. TPN should be given intermittently rather than continuously (51). When cholestasis develops, a trial of metronidazole is worth considering (51,57).

Finally, dependence on long-term TPN may limit a person's lifestyle and cause psychosocial problems (61). The complexity of the problems encountered with TPN emphasizes the need of a multidisciplinary nutrition team.

1.6 Surgical treatment of the short bowel syndrome

Many surgical procedures have been described that slow intestinal food transit or increase the absorptive surface area in patients with severe SBS. Anecdotal reports describe the construction of intestinal valves, colon interposition, recirculating loops, intestinal pacing and growing neomucosa (62,63). Two procedures deserve mention: antiperistaltic small bowel segments and intestinal loop lengthening. The antiperistaltic procedure isolates a short segment of small bowel, which is anastomosed in the reverse direction (64). In the intestinal loop lengthening procedure the remaining intestine is divided longitudinally and each loop is anastomosed in an isoperistaltic direction, thus doubling the length of the

segment (65). Limited clinical successes have been reported with both procedures, which have not become widely used.

In the (near) future, transplantation of small bowel may offer the most reasonable alternative in patients with loss of the small intestine and therefore no hope for adaptation, and in those with intolerance to prolonged TPN (48,66).

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CHAPTER 2

SMALL BOWEL TRANSPLANTATION

2.1 Introduction

In 1959 the first experimental small bowel autotransplantation was performed (1). During the 1960s and the early 1970s many experimental and even a few clinical small bowel transplantations were performed (2-9). Attempts to overcome rejection, graft-versus-host disease (GVHD) and sepsis proved to be not successful with conventional immunosuppressive regimens. By the mid-1970s enthusiasm for small bowel transplantation waned, because of poor results and - more importantly - because TPN was introduced as a valuable tool in the treatment of SBS (10).

During the early 1980s the discovery of cyclosporin A (CsA) and its success in kidney, heart, liver and pancreas transplantation renewed interest in small bowel transplantation (11-13). Recent experimental and clinical experiences with small bowel transplantation are promising, but rejection, infectious complications and lymphoproliferative disorders are still major problems (14-19). Although small bowel transplantation remains a demanding procedure from technical, physiological, and immunological points of view, it is expected to become the ultimate therapeutic treatment for patients with irreversible intestinal failure.

2.2 Experimental models

2.2.1 Animals

During the 1960s several investigators performed small bowel transplantations in large animals (1-8). Lillehei first described orthotopic small bowel transplantations in a canine model (1). Recipients of autografts survived permanently, whereas allografts were rejected within a few days (2). In the 1980s the long-term functional sequelae of small bowel transplantation were studied in preclinical dog and pig models (20-30). Although CsA treatment resulted in a statistically significant prolongation of graft survival compared with untreated animals, there were only a few long-term survivors. Small bowel autotransplantations were performed to study the effects of operative ischaemia and neural and lymphatic disruption without immunological reactions (31).

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Since the original report by Monchik and Russell, the rat model of small bowel transplantation has proved to be remarkably reproducible (32,33). The availability of inbred strains and their F1-hybrids has allowed the phenomena of rejection and GVHD to be studied separately in a controlled fashion that is not possible in large animal models. When the F1-hybrid is the donor and the parent the recipient, only rejection will occur; in the reverse combination only GVHD is possible. It remains questionable whether this is relevant for the clinical situation, in which both rejection and GVHD may occur (34,35). When fully allogeneic transplants were performed, only rejection was found (32).

2.2.2 *Heterotopic versus orthotopic position*

Small bowel transplantation can be carried out both heterotopically and orthotopically. In the heterotopic model the small bowel allograft constitutes an accessory graft, which is not in continuity with the recipient's gastrointestinal tract and remains defunctionalized. Heterotopic small bowel transplantations have been performed using small bowel segments as Thiry-Vella loops in the neck or abdomen (3-9,32). A heterotopic model has the advantage of accessibility to the graft for repeated histologic evaluation. A drawback of this model is the inability to assess the end point of graft rejection, because the rejection process may result in encapsulation and fibrosis of the graft rather than in death of the recipient (36). In addition, the mucosa of a defunctionalized small bowel segment will atrophy and may become permeable to bacteria (37-40).

In the orthotopic position, the small bowel graft is exposed to the faecal stream and is required to absorb nutrients administered enterally. Recipient survival depends on satisfactory preservation of small bowel graft function. Thus an orthotopic small bowel graft with markedly reduced functional absorptive capacity will lead to malnutrition and death of the recipient. Loss of function due to rejection will result in a weaker mucosal barrier function, an immediate and fatal effect of rejection upon the recipient (36). Compared with the heterotopic model of small bowel transplantation, the orthotopic model imposes more severe conditions for graft and recipient survival. This resulted in critical differences in postoperative weight gain, graft permeability and survival after heterotopic and orthotopic small bowel transplantation (37,42). As the orthotopic model, which represents a more physiological state of intestinal function, has a similar technical failure rate as the heterotopic model, it is the preferred preclinical model to examine immunological and functional aspects of small bowel transplantation (36,37,41).

Future clinical small bowel transplantation will entail orthotopic graft positioning, although heterotopic positioning may be employed as an initial step (36). If the intestinal graft is placed in continuity with the native bowel within several weeks of the heterotopic transplantation, the graft's function will return to normal (43).

2.2.3 *Portal versus systemic drainage*

In vascularized small bowel grafts venous outflow from the transplant may be conveyed into either the portal or the systemic venous circulation. Studies on a variety of organ allografts, including small bowel allografts, have suggested that portal venous drainage confers immunological benefits which favour prolonged allograft survival (44-47). However, these benefits appear to be present in heterotopic models only (48,49). When portal and systemic venous drainage were compared in an orthotopic small bowel transplantation model in rats, no significant increase in survival of the portally drained group was found (50).

Portal drainage re-establishes the physiological route of venous outflow, whereas systemic drainage creates a partial mesocaval shunt, which may result in metabolic alterations. Systemic drainage in rats resulted in higher blood levels of ammonia and some amino acids compared to portal drainage (51). However, these differences could be corrected with dietary modifications. Other rat studies also showed minor metabolic differences, which do not provide a compelling reason to favour portal over caval drainage (50,52). As the operative procedure of portal drainage is technically more difficult, venous drainage may be preferred.

2.2.4 *Graft composition*

Recent experimental small bowel transplantations have been performed in combination with liver transplantation (53,54). In a non-immunosuppressed simultaneous liver/small bowel transplantation model in rats it was shown that liver-induced immunosuppression suppresses both T- and B-cell immune function, IL-2 production, and causes a low titre of lymphocytotoxicity antibodies (53). In another non-immunosuppressed rat model a heterotopic small bowel transplantation was performed 17 days after an orthotopic liver transplantation, which resulted in small bowel graft survival of more than 150 days in five of six animals (54). The improved results after combined liver/small bowel transplantation may not be due to portal drainage, but to the fact that the liver could have a protective effect against rejection of other organs transplanted simultaneously. This effect was first reported by Calne, who was using combined liver and renal, or liver and skin grafts (55). Injection of soluble liver antigen caused a similar effect as transplanting the whole liver. This effect appeared to be donor-specific, which led Calne to suggest that the liver or liver extract releases histocompatibility antigens in a tolerogenic form, thus providing partial immune tolerance towards organs from the same donor (55). Kamada demonstrated a similar phenomenon in certain rat strain combinations: a liver recipient permanently accepted a simultaneous or subsequent heart, kidney or skin graft (56).

Multivisceral transplantations, including liver, small bowel, stomach and pancreas,

have been reported in both rat and large animal models (57-59). The results were poor, mainly due to rejection of the intestinal transplant, which seems to be the most immunogenic component of the procedure. Recently, however, better survival rates were obtained in a rat model of multivisceral transplantation using FK-506 as immunosuppressive drug (60).

2.3 Preservation

2.3.1 Harvest injury

Anoxic and reperfusion damage are the two principal mechanisms of harvest injury. In aerobic cells the energy necessary to maintain cell integrity is supplied by the mitochondrial cytochrome system through complete reduction of oxygen to water. This involves the generation of ATP, by which means energy is stored for later consumption (oxidative phosphorylation). These terminal reactions in aerobic glycolysis need a continuous supply of oxygen. As cells become anoxic, oxidative phosphorylation ceases and the stored ATP is consumed rapidly. Lack of ATP leads to cell injury by impairment of energy-dependent intracellular homeostatic functions. ATP can be saved by cooling the organ, which reduces the tissue's metabolic demands for nutrients and oxygen. Hypothermia, therefore, plays an essential role in the preservation of anoxic cells.

Many organs suffer from considerable damage induced immediately after a period of ischaemia at reperfusion (61). The ischaemic small bowel is particularly susceptible to reperfusion injury, and involvement of oxygen-derived free radicals in the pathophysiology of cell damage has been suggested (62). The major source of oxygen-derived free radicals is xanthine oxydase (61). Thus, direct prevention of superoxide formation by a xanthine oxydase inhibitor, such as allopurinol, or the use of oxygen-derived free radical scavengers, such as superoxide dismutase, could be important in protecting the organ against reperfusion injury (63,64).

2.3.2 Intestinal preservation

Developing an effective method for preserving the small bowel during the interval between harvesting and implantation is a key to successful clinical small bowel transplantation in the future. The small bowel, and particularly its mucosa, is susceptible to ischaemia, but possesses a great regenerative potential (65). Therefore, any method of intestinal preservation must maintain the integrity of the intestinal mucosa or minimize ischaemic damage so that regeneration may occur within a few days after the ischaemic period. Structural damage of the intestinal mucosa starts rapidly after the onset of

ischaemia. After a total ischaemia period of 30 minutes in the rat or 60 minutes in the dog, the upper two-thirds of the villi are completely denuded of epithelial cells, whereas the lower parts of the villi and the crypts remain relatively intact (65). If circulatory arrest persists for one to two hours, the crypt epithelium in rats will sustain ischaemic damage as well (66). The small bowel mucosa may recover from an ischaemic event, depending on the duration of ischaemia. In the rat, after ligation of the arterial flow for one hour, epithelial injury reverses within 48 to 72 hours (67). When the ischaemic episode is prolonged to two hours, the structure of both villi and crypts requires seven days to return to normal (68). After an ischaemic period of one hour, the villi of canine ileum are again covered with epithelial cells within 24 hours (65).

The first studies of intestinal preservation were performed in 1959 by Lillehei, who demonstrated that the canine intestine could be successfully preserved in vitro for five hours by simple immersion of the graft in normal saline at 5°C (1). Manax demonstrated the supplemental effects of hypothermia (2°C) and hyperbaric oxygen (69). Preserved for 24 and 48 hours, ileal grafts were transplanted successfully in a heterotopic position in the neck. Since then, numerous techniques for preservation have been reported, such as pulsatile normothermic perfusion, or intravascular flushing with a cold (4°C) crystalloid solution followed by cold storage (70,71). The latter technique resulted in effective short-term (6 to 18 hours) preservation. Ricour showed that successful preservation for 20 hours could be achieved with this technique in piglets (71). Using a cold (4°C) modified extracellular fluid containing fructose and heparin for intravascular flushing and storage at 4°C, safe preservation of rat small bowel grafts could be achieved for 12 to 24 hours (72). Continuous intravascular perfusion preserved canine small bowel grafts for 24 hours (73). Allopurinol used during perfusion preservation appeared to be an important additive (74). Raju performed simple hypothermic storage of canine small bowel by perfusing with ice cold Ringers-lactate (75). Allografts preserved in this way for 12 hours resulted in a mean recipient survival of 45.4 days, and grafts stored for 24 hours survived 22.5 days. Raju underlined the beneficial effect of in situ flushing and intraluminal irrigation, which may reduce endotoxinaemia and damage from pancreatic enzymes (75).

Recently, several studies have reported improved small bowel preservation by modifying free radical metabolism (76-78). Naloxone administration during or after ischaemia had a beneficial effect (76). It was suggested that naloxone may have a scavenging effect on the formation of free radicals. Matsusaka demonstrated the protective effect of coenzyme Q (CoQ_{10}) after reperfusion (77). CoQ_{10} is indispensable to the production of ATP in mitochondria and may reverse impaired mitochondrial function by suppressing an increase in lipid peroxides in mitochondria after reperfusion. Pretreatment with CoQ_{10} significantly increased ATP recovery, and survival rates surged from 13 to 75% (77). Administration of superoxide dimutase has been proven beneficial

to the small bowel graft, probably by effectively catalyzing the dismutation of superoxide radicals (78).

Although important advances have been made in the understanding of intestinal preservation injury, there is at present no consensus about a uniform preservation technique or preservation solution (78-81). An interesting field of research may be ex vivo perfusion of small bowel grafts with monoclonal antibodies to deplete T cell subsets (82-84). Stangl showed that preservation with monoclonal antibodies for three hours resulted in sufficient binding to dendritic cells, macrophages and class II antigens (82). Recipients of small bowel grafts perfused with these monoclonal antibodies survived significantly longer than control rats.

2.4 Immunology

2.4.1 The immunological problem

Small bowel transplantation between two unrelated individuals causes two distinct immunological reactions. First, cell-mediated graft rejection by the recipient and second, GVHD in which lymphocytes of the small bowel graft attack the recipient. Besides bone marrow transplantation, small bowel transplantation is the only type of organ transplantation in which GVHD plays a role. This may be due to the large amount of lymphoid cells in the lamina propria, in Peyer's patches and in mesenteric lymph nodes, collectively called gut-associated lymphoid tissue. In man, this lymphoid tissue represents 25% of the gastrointestinal mucosa, and every gram of small bowel mucosa contains 6 x 10^6 lymphoid cells (85).

It has been suggested that an immunological balance exists between rejection and GVHD (86-90). Cohen found that irradiation of canine small bowel with 150 rads before transplantation resulted in rejection without GVHD in 9.2 days. After pretreatment with 50 rads, graft survival was prolonged to 28 days with less aggressive rejection than in the heavily irradiated grafts (86). Grant postulated that GVHD following small bowel transplantation damages the host's lymphoid tissue, thereby producing profound immunosuppression (87). Gundlach found that mesenteric lymphadenectomy led to chronic rejection, probably caused by the absence of the immunosuppressive effect of GVHD (88). Saat and De Bruin showed that pretreatment of the donor with irradiation or anti-lymphocyte serum (ALS) accelerates rejection (35,89,90). It is still obscure whether this immunological balance has an impact on clinical small bowel transplantation.

2.4.2 Rejection

Rejection of small bowel grafts appears to proceed like in other vascularized organs. T-lymphocytes are thought to be the main cell population mediating the immune response (91). In the afferent phase, T-lymphocytes will recognize foreign antigens associated with Major Histocompatibility Complex (MHC) antigens and become sensitized, after which they develop into lymphoblasts-secreting lymphokines. In the efferent phase these sensitized T-cells will recognize antigen-presenting cells that express specific MHC-molecules on the cell membrane. This results in the production of interleukin-2 (IL-2) by helper T-cells, and cytolysins by suppressor T-cells. Activated suppressor/cytotoxic T-cells express the CD8 molecule and kill cells expressing foreign antigen bound to MHC class I molecules, which are present on almost all cells in the body. Helper T-cells express the CD4 molecule and recognize foreign antigen bound to MHC class II molecules, which are expressed by relatively few cells, including macrophages, B cells and endothelial cells. In response to cytokines secreted by helper T-cells, macrophages become cytocidal and B cells produce antibodies, both of which will contribute to the rejection process. Dendritic cells express both MHC class I and II molecules and can present antigen to resting T-cells as well as sensitized ones, unlike other antigen-presenting cells. Dendritic cells in organ grafts sensitize the recipient to graft antigens and initiate the rejection process.

The process of small bowel rejection has been most carefully examined in rats, in which the availability of inbred strains allows repetitive controlled experiments. However, there are slight differences between the rat strains studied (92,93). In the rejection model ((Lew x BN)F1 to Lew) the graft is rejected six to nine days after transplantation (32,92). Three days after transplantation there is only slight mucosal damage, restricted to the crypts (92). Endothelial cells of the microvasculature, particularly at the junction of the mucosa and submucosa, show vacuolization or endothelial cell swelling. Villus shortening is found by day six, but the villous epithelial cells are morphologically normal. The crypts are elongated with frequent foci of epithelial damage. The lamina propria becomes distended with a mononuclear lymphoid cell infiltrate. After nine days gross mucosal oedema and sloughing with marked shortening of villi and crypts is seen, and the graft is densely infiltrated with mononuclear polymorphonuclear cells. Some of the fine vessels of the microvasculature are occluded by swollen endothelium. As villi originate from crypt cells, the villous damage may be secondary to crypt damage. In addition, ischaemia secondary to vascular occlusion may play a role (92). The native small bowel appears essentially normal.

Histology of small bowel rejection in large animals has been less well studied (94,95). In a pig study the earliest features indicating rejection were seen at six days (95). Rejection is characterized by a decrease in mucosal depth accompanied by shortening of

absorptive cells and a dense infiltrate of mononuclear cells in the lamina propria. Multifocal perivascular cuffing of mononuclear cells can be seen in vessels adjacent to the muscularis mucosae. As rejection progresses, erosion of the mucosal surface with loss of all epithelial cells occurs in a patchy fashion.

2.4.3 Prevention of rejection

A cardinal problem in small bowel transplantation rests with the rejection process and its suppression. Rat studies have shown that rejection of a small bowel graft causes a weaker barrier function of the intestinal mucosa, which results in translocation of endotoxins and bacteria (96,97). The high rate of sepsis after small bowel transplantation is partly due to excessive immunosuppression. Even if the recipient survives rejection episodes accompanied by septic events, developing graft fibrosis and obstruction will eventually lead to malnutrition and loss of the graft. Rejection must therefore be detected and treated as early as possible.

Many strategies have been designed to prevent rejection of small bowel grafts either by host immunosuppression or by reducing graft immunogenicity. Disadvantages of immunosuppressive drugs are the compromised general immune status of the host with subsequent increased risk of infectious complications, and the toxic and lymphoproliferative side-effects of some immunosuppressants.

Host immunosuppression

Conventional immunosuppressive drugs such as azathioprine and prednisone proved relatively ineffective in small bowel transplantation. Ruiz documented a mean survival of 21 days in dogs after orthotopic small bowel transplantation using azathioprine and prednisone (7). Similar results using a heterotopic model have been reported as well (3,4,98). Monotherapy of ALS, or a combination of ALS, azathioprine and prednisone did not result in long-term survivors (5,99).

Since the availability of CsA numerous experimental small bowel transplantations have been performed in rats, pigs and dogs. In rat models CsA (15 mg/kg/day) resulted in long-term graft survival, even with short courses (92,96,100). However, this beneficial effect of CsA is rat-strain-dependent, as shown by De Bruin (93). In large animal models monotherapy of CsA is not as effective as in rat models, and only a few long-term survivors have been reported (20,21,23,26-28). Steroid therapy in addition to CsA did not prolong graft survival significantly compared to single CsA treatment (22-24).

In the early studies CsA was given either orally or intramuscularly in orthotopic models. It was shown that oral CsA is poorly absorbed in the early posttransplant period, which may result in low plasma CsA levels and thus cause rejection (21,101). CsA is a

liposoluble drug; when ingested orally, its intestinal absorption and lymphatic transport imply functional enterocytes and lymphatic pathway. This led to the suggestion that oral CsA treatment in the peritransplant period might be better avoided, because regeneration of disrupted lymphatics may take two to four weeks (102-104). Indeed, intravenous CsA treatment resulted in long-term survival in pigs (25,29,105). Grant showed that continuous intravenous CsA (15 mg/kg/day) for one week, followed by oral CsA (30 mg/kg/day) in tapering doses, prevented graft rejection and permitted long-term survival (121 $\pm$ 32 days) in an orthotopic small bowel transplantation model in pigs (29). However, in other intravenous CsA studies a substantial number of late deaths from sepsis was reported, probably due to high plasma CsA levels (25,105).

Since 1990 much attention has been focused on FK-506 as an immunosuppressant for small bowel transplantation. In the histo-incompatible ACI-to-Lew rat strain combination 2 mg/kg/day FK-506 more effectively prevented acute rejection than 15 mg/kg/day CsA (14). In other rat strain combinations low-dose FK-506 also showed superior immunosuppressive capacities compared to CsA (106,107). Using the BN-to-Lew model, Stangl demonstrated that FK-506 may be able to reverse established small bowel rejection (108). Also in multivisceral transplants FK-506 proved valuable to prevent rejection (60). In the WAG-to-BN rat strain combination, however, FK-506 led to reduced graft survival compared to CsA and permitted the development of fulminant GVHD (109).

Experiments with other immunosuppressive agents have been reported recently. Kim examined the immunosuppressive effects of CsA metabolites in vitro and in vivo in a rat model (110). It was demonstrated that CsA metabolites have similar immunosuppressive effects as CsA, but appear to have no hepato- and nephrotoxic side-effects, which may occur with CsA treatment. Several studies have reported the immunosuppressive efficacy of low-dose rapamycin, which may suppress both rejection and GVHD (15,111,112). Rapamycin combined with CsA may be more effective than rapamycin alone (112). However, Marquet found that low-dose rapamycin combined with CsA results in a similar immunosuppressive effect as CsA alone in BN-to-WAG and WAG-to-BN small bowel transplantations (113). Using the same immunosuppressive protocol, prolonged survival of heart allografts was found, which demonstrates the immunogenicity of small bowel grafts. At present, the value of RS-61443 and Brequinar sodium as immunosuppressants in small bowel transplantation are being investigated (114-117). It may be expected that a combination of these immunosuppressants has a potent immunosuppressive effect, thus circumventing toxic side-effects.

Theoretically, recipient splenectomy may be considered as immunosuppressive adjuvant therapy in small bowel transplantation. In a rat model of kidney transplantation, splenectomy prior to transplantation led to a suppressed lymphocytotoxic antibody

response to the graft, which resulted in prolonged graft survival (118). Schraut reported a beneficial effect of splenectomy in a F1-to-P rat model, but this could not be confirmed in a fully allogeneic combination nor in a pig model (26,33,47,49). Therefore, this form of therapy remains controversial.

Preconditioning of the host with donor antigen (active enhancement) or antidonor antiserum (passive enhancement) has been attempted to achieve specific suppression of allograft rejection (119,120). Although donor-specific blood transfusions (DST) proved very effective in prolonging heart and kidney survival in the BN-to-WAG rat combination, they had no effect on small bowel allograft survival (119,121). DST combined with CsA or FK-506 produced a synergistic effect (122,123). However, the role of DST is still uncertain, because other studies could not confirm this synergism of DST and immunosuppressive agents (121,124). Portal injection of donor splenocytes 14 days before transplantation led to prolonged small bowel graft survival, but the clinical relevance of this experiment is obscure (125). Active enhancement has also been achieved by transplanting a liver before or simultaneously with a small bowel in a rat model (53,54). The exact mechanism of immunological protection by the liver has not yet been elucidated, but it may already have proved its value in clinical small bowel transplantation (16). Another form of active enhancement is the achievement of chimerism by allogeneic bone marrow transplantation (126). Mechanisms to explain the immunological effect of chimerism include clonal deletion, clonal anergy and proliferation of donor-specific suppressor cells. In a rat model is was shown that chimeras accepted small bowel allografts from the bone marrow donor permanently (more than 300 days) without any evidence of GVHD when the small bowel transplantation was performed 60 or 150 days after the transplantation of irradiated bone marrow (127). Third-party small bowel allografts were acutely (six to nine days) rejected, thus demonstrating the specificity of this tolerance induction.

Passive enhancement by ALS resulted in a minor, but significant increase in small bowel graft survival in dogs and rats (5,128).

Reduction of graft immunogenicity

Another approach to prevent rejection of a small bowel allograft is reducing its pronounced immunogenicity, which is particularly caused by the many immunocompetent lymphocytes contained in Peyer's patches, in lymphatic tissue distributed in the small bowel mucosa, and within mesenteric lymph nodes. In rat models, neither length nor site of origin of small bowel grafts had any effect on the moment of onset of rejection (121,129,130). However, Kimura reported longer host survival when shorter segments and segments of proximal sites were transplanted in a heterotopic fashion (130). Thus, a

direct relationship was suggested between the severity of rejection and the amount of small bowel transplanted. In another study, Kimura showed that significantly lower doses of CsA were necessary for rats that received shorter grafts (131).

Donor or allograft irradiation intended to reduce the leukocyte count effected improved survival in experimental kidney and heart transplantation (132). In a rat model of small bowel transplantation, irradiation immediately or four days before transplantation did not prolong graft survival (33). However, histologic examination showed that graft lymphocyte destruction was not completely achieved. In dog studies ex vivo irradiation of the small bowel allograft did not prolong survival of the recipients either (27,133). In contrast, Williams reported a significant increase in survival after irradiating the small bowel graft with 750 rads (134). Nevertheless, the recipient dogs died either of chronic rejection or infection. Pretreatment of the donor with ALS to reduce graft immunogenicity also did not prevent rejection (5,27,99). Pretreatment by ex vivo perfusion with monoclonal antibodies may turn out to be a valuable tool to reduce graft immunogenicity. Stangl reported prolongation of rat small bowel graft survival after pretreatment of the graft with monoclonal antibodies directed against T-cell subsets (82,135).

A different approach to achieve reduced graft immunogenicity is selecting a donor-recipient combination by matching for MHC antigens (136,137). One of the dogmas of immunology dictates that the severity of allograft rejection is significantly influenced by the degree of histocompatibility between donor and recipient. Remarkably, the effect of MHC matching has rarely been investigated in experimental and clinical small bowel transplantations. Incompatibility for different MHC regions has been examined in a rat study (138). It was suggested that the absolute amount of classes I and II MHC antigens in the transplanted tissue has a major influence on the graft's immunogenicity and immune response. Lee showed the importance of MHC antigens in relation to small bowel graft rejection (139). When MHC-different and non-MHC-different rat strain combinations were compared, MHC-difference resulted in acute rejection in 12 to 14 days, whereas non-MHC difference led to rejection in 20 days (139). In the pre-CsA era, Westbroek demonstrated the significance of histocompatibility testing on the survival of small bowel allografts in dogs without using immunosuppressive drugs (6,140). Heterotopic small bowel allografts survived 45.3 days in identical littermate combinations, but only 15.3 days in non-identical ones; in non-littermate donor-recipient combinations matched grafts survived 27.5 days, mismatched grafts only 8.1 days. The beneficial effects of MHC matching could well have an impact on future clinical living-related small bowel transplantations.

2.4.4 Graft-versus-host disease

GVHD is caused by migration of immunocompetent T-lymphocytes from the donor to the recipient (141). It does not occur after transplantation of small bowel from T-cell depleted parental donors into F1-hybrid recipients (100). Reconstitution of the donor with syngeneic T-cells prior to graft donation restores GVHD (100). This confirms that donor T-lymphocytes mediate GVHD. Both helper and suppressor T-cell populations can cause GVHD, but only helper T-cells produce strong, acute GVHD (142). The intensity further depends on the number of transplanted graft lymphocytes; the smaller the intestinal allograft and the less the lymphoid tissue transplanted, the longer host survival (143). The local damage accompanying GVHD is a non-specific phenomenon and is probably caused by cytokines released by immunological interactions between graft T-cells and host leukocytes (144). The cytokines involved are tumor necrosis factor (TNF) and gamma interferon (144,145).

Small bowel transplantation from parents to F1-hybrids in inbred rat strains provides a useful model to study GVHD (32). In this model the recipient does not reject the graft, because it does not recognize foreign antigens. However, the small bowel allograft starts an immunological reaction against foreign recipient antigens. In contrast, in clinically more relevant allogeneic small bowel transplantation, rejection may predominate over GVHD (32,96). Macroscopically, GVHD is characterized by weight loss, dermatitis, alopecia, diarrhoea, and a hunched posture. These signs are evident approximately ten days after transplantation and eventually result in recipient death by day 20. Microscopically, the graft reveals no abnormalities, but the transplanted Peyer's patches and mesenteric lymph nodes show progressive lymphoid atrophy. In the host, the native small bowel shows reduced villus height and eventually villous sloughing, and loss of normal architecture of the spleen, lymph nodes and thymus (87,96,146). The skin shows a polymorphonuclear infiltrate at the dermal/epidermal junction with basal cell vacuolization and keratinocyte necrosis (146).

In outbred large animals GVHD is not seen as frequently as in rat models of small bowel transplantation (105). Although Lillehei and Cohen reported GVHD as an inevitable consequence of small bowel transplantation in the large animal model, at present it is believed that rejection predominates (2,33,86,147).

2.4.5 Prevention of graft-versus-host disease

GVHD may be prevented by host immunosuppression or reduction of the number of immunocompetent T-lymphocytes in the small bowel graft in vivo or in vitro prior to grafting. In unidirectional small bowel allograft studies, Kirkman showed that doses of CsA that prevent rejection are less effective in preventing GVHD (100). In some rat

strains a short course of CsA is able to prevent rejection, but continuous CsA is necessary to prevent GVHD (33,143,148). In large animals, even continuous CsA therapy does sometimes not prevent GVHD (105). Recently, the superior effects of FK-506 compared to CsA in preventing GVHD have been reported (14). However, these could not be confirmed by others, who reported an increase in GVHD with FK-506 (109,149).

Another avenue to prevent GVHD may be reduction of immunocompetent T-lymphocytes in the graft. In rats, T-cell depletion by donor or allograft irradiation has been shown to prevent GVHD (32,35,143,150). A disadvantage of irradiation in a clinical setting may be the long-term nutritional consequences of radiation to human small bowel. Donor pretreatment with ALS at two and one day before transplantation prevented GVHD without impairing subsequent allograft function as measured by absorption of dietary energy and nitrogen, weight gain, and small bowel morphology (90,151). Donor pretreatment with recipient blood transfusions reduced the likelihood of rejection, but increased the incidence of GVHD by pre-sensitization to host antigens (152). Mechanical reduction of the lymphoid load by mesenteric lymphadenectomy and reducing the length of the small bowel graft prevented GVHD in rat studies (88,130,143,153,154). Depletion of graft T-lymphocytes may also be achieved by treating the donor or allograft with monoclonal antibodies (83,84). In rats, elevated levels of TNF have been measured during GVHD after small bowel transplantation (155). Therefore, anti-TNF therapy may be useful in preventing GVHD, as has already been shown after experimental bone marrow transplantation (145).

In the rat model of small bowel transplantation, several authors have suggested an immunological balance theory, in which rejection is counteracted by GVHD (86-90). Indeed, prevention of GVHD resulted in non-opposed rejection of the small bowel grafts. However, at present it remains unclear whether this immunological balance will be clinically relevant.

2.5 Physiology

Intestinal physiology includes enteric motility, hormonal, immunological and nutritional aspects, and intestinal barrier function. The surgical procedure of small bowel transplantation inevitably involves transection of the intestinal wall with disruption of the intrinsic neural continuity, extrinsic denervation, interruption of lymphatic drainage, and preservation-induced injury. Each component of this procedure may alter intestinal physiology adversely. In addition, immunological reactions and immunosuppressive agents may further compromise normal intestinal functions after small bowel transplantation. In order to be of benefit to a patient with SBS, transplanted small bowel must be fully functional and capable of sustaining the recipient without parenteral nutrition.

2.5.1 Motor function

The gastrointestinal tract has a cyclic motor activity (156). In the small bowel, this activity generally begins in the duodenum and migrates in a distal direction, and is therefore called migrating motor complex (MMC). In most non-ruminants the MMC is present only in the fasted state, whereas in ruminants it is present in both fasted and postprandial states. Both the initiation and migration of these complexes seem to be controlled by intrinsic neural mechanisms, and may be modulated by extrinsic neural mechanisms (central nervous system) and by circulating hormonal substances (156). Feeding interrupts the MMC and induces a less well-defined, non-cyclic pattern of intermittent, low-amplitude contractions persisting a variable period, the length of which is related to type and amount of luminal substances. The physiological role of the MMC is to clean the small bowel of residual food, secretions, and cellular debris, and to empty them into the colon. In the postprandial state, the changed motor pattern results in optimal mixing and facilitates absorption of nutrients.

The motor function of transplanted small bowel has been investigated by several groups. In autotransplanted dogs, Ballinger demonstrated altered small bowel motility by barium X-ray studies (157). Schiller showed that smooth muscle contractile activity is enhanced as a result of extrinsic denervation following allotransplantation of a jejunal segment in the neck (158). In a syngeneic rat model, Taguchi evaluated the effect of small bowel transplantation on intestinal smooth muscle contractility and the associated neural control mechanisms (159). He found that neither the contractile properties of smooth muscle, nor the responses to several neuropeptides, nor the excitatory response to electrical stimulation had changed. Inhibitory innervation, however, underwent a change, probably reflecting the degeneration of adrenergic nerve endings as a result of extrinsic denervation. Taguchi suggested that loss of one aspect of inhibition does not interfere with motility, because neural control of the small bowel appears to be predominantly intrinsic.

Working with a dog model, Dennison reported normal intestinal myoelectrical activity by day two after auto- and allografting, and recovering of intestinal transit ten days after autografting (28,160). In contrast, others showed that motor activity is absent during several weeks following autotransplantation in dogs (161-163). After this period the autografted small bowel recovers the ability to generate normal MMCs, but the postprandial myoelectrical activity remains consistently and permanently absent. This may confirm that the fed response is dependent on the integrity of the extrinsic nerves (156,161). However, infusion of the postprandial hormones cholecystokinin (CCK) and pentagastrin induced a normal fed pattern of contractions, suggestive of a modulating effect by hormones without imput from the extrinsic nerves (161). Changes in motility after small bowel transplantation in rats have also been reported (164,165). Isograft

electrical activity was not observed until at least 40 hours after transplantation and never reached the level of basal electrical rhythm of the native small bowel. MMCs were observed in the isografts only after 11 days (165).

These alterations in fasting and/or postprandial responses could result in luminal bacterial overgrowth, which may contribute to failure of the small bowel graft and infectious complications (166).

2.5.2 Hormone function

The gastrointestinal tract is innervated by a network of intrinsic and extrinsic neurons containing peptides. Neuropeptides modulate the action of neurotransmitters, such as norepinephrine and acetylcholine, thereby influencing blood flow, smooth muscle contractility, secretion, absorption, gastrointestinal transit, and pancreatic function (167). Changes in the content and amount of these neuropeptides in response to denervation may have local and distant effects on small bowel functions. These observations have a great impact on small bowel transplantation, the more so because the gut is the largest endocrine organ of the body (168).

Gebhardt examined the patterns of hormone distribution after heterotopic and orthotopic small bowel transplantation in rats (169). Heterotopic transplantation resulted in mucosal atrophy and a decrease in vasoactive intestinal peptide (VIP) and CCK levels. However, when the heterotopic graft was brought into the orthotopic position, these levels returned to normal. LaRosa reported that small bowel transplantation, and thus extrinsic denervation, does not affect base-line intraluminal levels of substance P and serotonin, nor the physiological responses of these neuropeptides to intraluminal stimuli (170). Similar results were reported by Teitelbaum concerning somatostatin, VIP and substance P after syngeneic and allogeneic small bowel transplantation in rats. Progressive reductions in neuropeptides were only seen in case of small bowel graft rejection (171). In contrast, Nelson showed that in vivo neural isolation of canine jejunoileum results in temporal adaptation of enteric neuropeptides (172). After extrinsic and intrinsic denervation he found increased VIP and substance P levels, but decreased neuropeptide Y levels in both tissue and plasma. In addition, VIP and substance P levels progressively increased with time after surgery (198% and 217% mean maximal increases, respectively). As VIP and substance P induce a prosecretory effect and neuropeptide Y a proabsorptive effect, this may have contributed to the profuse watery diarrhoea observed in this model (172). Nelson ascribed the discrepancy between his findings and other reports to the relatively short follow-up of earlier reports. The altered neuropeptide levels after denervation may also modulate smooth muscle contractility and cause altered motility, as reported earlier by Sarr (162,163).

The effect of denervation on gastrointestinal hormone levels has not been precisely

delineated yet, but adaptive changes in the neuropeptides of the gut may possibly alter the enteric physiology after small bowel transplantation. An interesting field of research could be the influence of neuropeptides in modulating the gastrointestinal neuroimmune axis. It has been shown that VIP exerts potent immunosuppressive actions via T-cell specific mechanisms, which may play a role after small bowel transplantation (173).

2.5.3 Immune function

The small bowel is a major organ in the immune defense system. The exposed surface of the intestinal mucosa is constantly challenged by ingested foreign antigens in micro-organisms, products of food digestion and drugs (174,175). Therefore it is not surprising that the small bowel contains a large accumulation of lymphoid tissue in mesenteric lymph nodes, Peyer's patches, lamina propria and epithelium. Much attention has been paid to the stimulating effect of lymphoid tissue on rejection and GVHD. Little is known, however, about the immune function of the transplanted small bowel, although it is obvious that long-term survival with a small bowel graft necessitates a well-functioning local immune system (176).

The secretory immunoglobulin (Ig) system is the best defined effector mechanism of mucosal immunity (175). In man and mice, approximately 80% of Ig-producing cells (B-lymphocytes) are located in the intestinal mucosa. The majority of secretory Ig, sIgA, is produced by plasma cells in the villi and translocated to the gut lumen at a rate of 40 mg/kg body weight per day. After antigenic stimulation, B-lymphocytes leave the Peyer's patches via the mesenteric lymph nodes and the thoracic duct to the peripheral circulation. These B-lymphocytes home to the lamina propria of mucosal tissues, in which they differentiate into plasma cells.

In an effort to understand the immune function of transplanted small bowel, Xia and Kirkman reported a consecutive series of studies about specific sIgA production against cholera toxin (CT) in a rat small bowel transplantation model (177-181). They found that there is no difference in total sIgA production after syngeneic or allogeneic transplantation, but that allografts are less effective in producing a primary sIgA response against CT than isografts (177). However, after priming the donor, small bowel allografts are capable of producing a secondary sIgA response to a booster of CT seven days posttransplant (178). CsA treatment has no effect on total sIgA production, but it suppresses the sIgA response to CT in both allografts and isografts (179). This inhibitory effect of CsA lasts as long as the recipients are receiving CsA. Another experiment revealed that CsA only inhibits sIgA production against a T-cell dependent antigen such as CT, but not against a T-cell independent antigen (trinitrophenyl-lipopolysaccharide)(180). Xia and Kirkman suggested that this unresponsiveness against T-cell dependent antigens in CsA treated recipients explains the high incidence of septic

complications after small bowel transplantation. Recently, they demonstrated that priming of the recipient with CT before transplantation abrogates the inhibitory effect of CsA on specific sIgA production against CT (181). This finding may be relevant to clinical small bowel transplantation, since immunity against an infectious agent may be obtained after priming the transplantation candidate. They proposed that functional recipient lymphocytes repopulate the small bowel graft and produce the secondary immune response after boosting with CT. This phenomenon of repopulating the small bowel graft with recipient lymphocytes without causing rejection of the graft has been described in rats and in man (182,183).

Yagi examined the effect of rejection and GVHD on IgM production by the intestinal mucosa in semi-allogenic small bowel transplantation in rats (184). In the rejection group, decreased IgM levels of the graft were associated with rejection, but IgM levels of the native small bowel remained stable. In the GVHD model, mucosal IgM levels in the graft were higher, whereas IgM levels in the native intestine were lower. Treatment with FK-506 could not prevent the drop of mucosal IgM levels of the native intestine (184). This suggests that the local mucosal immunity is compromised after small bowel transplantation.

2.5.4 Nutritional function

Obviously, small bowel transplantation is only meaningful if the graft is capable of maintaining adequate nutrition in adult recipients, and normal growth and development in children without the support of parenteral nutrition. A number of - sometimes conflicting - studies have been reported concerning growth and development after small bowel transplantation in both rats and large animals. After an initial postoperative weight loss, rats regain normal weight after total small bowel iso- and allografting (185-187). Some investigators found normal weight gain even after a segmental small bowel graft consisting of either jejunum, ileum or only part of jejunum (185,188,189). This may suggest that transplanted small bowel has the ability to adapt, which indeed has been confirmed by Kirsch, who found an increased diameter of the graft with hypertrophied villi (189). Kimura designed a fatal model of SBS, in which transplantation of 40 cm of jejunum resulted in normal growth, whereas rats with a 20 cm jejunal graft showed suboptimal weight gain (190). Suboptimal growth after segmental small bowel grafting has also been found by De Bruin and Oki (186,191).

Small bowel transplantation in large animals has yielded more controversial data, probably because only occasionally long-term survivors have been reported. Dogs with a total small bowel autotransplant regained their pretransplant weight either soon or after a prolonged period (31,161,192). However, several authors reported a 10 to 15% weight loss after total small bowel or 100 cm ileal autografting (160,193). Long-term surviving

dogs with a total small bowel allograft maintained their body weight, but after one year the weights of the two long-term survivors reported by Diliz-Perez fell to a lower but eventually stable level (20,22). Transplantation of 100 cm ileal allografts in dogs by Collin resulted in 88% of pretransplant weights (194). In contrast, pigs with a total small bowel allograft show a weight gain comparable to that of control pigs (29,105). Kimura showed that pigs transplanted with a segmental jejunal allograft (25% of total small bowel length) increased their body weight by almost 40% after six months, whereas enterectomized control pigs uniformly suffered from malnutrition and show progressive weight loss (30). Kimura concluded that porcine segmental small bowel allografts maintain their compensatory capacity and hypertrophy after massive small bowel reduction.

Nutrient absorption

With regard to clinical small bowel transplantation, the absorptive function of the transplanted small bowel is the most important physiological determinant of graft function. It may be expected that after small bowel transplantation the absorptive function will have changed - at least temporarily - due to harvest injury, extrinsic denervation, transection of intrinsic neural continuity, and disruption of lymphatics. At present, however, intestinal absorptive function after small bowel transplantation is not fully understood and inconsistent studies have been reported. The different results may be explained by the large variety of experimental protocols used. In addition, differences may be related to the transplantation procedure itself and/or the subsequent immunological phenomena. Using a syngeneic or autotransplant model the latter problems can be circumvented, while it allows investigation of the functional effects of harvest injury and disrupting neural and lymphatic continuity.

Nutrient absorption studies after small bowel transplantation in rats showed prolonged malabsorption of fat and fat-soluble vitamins (A and E), reduced uptake of glucose and glycine, and reduced glucose-stimulated electrophysiological parameters (185,187,195). Other rat studies, however, demonstrated normal faecal fat excretion, normal glucose and vitamin B_{12} uptake, and relatively normal electrophysiological characteristics in non-rejecting small bowel grafts (92,100,186,196).

In dogs and pigs, absorptive functions of jejunum (D-xylose, folate), ileum (vitamin B_{12}) and jejunoileum (fat, fat-soluble vitamins, CsA) have been studied in auto- and allotransplants. D-xylose is a simple sugar absorbed primarily in the duodenum and jejunum, which does not utilize other active transport systems that facilitate absorption of other sugars (199). Folate absorption occurs primarily in the jejunum (200). In a modified autotransplantation model without peritransplant ischaemia, Sarr showed normal D-xylose and folate absorption (193). Normal D-xylose absorption has also been reported in

allotransplant experiments (25,197,198). This may suggest that absorption of D-xylose and folate does not require neural or lymphatic continuity. In contrast, several others reported reduced D-xylose absorption by small bowel allografts, but this could be related to rejection of the graft (20,22). The reduced D-xylose absorption found by Raju in autotransplants may be ascribed to bacterial overgrowth, because a similar phenomenon was found by other investigators in canine autotransplants surviving more than one year (31,161,192). This late malabsorption was correlated to significant bacterial overgrowth, probably due to permanently impaired small bowel motility (192).

Vitamin B_{12} absorption and faecal fat excretion have been reported to be normal in auto- and allografts in both dogs and pigs (29,105,161,193,198). This suggests that vitamin B_{12} absorption occurs without extrinsic and intrinsic neural and lymphatic continuity. An interesting finding is the normal fat absorption in the presence of disrupted lymphatic drainage, even in the early posttransplant period, as reported by Sarr (193). Re-establishment of lymphatic drainage has been shown to occur two weeks after small bowel transplantation and is fully achieved by four to six weeks (101-103,201). An explanation for this early fat absorption may be the opening of lymphovenous communications in the mesentery if the lymphatic drainage is obstructed (202). This allows a pathway for absorption of fat until the regenerated lymphatics take over. Nevertheless, Diliz-Perez found that faecal fat excretion after total small bowel transplantation was higher than that in control dogs (22). However, in the latter study the control dogs did not receive CsA, which can induce decreased fat absorption by itself or by the oily solvent. A delayed effect in decreased intestinal absorption of fat has been reported by Thompson in canine small bowel autotransplants more than 12 months after transplantation, correlated to significant bacterial overgrowth in transplanted jejunum and ileum (192).

Adequate CsA absorption, a process that takes place in the small bowel, is of vital importance for a recipient with a small bowel allograft (101,186,203). CsA is a lipophilic agent and is absorbed through the lymphatic drainage. Schraut postulated that CsA absorption in the early posttransplant period may be compromised due to disrupted lymphatics (33). This has been underscored by investigators who found a low incidence of small bowel graft rejection if CsA was administered parenterally in the peritransplant period (29,105). However, other investigators found normal CsA absorption after oral or intraluminal treatment, even in the first week posttransplant (23,186,204). Cohen explained this by CsA absorption via the peritoneal membrane (205). According to Cohen, CsA leaks into the peritoneal cavity through the disrupted lymphatics, after which it is absorbed. Another explanation may be that CsA is absorbed via lymphovenous anastomoses in the mesentery. However, during the early posttransplant period it seems justified to treat the recipient with parenteral CsA, because plasma CsA levels may be unpredictable in the early posttransplant period and rejection episodes of the small bowel

graft will impair the absorptive capacity for CsA (203,206). Complicating factors may be that CsA itself reduces nutrient absorption and adversely affects small bowel microvasculature (207,208).

Water and electrolyte absorption

At least in the early posttransplant period, regulation of water and electrolyte transport can be disturbed, as is evidenced by (temporary) watery diarrhoea after small bowel transplantation. Several rat studies have shown that net absorption of electrolyte solutions is decreased early after small bowel transplantation (195,209). Extrinsic denervation may be the underlying cause, for it is known that denervated small bowel secretes water and chloride from the crypts as a consequence of loss of sympathetic imput (210,211). It has been suggested that extrinsic denervation after small bowel transplantation plays a much more important role in the fluid and electrolyte balance than in the absorption of nutrients. Watson found that glucose is able to reverse the secretory state found with isotonic saline, which implies that glucose electrolyte solutions may be clinically useful in promoting electrolyte absorption after small bowel transplantation (195). Sigalet observed an increase in sodium/glucose cotransporter activity after small bowel transplantation, which may be compensatory and restore the electrolyte balance (187). However, recent rat and canine studies demonstrated no significant effect on jejunal absorption of a balanced physiological saline solution pre- or posttransplant (212,213). At present, therefore, it remains uncertain whether regeneration of extrinsic neural continuity or adaptation to chronic denervation takes place.

2.5.5 Barrier function

Under normal conditions, the small bowel mucosa is impermeable to macromolecules and provides a barrier against luminal bacteria and their toxins. The mucous layer of the small bowel and the composition and concentration of bacteria within the small bowel protect the underlying small bowel mucosa from pathogenic invasion. Normal enterocytes and intact intercellular tight junctions provide a physical barrier, supplemented by immunological factors such as gut-associated lymphoid tissue and sIgA production. It is known that an impaired gut barrier function is a possible etiological factor in the development of multi-organ failure (214). Therefore, the high incidence of infectious complications and sepsis after small bowel transplantation may be due to excessive immunosuppression, but a compromised barrier function of native or grafted small bowel may be another important factor (215).

In a rat study, Grant showed that rejection of orthotopic small bowel grafts leads to increased intestinal permeability to ^{51}Cr-EDTA and increased bacterial translocation

from the lumen of the graft to the host's mesenteric lymph nodes, liver and spleen (216). He proposed that bacterial translocation may stimulate immune responses, which will contribute to the intensity and rapidity of small bowel graft rejection. In a heterotopic rat model, Fabian reported increased bacterial translocation not only in allografts, but also in isografts, suggesting that the transplantation procedure itself affects the intestinal permeability independently of rejection (217). Browne demonstrated that the transplantation procedure itself leads to Gram-negative aerobic overgrowth within the transplanted small bowel segment and ascending colon (218). Significant translocation of bacteria to the mesenteric lymph nodes occurred only when CsA was added. Remarkably, Gram-negative organisms were more commonly isolated from the native mesenteric lymph nodes than from the graft, which suggests translocation through both native and transplanted small bowel (218). It was also found that CsA facilitates distant dissemination to liver, spleen and lung once translocation has occurred. Therefore, both bacterial overgrowth and immunosuppression are associated with translocation and sepsis after small bowel transplantation.

Using several permeability probes, Sigalet examined the effects of small bowel transplantation and CsA on intestinal permeability in non-rejecting small bowel grafts. The surgical procedure itself influences the transmembrane pores (increased mannitol uptake), CsA treatment affects ^{51}Cr-EDTA permeability via intercellular tight junctions, and allogeneic small bowel transplantation results in increased permeability to lactulose and ^{51}Cr-EDTA via intercellular tight junctions (219).

Therefore, the interaction between the small bowel microflora and immunosuppressive therapy will play a pivotal role in clinical small bowel transplantation. The transplantation itself alters the bacterial flora and increases the risk of sepsis when immunosuppression is added. Rejection episodes may further facilitate bacterial translocation. Strategies to enhance the mucosal barrier of the small bowel graft may improve morbidity and mortality after small bowel transplantation. Selective gut decontamination, early enteral nutrition, and modified parenteral nutrition may prove to be important to achieve this goal.

2.6 Monitoring

Monitoring of the small bowel allograft for early recognition and treatment of rejection is mandatory. At present the gold standard to diagnose rejection is histological investigation of the grafted tissue (94,95,220,221). However, recent studies have revealed several shortcomings of histological monitoring of small bowel allografts (222-224). Mucosal biopsies provide too little diagnostic information, while full-thickness biopsies carry the risk of perforation of the graft. Moreover, a series of consecutive biopsies is necessary to

evaluate rejection, because of the patchy character of morphological alterations (222,224). In addition, access to the small bowel allograft may be hampered when the graft is placed in continuity with the native small bowel. Heterotopic loops of small bowel may be readily accessible to a biopsy forceps, but defunctioning of small bowel loops results in atrofied small bowel mucosa, which may mimic the histological pattern of rejection.

To gain more diagnostic value of histological monitoring, several functional tests and serum parameters have been suggested to diagnose early rejection. The absorption of glucose, glycine, alanine, and fatty acids (lauric acid and oleic acid) has been shown to decrease during a rejection episode (28,225-227). However, none of these tests can be used as an early marker of rejection as morphological alterations of rejection precede changes of these functional tests. In addition, Holmes found that rejected small bowel is freely permeable to glucose, suggesting that a glucose absorption test is not useful to evaluate active glucose transport (8). Billiar evaluated the digestion of maltose and subsequent absorption of its split product, glucose. Early in the course of rejection, maltose absorption is diminished and may precede morphological changes of rejection by one or two days (228).

Hatcher noted increased permeability to polyethylene glycol (PEG) during small bowel graft rejection, but the development of an abdominal mass, suggesting rejection, occurred earlier than functional alterations (229). Teitelbaum found elevated permeability to PEG-900 two days preceding histological signs of rejection (230). However, he emphasized that an elevated urinary PEG level may not be diagnostic of rejection as a number of forms of intestinal injury may lead to PEG leakage. Grant reported increased intestinal permeability to ^{51}Cr-EDTA during small bowel graft rejection and concluded that a permeability change can be expected with minimal histological signs of rejection (231). In addition, Grant used this permeability probe to diagnose the early onset of rejection in a clinical case of small bowel transplantation (16). Recently, Sigalet examined different permeability probes (mannitol, lactulose, PEG-400, ^{51}Cr-EDTA) and he suggested that a combination of these markers may be necessary to predict rejection (219).

Teitelbaum showed that disaccharidase activity of the intestinal brush border, and mucosal levels of VIP, substance P, and somatostatin are reduced simultaneously with the first histological signs of graft rejection (171,232). However, full-thickness biopsies are necessary with the accompanying risks of sampling error and perforation of the graft. Schroeder used monoclonal antibodies to determine the distribution of brush border enzymes (maltase, sucrase) and alkaline phosphatase in both rat and human small bowel biopsies. Using this antibody technique, reduced maltase levels correlated with early rejection in the rat model, but this technique was useless to detect rejection in the human biopsies (233).

Monocyte/macrophage procoagulant activity (PCA) has been described to monitor

small bowel graft rejection (234,235). PCA is a serological correlate of alloantigen-induced activation of the immune system and may be a measure of immune activation of mononuclear cells (234). Elevated levels of PCA in peripheral blood mononuclear cells correlate well with early morphological changes of rejection, and PCA levels remain elevated during rejection. Kim concluded that PCA measured in peripheral blood mononuclear cells appears to be a sensitive serological marker of rejection (235). Maeda evaluated the usefulness of the lysosomal acid hydrolase, N-acetyl hexosaminidase (NAH), in detecting small bowel allograft rejection (236). In a rat model he found that in 40% of rejections serum NAH levels were elevated before morphological signs of rejection were evident, and thus he proposed to use serum NAH as a rapid and simple serum assay to detect small bowel rejection. However, it remains to be determined whether serum NAH is diagnostic to detect small bowel rejection, because it has been shown to be elevated in association with intestinal ischaemia or necrosis (237).

Kirkman and Madara investigated the effect of rejection on the electrophysiological function of small bowel allografts in a rat model by in vitro techniques (92,100). They found that decreased electrophysiological responses to stimulating solutions (glucose and theophylline) correlate with rejection. Using this technique it was found that crypts are damaged earlier than villi in the course of rejection. Lee reported an in vivo electrophysiological technique to detect small bowel graft rejection (238). Unfortunately, this method was too invasive to be recommended as a practical clinical tool.

In summary, none of the functional tests and serum parameters has been proven to replace histology as the gold standard to diagnose small bowel graft rejection. At present, therefore, it seems advisable to use a combination of both morphology and functional or serum tests to monitor a small bowel allograft. To assure small bowel transplantation as a clinical reality, future research efforts should be directed to finding a reliable marker to early detect the onset of rejection.

2.7 Clinical small bowel transplantation

2.7.1 The pre-CsA period

Seven clinical small bowel transplantations have been reported between 1964 and 1970 (104,239-241). The ages of the recipients ranged from 8 to 46 years. Two transplantations were living-related (mother to child, sister to sister), the others were from cadaveric donors. The living-related transplants consisted of 100 cm and 170 cm of jejunoileum, the cadaveric transplants comprised the entire small bowel, in one case supplemented with the right colon. Immunosuppressive treatment consisted of triple therapy with azathioprine, steroids and anti-lymphocyte globulin (ALG). All seven

recipients died of technical failures, rejection, suspected GVHD, or systemic sepsis between 12 hours and 76 days after transplantation. The one who survived longest was a 37-year-old woman who received 170 cm jejunoileum from her HLA-identical sister (240). Semiformed stools were present once or twice daily from the seventh posttransplant day and she was able to eat for two months. However, after 76 days she died from an E coli sepsis probably due to GVHD. Because of these poor results and the development of TPN, interest in clinical small bowel transplantation waned for several years.

2.7.2 Non-successful clinical experiences between 1983 and 1988

With the advent of CsA clinical small bowel transplantation started again and in the period 1983 until 1988 eleven clinical small bowel transplantations have been described (242-247). The ages of the recipients ranged from 9 months to 26 years. Only one living-related transplantation was performed (one HLA mismatch at each of the A, B, and DR loci), the others were cadaveric transplants. Tissue typing was not performed in most instances. Donor and recipient were of the same blood group, except in one case which resulted in a haemolytic anaemia 5 days posttransplant. Cross matches were negative, except in two cases. Seven patients received a transplant consisting of small bowel alone: 80 cm in the living-related transplantation, 110 cm jejunoileum in all three children transplanted by Goulet and the entire small bowel in the remaining recipients. Four recipients received a composite graft comprising liver, stomach, pancreas and entire small bowel (242,244). All patients were treated with intravenous CsA and steroids from the day of transplantation. Three patients also received ALG during the first five days, one patient was also treated with azathioprine for five days.

Two of the composite graft recipients and one of the small bowel graft recipients died from technical failures. In almost all other recipients early rejection necessitated graft removal. Two composite graft recipients died of lymphoproliferative disorders four and six months after transplantation (242,244). In one child the small bowel graft was brought into continuity with the residual gastrointestinal tract in the eleventh month (246). Unfortunately, she developed chronic rejection 17 months after transplantation, but survived after removal of the graft. Thus, between 1983 and 1988 none of the clinical transplantations proved to be successful.

2.7.3 Clinical experiences since 1988

The first successful clinical small bowel transplantation was performed by Deltz in 1988 (248). A 42-year-old woman received a living-related small bowel graft from her sister, consisting of 60 cm jejunoileum. Tissue typing revealed no HLA mismatches from the recipient to the donor, and only two HLA mismatches from the donor to the recipient

(A1,B8,DR3 and A1,B8,B44,CW4,DR3,DR7 for donor and recipient, respectively). Immunosuppressive treatment comprised intravenous CsA, methylprednisolone and anti-thymocyte globulin (ATG). The graft was placed into a heterotopic position with both ends exteriorized as cutaneous stomata. At day 41 the graft was brought into the orthotopic position. Oral food intake was started 14 days after the second operation and at the end of the fourth month parenteral nutrition was stopped. The patient faced at least five rejection episodes and prolonged haemolytic anaemia, probably caused by GVHD. Rejection could be treated with high doses of methylprednisolone during four to ten days. At present the patient is clinically well and produces three to four fluid stools daily.

Since 1988 Grant performed three combined small bowel/liver transplantations and two multivisceral transplantations consisting of stomach, duodenum, and pancreas in addition to small bowel and liver (16,19,252). The recipients were aged from 27 to 47 years and all received HLA-mismatched cadaveric grafts. The donors were treated with OKT3 and ALG over six hours before organ retrieval. Immunosuppressive treatment included OKT3 (seven to ten days), CsA, azathioprine and methylprednisolone. There was only one episode of small bowel rejection, which responded to high-dose steroids followed by a course of OKT3. No liver graft rejections occurred. One episode of GVHD (a skin rash and donor lymphocytes in peripheral blood) was noted, which disappeared spontaneously. Frequent bacterial, fungal and cytomegalovirus infections were seen. Within one year after operation both patients with a multivisceral graft died from a lymphoproliferative disorder. One recipient of a small bowel/liver graft died from renal failure and a stroke at three months. Intestinal and liver graft functions in both long-term survivors were normal two and three years after transplantation (19). Both recipients maintained their weight on a normal diet without fluid or nutritional supplementation. However, the clinical small bowel transplant program was stopped because of the high rate of infections and lymphomas, probably as a consequence of the vigorous immunosuppressive treatment (personal communication). Grant will continue clinical small bowel transplantations, either as isolated grafts or in combination with liver grafts, when appropriate immunosuppressive agents become available. Margreiter performed a multivisceral transplantation in a patient with an extended pancreas head carcinoma in December 1989 (249,250). The recipient suffered from two rejection episodes, a prolonged episode of pneumonitis necessitating ventilation and a cytomegalovirus enteritis, which were all treated successfully. The patient died, however, from recurrent malignancy seven months posttransplant.

Revillon, Wallander and D'Alessandro have reported four small bowel transplantations and one small bowel/liver transplantation in neonates and children since 1989 (251,253,254). There were four cases of acute rejection, of which two children died and two survived after removal of the small bowel graft. The only long-term survivor ("Virginie") received at the age of five months a 95 cm small bowel graft from an iso-

blood group O, HLA-mismatched anencephalic neonate (253). The small bowel graft was flushed with ALG before transplantation. Immunosuppressive therapy consisted of CsA, azathioprine and methylprednisolone. A heterotopic small bowel transplantation was performed and intestinal continuity was established after eight months. Three rejection episodes were successfully treated with OKT3, anti-IL2 monoclonal antibodies and ALG respectively. Starting from the third week after transplantation, enteral feeding was progressively increased and TPN was stopped ten months after transplantation. Three years after transplantation, she is growing normally, has a normal diet with enteral infusions at night, and fat absorption is currently higher than 95%. The origin of the small bowel allograft could have played a role in this favourable outcome.

In the period May 1990 until August 1992 Starzl performed 23 small bowel transplantations: 9 isolated small bowel transplantations, 12 small bowel/liver transplantations, and 2 multivisceral transplantations (18, oral report at the XIVth International Congress of the Transplantation Society, Paris, August 1992). The transplantations were performed in 11 children and 12 adults. Only cadaveric grafts from ABO-identical and HLA-mismatched donors were used. Immunosuppressive treatment was basically FK-506, in several cases supplemented with prednisone and/or azathioprine. During follow-up, which was extremely short in the last ten cases, three recipients died from sepsis and one from a lymphoma. All recipients with a longer follow-up developed at least one rejection episode, which presented with fever, malaise, dysmotility of the small bowel graft (ileus or diarrhoea), and malabsorption of FK-506, D-xylose, vitamin E and fat (255,256). Episodes of rejection were often accompanied by bacterial translocation. Treatment of rejection was successful with increased FK-506 and steroids with or without OKT3 in combination with systemic antibiotics. All patients received TPN support immediately after transplantation, and oral and enteral feeding were started as soon as gastrointestinal ileus had resolved. The achievement of full oral alimentation was a slow process requiring four weeks to nine months. At the time of reporting some of the recipients still required night-time TPN. These remarkable results demonstrate the feasibility of clinical small bowel transplantation and the therapeutic efficacy of FK-506. However, it is evident that long-term follow-up is needed before a definitive conclusion can be drawn.

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CHAPTER 3

SCOPE OF THE STUDY

3.1 Introduction

Small bowel transplantation poses two major problems. First, as a consequence of the transplantation procedure the small bowel graft faces several physiological obstacles: ischaemic and reperfusion injury, intrinsic and extrinsic denervation, and disruption of the lymphatic drainage. These factors may adversely influence small bowel motility, hormonal, immunological and nutritional functions of the small bowel, and the intestinal barrier (1-5). Although it is known that lymphatics are re-established within several weeks, less is known about the (long-term) effects of harvest injury on small bowel graft function. Nor do we know whether regeneration of neuronal continuity or adaptation to chronic denervation takes place (6-8).

The second problem is that small bowel transplantation between two unrelated individuals results in two different immunological reactions: host-versus-graft reaction (graft rejection) and graft-versus-host reaction. The large amount of transplanted lymphoid tissue is responsible for this highly immunogenic character of a small bowel allograft. When either reaction occurs in combination with immunosuppressive treatment, normal small bowel function will be further compromised (9). Using less immunosuppressants inevitably results in small bowel graft rejection and bacterial translocation with septic complications. Overimmunosuppression, on the other hand, endangers the general immune status of the host with subsequent infectious complications. Thus, the therapeutic window for immunosuppression is narrow (9,10). In addition, the transplantation procedure itself alters the bacterial flora and increases the risk of infectious complications when immunosuppression is added (11,12). At present, small bowel graft rejection with sepsis and - to a lesser extent - GVHD still hamper the introduction of routine clinical small bowel transplantation.

3.2 Questions to be answered

Several important questions remain to be answered. This thesis tries to answer the following:

- What is the effect of the transplantation procedure on the functions of transplanted small bowel? It can be expected that harvest injury, intrinsic and extrinsic denervation,

and disruption of lymphatic drainage alters small bowel physiology, at least temporarily.

- Is it possible to detect small bowel allograft rejection at an early stage in which the graft can be saved? To assure future clinical small bowel transplantation, it is of utmost importance to monitor the small bowel graft for early recognition of rejection. Apart from the available morphological evaluation with several disadvantages, it is at present impossible to monitor small bowel allografts and to timely detect rejection.

- What is the influence of MHC matching on the survival of small bowel allografts? A strategy to minimize graft antigenicity is selecting donor-recipient combinations by matching for MHC antigens. Thus the rejection reaction against tissue antigens is selectively suppressed without altering the recipient's immune status. Remarkably, in the CsA era the effect of MHC matching on small bowel allograft survival has not been evaluated in experimental or clinical studies.

- What is the effect of a segmental small bowel allograft on the nutritional status of the host? On the one hand, a segmental small bowel graft implicates less transplanted lymphoid tissue. On the other hand, it means less absorptive surface area, which may bring about malabsorption and malnutrition. Potential future living-related small bowel transplantation is only meaningful if a segmental small bowel graft is able to sustain a normal nutritional status of the adult recipient, or normal growth and development of the growing acceptor.

3.3 Objectives of investigation

To answer the above questions various functional and immunological aspects of canine small bowel transplantation were investigated in vivo. The aims of these experiments were:

- To study the effect of the transplantation procedure on mucosal small bowel function in an autotransplant model by using a non-invasive method for in vivo measurement of transepithelial potential difference (Chapter 5).

- To evaluate the significance of in vivo measurements of electrophysiological parameters for the detection of small bowel allograft rejection in non-immunosuppressed dogs (Chapter 6).

- To assess the effect of MHC matching on the survival of small bowel allografts in non-immunosuppressed dogs (Chapter 7).

- To examine the validity of serum N-acetyl hexosaminidase as a biochemical marker to monitor early small bowel allograft rejection (Chapter 8).

- To determine the effect of rejection on mucosal disaccharidase activity and intestinal permeability, and to evaluate whether these functional parameters are useful to monitor a small bowel allograft (Chapter 9).

- To investigate the combined effect of MHC matching and immunosuppressive therapy on the survival of dogs with an orthotopic segmental small bowel allograft (Chapter 10).

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PART B

MATERIALS AND METHODS

CHAPTER 4

MATERIALS AND METHODS

4.1 Animals

Adult male and female Beagles, obtained from the Central Institute of Laboratory Animals (Harlan, Zeist, The Netherlands), were used as donors and recipients. The dogs weighed 10 to 20 kg and their ages ranged from one to three years.

4.2 DLA matching

Tissue typing for antigens of the canine MHC, the DLA complex, was achieved by serological methods (class I antigens) and mixed lymphocyte cultures (class II antigens) as described previously (1,2). Typing for class I antigens was performed with a battery of 60 to 90 allo antisera using one- and two-stage microlymphocytotoxicity tests. These antisera define the antigens belonging to three closely linked series, DLA-A (alleles 1,2,3,7,9 and R20), DLA B (alleles 4,5,6,10 and 13) and DLA-C (alleles 11 and 12). Typing for class II antigens was done with a unilateral mixed lymphocyte culture harvested on day 6. Identity for class II antigens (DLA-D) was based on the absence of significant stimulation as compared with autologous controls. Only non-littermate donor-recipient pairs were selected.

4.3 Operative techniques

Anaesthesia
Premedication consisting of 0.5 mg atropin and 2 ml Thalamonal (0.05 mg Fentanyl and 2.5 mg droperidol per ml) was administered one hour prior to operation. During operation anaesthesia consisted of 200 mg thiopental (Nesdonal, Rhône-Poulenc Pharma, Amstelveen, The Netherlands) and 0.1 mg Fentanyl intravenously and endotracheal enflurane (Ethrane, Abbott BV, Amstelveen, The Netherlands) supplemented with nitrous oxide and oxygen. Thalamonal and Fentanyl were obtained from Janssen Pharmaceutica BV, Tilburg, The Netherlands.

Enterectomy to create a short bowel syndrome
Twice daily the dogs were given 500 mg of Neomycin orally and 500 IU of heparin subcutaneously for 2 days, and food was withheld for 36 hours prior to operation. Under

general anaesthesia with endotracheal intubation and mechanical ventilation, a laparotomy was performed through a midline incision. All dogs of the short bowel control group underwent an enterectomy from the ligament of Treitz to about 10 cm proximal to the ileocaecal valve. Intestinal continuity was restored (with Vicryl 4-0) by a single-layer end-to-end anastomosis of distal duodenum and terminal ileum (Figure 4.1C). All animals were treated with 5 ml depomycin subcutaneously at the day of operation and 2.5 ml depomycin and 2 x 500 IU heparin subcutaneously for 5 days postoperatively. The dogs were supported with parenteral fluids (0.9% saline and 5% glucose subcutaneously) until they were eating and drinking normally, which normally took two to three days. All dogs were maintained on standard kennel rations (Canex, Hope Farms, Woerden, The Netherlands) and water was freely available.

Heterotopic small bowel auto- and allotransplantation

A loop of ileum from the proximal ileum to 5 cm proximal of the ileocaecal valve was isolated on a vascular pedicle provided by a branch of the mesenteric artery and vein. This loop was removed and perfused with heparinized saline (5 IU heparin/ml 0.9% saline) at 20°C until the venous effluent became clear and the graft white. The right external iliac artery and vein of the recipient were dissected. The graft was revascularized by anastomosis (with Prolene 7-0) of the mesenteric vessels to the iliac vessels in an end-to-side fashion, after which the graft was divided into two parts: a blind-ending loop ileostomy (length 15 to 25 cm), and an isoperistaltic Roux-en-Y loop in continuity with the host's terminal ileum approximately 10 cm from the ileocaecal valve (length 55 to 75 cm, which is 25 to 30% of total small bowel length) (Figure 4.1A). The blind-ending loop was created for functional measurements, whereas the Roux-en-Y loop was constructed for long-term experiments; in a second-stage operation this heterotopic small bowel loops were placed in an orthotopic position. Both small bowel loops were used to obtain histological specimens. The small bowel loops were exteriorized as cutaneous ileostomies on the right side of the abdomen for monitoring purposes. Continuity of the residual small bowel was restored by a single-layer end-to-end anastomosis.

Orthotopic small bowel allotransplantation

Long-term surviving dogs treated with immunosuppressive drugs underwent a second operation 5 to 8 weeks after the heterotopic small bowel transplantation; the native small bowel from the ligament of Treitz to about 10 cm proximal to the ileocaecal valve was resected and the heterotopic Roux-en-Y loop was placed in an orthotopic position by end-to-end anastomosis with the distal duodenum (Figure 4.1B).

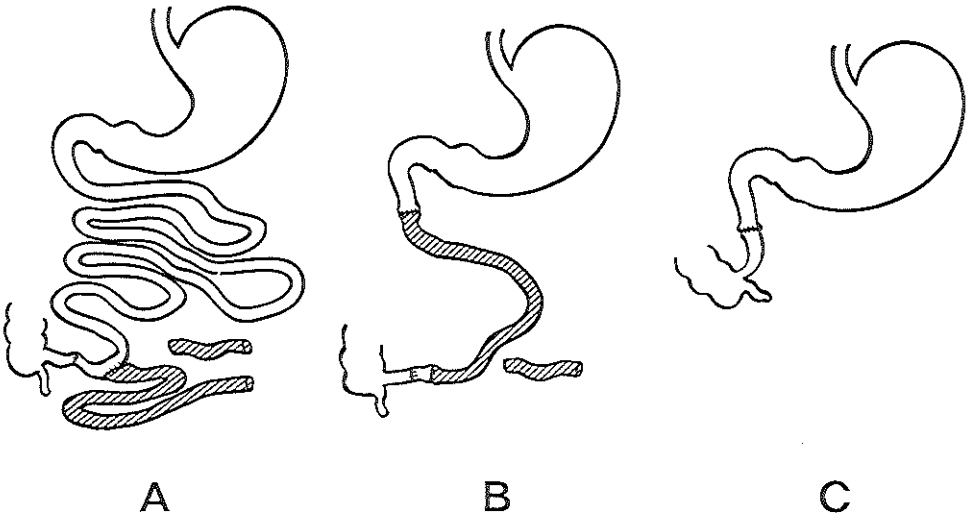

Fig.4.1. Model of canine small bowel transplantation: (A) Heterotopic small bowel transplantation consisting of a blind-ending loop ileostomy and an isoperistaltic Roux-Y loop in continuity with the host's terminal ileum; (B) Orthotopic position of small bowel graft after resection of the host's small bowel; (C) Enterectomy with end-to-end anastomosis of distal duodenum and terminal ileum.

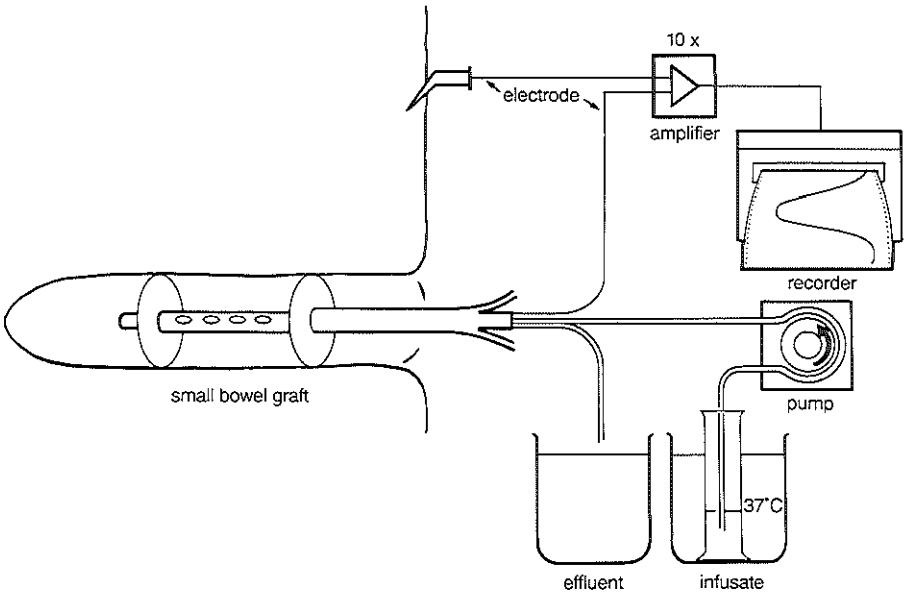

Fig.4.2. Schematic representation of the triple-lumen double-balloon catheter, which isolates a 2.5 cm segment of the blind ending ileostomy, and the equipment used for the electrophysiological measurements.

4.4 Animal care

The experimental protocols adhered to the rules laid down in 'The Dutch Animal Experimentation Act' (1977) and the 'Guidelines on the Protection of Experimental Animals' published by the Council of the European Committee (1986). Specific protocols were approved by the Committee on Animal Research of Erasmus University, Rotterdam, the Netherlands. Animals were killed if their general condition deteriorated or if they lost more than 30% of their preoperative body weight.

4.5 Electrophysiological measurements

The blind ileostomy was used for electrophysiological measurements. Using a triple-lumen, double-balloon catheter, a 2.5 cm segment of the blind-ending loop could be isolated (Figure 4.2). In this segment a continuous flow of test solution was maintained at a constant rate of 10 ml/min by a peristaltic roller pump (Minipuls 3, Gilson, Middleton, WI, USA). One lumen of the catheter introduced the test solution and a second served as an outflow channel. The third lumen contained a salt bridge (3% agar in 0.9% saline) connected to an $Ag/AgCl_2$ electrode (0.5 mm in diameter, 7 mm in length). A sterile Teflon needle filled with 0.9% saline inserted in the right lower leg of the dog was connected to an $Ag/AgCl_2$ reference electrode. The transepithelial potential difference (PD) of the isolated ileal segment was measured in reference to the latter electrode. Both electrodes were connected to an amplifier. The transepithelial PD was visualized on a paper recorder (Kratos, London, United Kingdom). To avoid electrophysiological disturbances, all dogs received 5 mg of Droperidol intramuscularly before the experiments. The dogs were placed in a Pavlov stand for the duration of the experiments and tolerated handling of their ileostomies well. The postoperative day on which electrophysiology no longer showed any stimulated PD responses or showed reversed PD responses was evaluated as parameter for the end-stage of graft survival.

Test solutions

During the electrophysiological measurements, three test solutions were used: 0.9% saline (275 mOsm/kg H_2O), 10 mmol/l theophylline in 0.9% saline (285 mOsm/kg H_2O), and 40 mmol/l of glucose in 0.9% saline (312 mOsm/kg H_2O). The test solutions were instilled in the ileal segment at a temperature of 37°C and at a constant rate of 10 ml/min. Spontaneous transepithelial PD, measuring baseline active ion transport, was recorded by perfusing the blind ileostomy with 0.9% saline during 30 minutes. After this equilibration period the ileal segment was challenged with 10 mmol/l theophylline in 0.9% saline during 30 minutes. Normally, this results in a luminal negative PD response (PD-theophylline) in reference to

the base-line PD due to theophylline-stimulated and cyclic-AMP-mediated chloride secretion by the crypt cells (3). Thereafter, 0.9% saline was again instilled in the small bowel loop for 15 minutes, after which it was perfused with 40 mmol/L glucose in 0.9% saline. This results in a sodium-dependent active glucose absorption by the villus cells, and thereby produces a luminal negative PD-response (PD-glucose) (4). The above mentioned electrophysiological responses to theophylline and glucose, respectively, are widely used indices of these specific transport processes (5,6). At the end of the electrophysiological test, the ileal segment was again perfused with 0.9% saline for 15 minutes.

4.6 Morphology

During operations full-thickness biopsies were obtained from native and transplanted small bowel. Later, biopsies were regularly obtained from both small bowel loops at approximately 5 to 7 cm from the cutaneous stomas. They were fixed in 10% buffered formalin, dehydrated, and embedded in paraffin. Haematoxylin azophloxin-stained sections (4 to 5 μm) were prepared and subsequently examined by light microscopy. At autopsy, full-thickness biopsy specimens of the allografts were taken, as well as samples of the vascular anastomosis, the recipient's own small bowel, lymph nodes, spleen, liver, lung, and skin.

4.7 References

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PART C

ORIGINAL STUDIES

CHAPTER 5

THE VALUE OF IN VIVO ELECTROPHYSIOLOGICAL MEASUREMENTS TO EVALUATE CANINE SMALL BOWEL AUTOTRANSPLANTS

5.1 Abstract

This study aimed to develop a non-invasive method for in vivo measurement of transepithelial potential difference (PD) in canine small bowel (SB) and to evaluate this parameter in SB autotransplants. In dogs of group 0 (control group, n=4) 2 intestinal loops were created without disturbing their vascular, neural and lymphatic supply. In group I (successful autotransplants, n=11) 2 heterotopic SB loops were constructed. Long-term functional sequelae of vascular, neural and lymphatic division were studied. Group II (n=6) consisted of non-successful autotransplants suffering thrombosis of the vascular anastomosis, which resulted in ischaemic SB autografts. In group I values of spontaneous transepithelial potential difference, an index of base-line active electrolyte transport, were significantly lower compared to group 0 ($p<0.05$), probably as a result of denervation of the autotransplants. Both theophylline- and glucose-stimulated PD responses, measuring cyclic-AMP-mediated chloride secretion and sodium-coupled glucose absorption respectively, showed luminal negative values in group I at all time points after transplantation. These transepithelial PD responses progressively diminished with time. From day 21 onwards both theophylline- and glucose-stimulated PD responses were significantly less than the corresponding responses at day 7 ($p<0.05$). Morphometric analysis showed that the reduction of transepithelial PD responses preceded degenerative mucosal alterations of the heterotopic SB autografts. In group II PD responses to theophylline and glucose showed luminal positive values ($p<0.01$ versus group I), probably as a result of passive potassium effusion from necrotic enterocytes.

This chapter is an adaptation of an original article published in Gut 1991;32:1329.

5.2 Introduction

In small bowel transplantation (SBT) monitoring of the SB allograft for early recognition and treatment of rejection is mandatory. The value of functional tests to diagnose rejection continues to be debatable (1-13). Therefore, at present rejection is evaluated by histological investigation only (14-16). However, recent studies have revealed several shortcomings of histological monitoring of SB allografts (17-19). Mucosal biopsies provide too little diagnostic information, while full thickness biopsies produce a risk of perforation of the graft (17). On top of that, a series of consecutive biopsies is necessary for the evaluation of rejection, because of the patchy character of morphological alterations (18).

To gain more diagnostic value of histological monitoring, Kirkman and Madara investigated the effect of rejection on the electrophysiological function of SB grafts in a rat model by in vitro techniques (20,21). The measurement of transepithelial potential differences (PD) of SB is based on active electrolyte transport by sodium-potassium-ATPase localized at the basolateral membrane of the enterocyte (22). It was found that alterations in the mucosal structure associated with rejection do correlate with a decreased spontaneous transepithelial PD (20). Furthermore, diminished responses to both sodium-coupled glucose absorption, which is an index of villus function (23,24), and theophylline-stimulated chloride secretion, an index, at least in part, of crypt cell function (25), were paralleled by structural abnormalities indicative for SB rejection (21). Recently Lee reported about in vivo transepithelial PD as a marker of rejection in a rat SBT model (26). He found that changes in transepithelial PD often occurred before histological changes. However, because of the invasive nature of their method, he did not recommend it as a practical clinical tool (25).

The aim of our study was to develop a non-invasive method for in vivo measurement of transepithelial PD in canine SB and to evaluate this parameter in SB autotransplants. This model was chosen to study the long-term consequences of vascular, neural and lymphatic interruption of the SB graft without the effects of rejection and immunosuppression. The electrophysiological parameters of the grafts were compared with the accompanying morphological characteristics.

5.3 Materials and methods

Experimental groups

The operative technique has been described in chapter 4. The animals were divided into 3 groups: Group 0 (n=4) was the control group. In all dogs of this group a blind and an open ileostomy were constructed without creating the vascular pedicle, consequently

without disturbing the vascular, neural and lymphatic supply. These SB loops, not challenged by an ischaemic episode during operation and with an intact neural and lymphatic system, gave us the opportunity to study SB characteristics in the normal physiological situation. Group I (n=11) consisted of dogs which had undergone successful SB autotransplantations. All these dogs received a blind and an open ileostomy as described in chapter 4. Cold ischaemic time averaged 36.1 minutes (range 30-43 minutes). In this group it was possible to study long-term functional sequelae of autotransplanted SB without the influence of transplant rejection and immunosuppression. Group II (n=6) consisted of dogs which had undergone non-successful SB autotransplantations. Autotransplantations were performed as in group I, but turned out to be technical failures as a result of thrombosis at the vascular anastomosis. Operative cold ischaemia averaged 37.3 minutes (range 33-43 minutes). Before sacrificing the dogs of this group electrophysiological parameters of ischaemic grafts were obtained at postoperative day 2 in two dogs and at day 3 in two other dogs.

Electrophysiological measurements

The electrophysiological measurements and test solutions have been described in chapter 4. The electrophysiological responses to theophylline and glucose are widely used indices of cyclic-AMP-mediated chloride secretion by crypt cells and sodium-coupled glucose absorption by villus cells, respectively (22-25). Measurements of transepithelial PD were performed between 12 and 44 days postoperatively in group 0, on days 3, 7, 10, 14, 21, 42 and 70 in group I and on day 2 or 3 in group II.

Morphology

At the day of electrophysiological measurements biopsies were obtained using a biopsy forceps from both the blind and the open ileostomy at approximately 5 to 7 cm from the cutaneous stoma. Morphometric analysis of villus height and crypt depth was performed on each histological section as described previously (27). Autopsies were performed on the dogs of the control group (group 0) at postoperative day 100 and on the dogs of group II at the day of sacrifice between 2 and 6 days after transplantation. The dogs of group I were not sacrificed, but used for long-term follow-up.

Statistical methods

All data are expressed as means $\pm$ the standard errors of the mean (SEM). Comparisons of electrophysiological parameters between the same animals at different time points were performed using the paired Student's *t* test. Differences of electrophysiological parameters between the various experimental groups were analysed using Student's *t* test for two means. Statistical analysis of mucosal morphometry between the same animals at different time points was performed using the Wilcoxon rank sum test and for comparisons

between group 0 and I the Mann-Whitney-U test was used. For all tests $p<0.05$ was considered significant.

5.4 Results

Postoperative course

All dogs of group 0 and 10 dogs of group I survived an observation period of 10 weeks. One dog of group I died 8 days postoperatively as a result of a wound abscess. Six dogs forming group II had to be sacrificed between 2 and 6 days after operation because of a deteriorating condition. At autopsy thrombus formation at the arterial and venous anastomosis, causing haemorrhagic ischaemic necrosis of the graft, was found. Although these dogs could be regarded as technical failures, electrophysiological parameters obtained in 4 dogs proved a valuable source of information for ischaemic SB grafts.

Table 5.1. Base-line transepithelial potential difference (base-line PD), theophylline-stimulated PD response (PD-theophylline), and glucose-stimulated PD response (PD-glucose)[a]

	base-line PD (mV)	PD-theophylline (mV)	PD-glucose (mV)
group 0[b]			
day 21	1.33 ± 0.42 n=4	-4.13 ± 0.45 n=4	-5.68 ± 0.81 n=4
group I[b]			
day 3	-1.41 ± 0.87 n=8	-3.23 ± 0.50 n=8	-2.20 ± 0.38[c] n=8
day 7	-2.79 ± 0.96 n=7	-3.56 ± 0.50 n=7	-4.02 ± 0.53 n=6
day 10	-1.61 ± 0.95 n=8	-2.47 ± 0.31 n=8	-3.16 ± 0.46 n=7
day 14	-0.67 ± 0.20 n=7	-2.41 ± 0.33 n=7	-3.10 ± 0.56 n=6
day 21	-1.83 ± 1.03[d] n=7	-2.17 ± 0.25[c,e] n=7	-2.69 ± 0.35[c,e] n=7
day 42	-1.24 ± 0.98 n=7	-1.61 ± 0.41[c] n=7	-1.45 ± 0.53[c] n=7
day 70	0.39 ± 0.89[c] n=8	-1.98 ± 0.25[c] n=8	-2.06 ± 0.41[c] n=8
group II[b]			
day 2/3	1.25 ± 1.11 n=4	0.83 ± 0.35[f] n=4	0.46 ± 0.31[f] n=4

[a]Results are expressed as mean ± SEM; [b]group 0: control group; group I: successful autotransplants; group II: non-successful autotransplants; [c]$p<0.05$ compared with corresponding PD response at day 7; [d]$p<0.05$ compared with group 0 at day 21; [e]$p<0.01$ compared with group 0 at day 21; [f]$p<0.01$ compared with group I at day 3.

Group 0: control group

In this group 10 series of electrophysiological measurements were obtained in 4 dogs between 12 and 44 days postoperatively. During this test period data of transepithelial PD were not collected systematically. As a result only electrophysiological data obtained at postoperative day 21 could be used for statistical analysis (Table 5.1).

Perfusion of the ileal segment with 0.9% saline provided a luminal positive base-line transepithelial PD at all time points after operation. Addition of 10 mM theophylline in 0.9% saline caused a luminal negative peak PD response in reference to the base-line PD. Instillation of 40 mM glucose in 0.9% saline also revealed a negative peak PD response in reference to the spontaneous transepithelial PD. The theophylline and glucose evoked potential increments disappeared within several minutes after perfusing the ileal segment with 0.9% saline.

Biopsies taken simultaneously from both SB loops were histologically identical and showed structurally normal small bowel architecture between 12 and 44 days after operation (Figure 5.1A). Autopsy at day 100 after operation revealed a considerable villus shortening of the ileal grafts (Table 5.2). However, due to the limited number of animals the difference with day 0 was not statistically significant ($p=0.07$). The deeper layer of the lamina propria of the ileal grafts showed a substantial amount of fibroblasts and fibrosis. At day 100 the crypt depth was not different compared with the situation at day 0 (Table 5.2).

Group I: successful autotransplant group

The results are shown in Tables 5.1 and 5.2 and Figure 5.2. In contrast to the control group, perfusion of the ileal graft with saline caused a luminal negative base-line transepithelial PD in this group at all time points with the exception of day 70. Throughout the test period these base-line PD measurements showed a considerable range. However, the base-line PD at day 70 differed significantly from the corresponding response at day 7 ($p<0.05$). Analysis of the base-line PD responses between groups 0 and I showed that the PD response differed significantly in group I from the PD response in group 0 at day 21 ($p<0.05$).

Stimulated active chloride secretion after exposure to 10 mM theophylline resulted in luminal negative peak PD responses at all time points after transplantation, but from day 7 onwards these responses progressively diminished during the remainder of the test period. From day 21 onwards the peak PD responses to theophylline were significantly lower than the corresponding response at day 7 ($p<0.05$). Compared with group 0 the PD response to theophylline was significantly reduced in group I at day 21 ($p<0.01$).

Exposure of the ileal graft to 40 mM glucose resulted in luminal negative peak PD responses at all time points. However, at day 3 the PD response was significantly reduced compared with the response at day 7 ($p<0.05$). From day 7 onwards the PD responses to

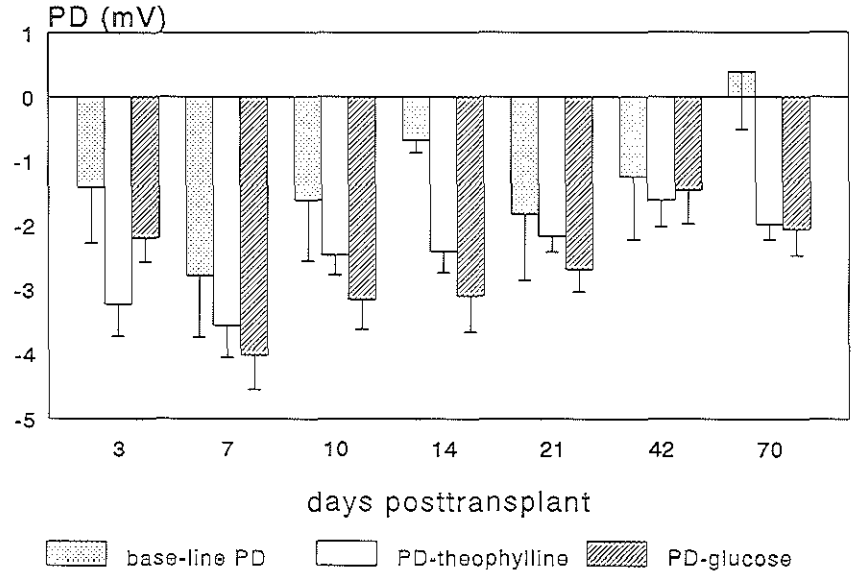


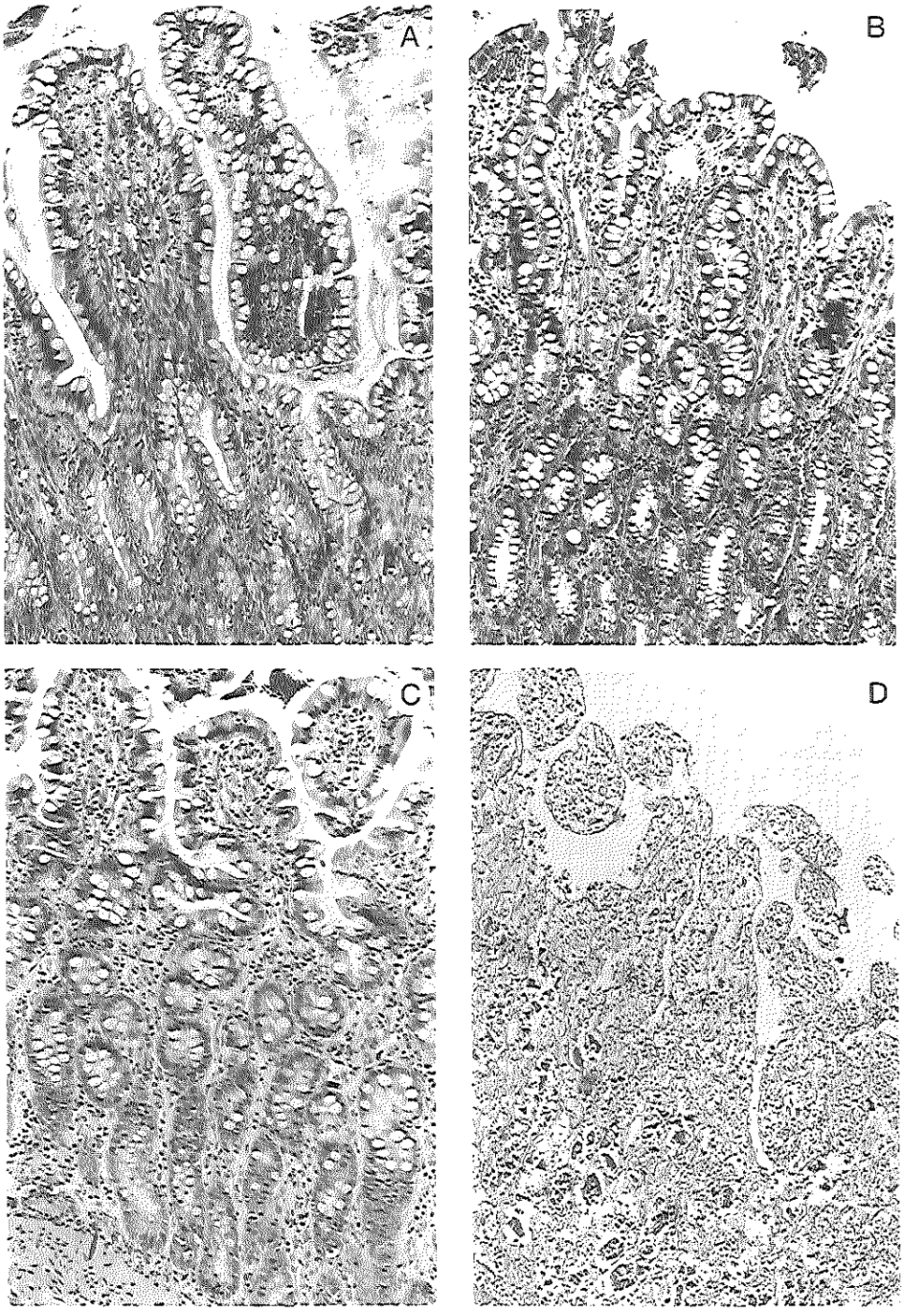
Fig.5.2. Base-line PD, theophylline-stimulated PD response (PD-theophylline) and glucose-stimulated PD response (PD-glucose) in group I. Results are expressed in mV (mean ± SEM).

Fig.5.1A. Normal histological appearance of small bowel architecture of blind ending ileostomy in group 0 at postoperative day 69 (H&A stain, magnification x 100).

Fig.5.1B. Histological appearance of ileal autotransplant in group I obtained on postoperative day 3. Reduced height of villi, dilated lymph vessels and edema are present (H&A stain, magnification x 100).

Fig.5.1C. Histological appearance of ileal graft in group I at postoperative day 51. Reduction in villus height and crypt depth and fibrosis of lamina propria are present (H&A stain, magnification x 100).

Fig.5.1D. Histological appearance of small bowel graft in group II showing ischaemic necrosis at postoperative day 3 (H&A stain, magnification x 100).


A
B
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glucose progressively decreased and after day 21 the response to glucose was significantly less than the PD response at day 7 ($p<0.05$). Compared with the control group the PD response to glucose was significantly reduced in group I at day 21 ($p<0.01$).

Histological and morphometric evaluation of the biopsies of the SB grafts demonstrated a significantly reduced height of the villi with dilated lymph vessels at day 3 after transplantation ($p<0.05$ versus day 0), whereas the crypt depth was not altered (Figure 5.1B and Table 5.2). After day 3 the height of the villi gradually increased to reach a normal morphological pattern at day 10. From day 42 onwards progressive alterations in mucosal structure were found: a significant reduction in both villus height and crypt depth ($p<0.05$ versus day 0) and substantial numbers of fibroblasts and fibrosis in the deeper layer of the lamina propria (Figure 5.1C). In some instances a polymorphonuclear cell infiltrate of the mucosa and cryptitis were found. The reduction of villus height in group I at day 70 was more pronounced than the reduction in group 0 at day 100 ($p<0.05$). At day 70 the crypt depth in group I was also significantly smaller compared to the crypt depth in group 0 at day 100 ($p<0.05$).

Table 5.2. Morphometric analysis of villus height and crypt depth in groups 0 and I.[a]

	villus height		crypt depth	
	(mm)	(%)[b]	(mm)	(%)[b]
group 0[c]				
day 0	0.831 ± 0.055	100.0	0.391 ± 0.020	100.0
day 100	0.530 ± 0.015	63.8	0.448 ± 0.035	114.6
group I[c]				
day 0	0.745 ± 0.027	100.0	0.368 ± 0.031	100.0
day 3	0.443 ± 0.025[d]	59.5	0.316 ± 0.021	85.9
day 7	0.623 ± 0.021[d]	83.6	0.363 ± 0.023	98.6
day 10	0.693 ± 0.043	93.0	0.425 ± 0.022	115.5
day 14	0.738 ± 0.029	99.1	0.401 ± 0.030	109.0
day 21	0.689 ± 0.047	92.5	0.317 ± 0.024	86.1
day 42	0.504 ± 0.051[d]	67.7	0.305 ± 0.015[d]	82.9
day 70	0.355 ± 0.043[d,e]	47.7	0.308 ± 0.019[d,e]	83.7

[a]Results are expressed as mean ± SEM; [b]Values at day 0 were considered 100%; [c]group 0: control group; group I: successful ileal autotransplants; [d]$p<0.05$ compared with group I at day 0; [e]$p<0.05$ compared with group 0 at day 100.

Group II: non-successful autotransplants

Results are shown in Table 5.1. Because of the deteriorating condition of the dogs in group II electrophysiological parameters could only be obtained at day 2 in two dogs and at day 3 in two other dogs. The cutaneous stomas of the grafts had a brown and dry appearance and produced blood stained secretions at day 2 or 3. The spontaneous

transepithelial PD had a luminal positive value at day 2 or 3. However, this PD response was not statistically different from the base-line PD of group I. Both peak PD responses to theophylline and glucose stimulation showed luminal positive values and were different compared with the corresponding responses in group I ($p < 0.01$). At autopsy histological examination showed changes comparable with haemorrhagic ischaemic necrosis of the grafts (Figure 5.1D).

5.5 Discussion

The results of our study demonstrate that in vivo recording of electrophysiological parameters of canine SB is technically feasible. By using an autotransplant model long-term electrophysiological and morphological consequences of vascular, neural and lymphatic division could be studied. Furthermore, long-term effects in non-functional SB loops were evaluated.

The intestinal mucosa can be divided into 2 complementary entities: the crypts, in which electrogenic chloride secretion occurs (24) and the villi, in which nutrient absorption, including sodium and glucose, takes place (23). The spontaneous transepithelial PD, an index of base-line active transport, results from electrogenic sodium absorption and chloride secretion.

Saline perfusion of the control grafts, in which the vascular, neural and lymphatic supply was not disturbed, resulted in luminal positive base-line PD responses at all time points after operation. Addition of a theophylline solution resulted in luminal negative PD responses in reference to the base-line PD. This is due to theophylline-stimulated and cyclic-AMP-mediated chloride secretion by the crypt cells (22). It is based on a Na-K-Cl_2-cotransporter in the basolateral membrane which leads, driven by Na-K-ATPase, to a cellular accumulation of chloride that subsequently leaves the crypt cell through the apical membrane. Perfusion of the control grafts with glucose in saline caused luminal negative peak PD responses as well. This is a result of active glucose absorption by the villus cells with a cotransport of sodium leading to an increase in transmural net sodium absorption (24). In the control group the results of base-line PD and stimulated PD responses obtained between 12 and 44 days postoperatively did not vary considerably. Unfortunately, data collection was not complete in group 0. As a consequence, only data obtained in all four dogs at day 21 could be used for statistical analysis. Because the biopsies of the control grafts revealed a normal small bowel architecture between 12 and 44 days postoperatively, the electrophysiological responses obtained at day 21 may well reflect a physiological situation.

In contrast to the control group, the base-line transepithelial PD in group I showed luminal negative values at all time points with the exception of day 70. It is likely that

this difference is due to an enhanced secretory activity of the crypts as a result of denervation, since the crypts are under much greater autonomic neurological control than the villi (28,29). Furthermore, nerve stimulation inhibits electrogenic chloride secretion and stimulates fluid absorption (30). Thus, the observed luminal negative base-line PD may have been due to net chloride secretion by crypts no longer controlled by the autonomic nervous system. Our findings are in accordance with the results of Watson, who found chloride secretion in denervated Thiry-Vella loops, whereas innervated control Thiry-Vella loops absorbed chloride (31). This effect mave have considerable implications for clinical SBT; the findings reported in this paper demonstrate that even without the deleterious influence of rejection and complications of immunosuppressive drugs, denervation in intestinal autotransplants results in functionally altered SB grafts. Unless reinnervation occurs, an abnormal digestive and absorptive intestinal (auto-)transplant can be expected (31,32).

In group I the glucose stimulated response, assessing the sodium coupled glucose transport, was substantially diminished at day 3 compared to day 7. This decreased absorptive response correlated well with histology, which showed a significantly reduced height of the villi at day 3 as compared to day 7. This is probably a result of the short ischaemic period during transplantation. It is known that the intestinal mucosa is highly sensitive to ischaemic injury (33). Reperfusion injury generated by oxygen-derived free radicals also has a damaging effect on intestinal mucosa (34). Nevertheless, SB has an enormous regenerative potential after a short period of ischaemia (33). This regeneration starts in the crypts and regenerated enterocytes gradually migrate from the crypts to the villus tips. This could explain that the glucose stimulated response, which is a villus function, was still reduced at day 3, whereas at the same time the regenerated crypt cells caused a normal PD response after theophylline stimulation.

In group I both theophylline and glucose stimulated PD responses showed luminal negative values, but these responses progressively decreased with time: from day 21 onwards both theophylline and glucose responses were significantly less than the corresponding responses at day 7. Morphometric parameters followed these electrophysiological changes with some delay: from day 42 onwards a significant reduction in both villus height and crypt depth and progressive fibrosis of the deeper layer of the lamina propria were found. These morphological changes indicative for mucosal atrophy are known to occur when the SB is deprived of intraluminal nutrition (35-37). Furthermore, our results are supported by the finding that blind loops are characterized by impaired active ion transport processes and an increase in epithelial and subepithelial resistance as reported by Schulzke (38). After day 42 we found in some instances a polymorphonuclear cell infiltrate of the mucosa and cryptitis suggesting a bacterial infection. This could be explained by bacterial overgrowth in the SB loops with a damaging effect on the mucosa.

It is interesting to note that the reduction of theophylline and glucose stimulated PD responses, which are caused by stimulated active ion transport processes, precede the degenerative morphological alterations of the SB mucosa. However, the significance of the base-line PD responses, measuring the base-line active transport processes, remains uncertain. Only at day 70 the base-line PD showed a significantly different value compared to the value of day 7. This is most likely due to the considerable range in base-line PD responses suggesting a substantial physiological variation in base-line ion transports by enterocytes.

Our findings demonstrate that the mucosal atrophy in the autotransplants is more advanced than in the control group. Comparison between groups 0 and I showed significantly smaller PD responses to theophylline and glucose in group I at day 21. Morphometric analysis in group 0 showed no mucosal alterations before day 44, whereas reduction of both villus height and crypt depth was evident in the autotransplanted SB loops of group I from day 42 onwards. In addition, comparison between groups 0 and I showed significantly smaller villi and crypts in group I at day 70 compared to group 0 at day 100. This discrepancy cannot be explained by lymphatic disruption as lymphatic reconnection to SB autotransplants occurs within a few weeks after surgery (39). Thus, the degenerative changes of the SB mucosa may be accelerated by denervation of the graft.

Although in group II, which suffered acute ischaemia as a result of thrombosis at the vascular anastomosis, the base-line PD responses showed luminal positive values, the difference with group I was not significant at day 3. However, the theophylline and glucose stimulated PD responses in group II, were completely different from the results of group I. Because one cannot expect any active electrolyte transport mechanism in ischaemic necrotic cells, these electrophysiological results may reflect passive ion diffusion processes, probably potassium effusion from necrotic cells resulting in a luminal positive PD response. It has been suggested that focal endothelial cell injury of the microvasculature is an important factor in acute rejection of the SB transplant (21). If the rejection episode is not reversed, swelling of the endothelial cells will result in persistent ischaemia which will eventually damage the transplant. Thus, although we have no direct evidence, the electrophysiological parameters of group II may reflect those of an end-stage rejected SB graft.

In summary, this study showed that in vivo evaluation of electrophysiological parameters of canine SB is feasible and provides an elegant, practical tool in the functional assessment of SB autotransplants. We found that denervation in intestinal autotransplants causes functionally altered SB mucosa. Unless reinnervation occurs, an abnormal digestive and absorptive transplant will result. Our findings demonstrate that decreases of transepithelial PD responses precede degenerative mucosal alterations of autotransplanted SB. Since in vitro studies have clearly demonstrated the correlation

between electrophysiological and structural alterations in intestinal allograft rejection, our in vivo method could be employed for indirect monitoring of functional and structural changes of the SB allograft.

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CHAPTER 6

DETECTION OF CANINE INTESTINAL ALLOGRAFT REJECTION BY IN VIVO ELECTROPHYSIOLOGICAL MONITORING

6.1 Abstract

The aim of this study was to evaluate the significance of in vivo measurements of electrophysiological parameters for the detection of canine small bowel (SB) allograft rejection. In dogs of group I (n=17) a heterotopic SB autotransplantation was performed. Dogs of group II (n=8) received a heterotopic SB allograft in a fully mismatched donor-recipient combination. No immunosuppression was given. All grafts were monitored regularly by in vivo measurements of transepithelial potential differences (PD's) and by biopsies of the grafts. The overall technical failure rate was 36% caused by thrombosis at the vascular anastomosis in most cases. All successful autografts survived the experimental period and showed physiological PD responses after stimulation by both a theophylline solution and a glucose solution. The successful allografts survived 5.5 $\pm$ 0.2 days (mean $\pm$ SEM); the transepithelial PD's showed normal responses at postoperative day 3, but showed decreased responses at day 5 ($p<0.05$) and reversed responses at day 6 ($p<0.05$). The diminished PD responses correlated well with the onset of histological alterations characteristic of rejection. This study demonstrates that serial monitoring of transepithelial PD responses is a non-invasive method to detect acute SB allograft rejection.

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6.2 Introduction

Transplantation of the SB, an organ with immunological, nutritional, motor and hormonal functions, presents a number of obstacles. Despite immunosuppression, long-term survival of experimental and clinical SB transplants is hampered by rejection, graft-versus-host disease (GVHD) and sepsis (1,2). Therefore, monitoring of the SB allograft is of the utmost importance, since it enables to initiate treatment before irreversible damage to the graft or the recipient occurs. Although histological examination of the SB allograft has several disadvantages (3-6), it still remains the gold standard to diagnose allograft rejection.

Madara and Kirkman investigated the effect of rejection on the electrophysiological functions of SB allografts in a rat model by in vitro techniques (7,8). They found that decreased transepithelial potential differences (PD) caused by diminished active ion transport of the enterocytes correlate with mucosal changes resembling rejection of the graft. Recently we described a non-invasive method for in vivo measurement of transepithelial PD in canine SB autotransplants (9); it revealed a distinct correlation between electrophysiological responses and the condition of SB autografts. We also demonstrated by long-term follow-up that heterotopic SB autotransplantation results in mucosal atrophy of the SB graft; the decreased PD responses reflected the degree of atrophy of the mucosa (10).

The aim of the present study was to evaluate the significance of in vivo measurements of electrophysiological parameters in detecting canine SB allograft rejection. The electrophysiological parameters were compared with the histological characteristics of the SB grafts.

6.3 Materials and methods

Experimental groups

In total thirty-one healthy adult Beagle dogs weighing 10 to 20 kg underwent a heterotopic small bowel transplantation (SBT) as described in chapter 4. The animals were grouped as follows: in 17 dogs forming group I a heterotopic SB autotransplantation was performed. Group II consisted of 8 dogs, which received a heterotopic SB allograft in a fully mismatched donor-recipient combination; 6 of these 8 dogs received a SB allograft from 6 other dogs, which served as donors and were sacrificed at operation. Two other dogs of group II served both as donor and recipient of a SB allograft. Tissue typing for antigens of the canine major histocompatibility (DLA) complex was achieved by serological methods (class I antigens) and mixed lymphocyte cultures (class II antigens) (11,12). The selection of donor-recipient pairs was based on a 2 haplotype difference resulting in a model in which acute rejection of the SB allograft could be

expected. No immunosuppression was given.

Electrophysiological measurements
The electrophysiological measurements and test solutions are described in chapter 4. The electrophysiological responses to theophylline and glucose are widely used indices of cyclic-AMP-mediated chloride secretion by crypt cells and sodium- coupled glucose absorption by villus cells, respectively (13,14). In group I measurements of the transepithelial PD were performed on postoperative days 2 or 3, 7, 10 and 14, and in group II on days 3, 5 and 6. The postoperative day on which electrophysiology did no longer reveal any stimulated PD responses or showed reversed PD reponses was taken as the end-stage of graft survival.

Morphology
At the day of electrophysiological measurements biopsies were obtained from both SB loops at approximately 5 to 7 cm from the cutaneous stomas. The biopsies were prepared as described in chapter 4. Autopsies were performed on 7 dogs of group I and on all dogs of group II at the day of sacrifice. At autopsy full-thickness biopsies of the autografts or allografts were taken, as well as samples of the vascular anastomosis, the recipient's own SB, lymph nodes, spleen, liver, lung and skin.

Statistics
All data are expressed as means $\pm$ the standard errors of the mean (SEM). Statistical analysis of the electrophysiological parameters was performed by using Student's t test; $p < 0.05$ was considered significant.

6.4 Results

Postoperative course
Ischaemic times in groups I and II did not differ significantly (36.7 $\pm$ 1.0 minutes (mean $\pm$ SEM) and 35.9 $\pm$ 1.9 minutes respectively). The overall technical failure rate was 36% (Table 6.1). One dog of group I died 8 days after transplantation as a result of a wound abscess. Six dogs of group I and 2 dogs of group II had to be sacrificed at postoperative day 2 or 3 because of a deteriorating condition. At that time the cutaneous ileostomies of the grafts of these 8 dogs appeared brown and produced blood stained secretions. Before sacrificing these dogs, electrophysiological parameters were obtained in 4 dogs with autografts (subgroup Ib) and 1 dog of group II. Autopsy revealed thrombus formation at either the arterial or venous anastomosis, causing haemorrhagic ischaemic necrosis of the graft.

Ten dogs of the successful autotransplant group (subgroup Ia) survived the experimental period. During the first postoperative day the cutaneous ileostomies produced mucosal sludge, but throughout the test period both stomas had a pink appearance and secreted a small amount of clear fluid. Graft survival of the 6 successful allotransplants (group II) was 5.5 ± 0.2 days (mean ± SEM). These dogs initially showed a postoperative course comparable to the dogs of the successful autotransplant group. However, at day 5 the cutaneous stomas showed purple discolorations and the dogs developed fever. At day 6 the cutaneous stomas appeared brown and dry and the general condition of the dogs deteriorated. Autopsy revealed peritonitis and necrosis of the grafts and lymph node enlargement in the allograft mesentery.

Table 6.1. Technical failures

group[a]	n	survival (days)	technical failure
I	5	2, 2, 2, 3, 3	arterial thrombosis
I	1	3	arterial thrombosis and intussusception
I	1	8	wound abscess
II	1	2	arterial thrombosis
II	1	3	arterial and venous thrombosis

[a]group I: ileal autotransplants; group II: ileal allotransplants.

Electrophysiology

Results of the electrophysiological measurements are shown in Table 6.2. In group Ia, the successful autotransplant group, perfusion of the blind ending ileostomy with 0.9% saline caused a luminal negative base-line PD response. Throughout the test period these base-line PD measurements showed a considerable range. Stimulated active chloride secretion after exposure to theophylline (Figure 6.1) and active sodium absorption after exposure to glucose (Figure 6.2) also showed luminal negative peak PD responses. However, the PD response after glucose stimulation was diminished at day 3 compared with day 7 ($p < 0.05$), whereas the PD response after theophylline exposure at day 3 did not differ statistically from the corresponding response at day 7.

In the dogs of group Ib, suffering acute ischaemia as a result of thrombosis at the vascular anastomosis, the results of the electrophysiological measurements were completely different compared to group Ia. At day 2 or 3 the base-line PD showed a positive value and peak PD responses to both theophylline and glucose differed significantly from the corresponding responses in group Ia and II ($p < 0.001$). In 1 dog of the allotransplant group, which suffered also from an ischaemic graft, the electrophysiological parameters obtained at day 2 showed results identical to group Ib

(0.28 mV, 0.55 mV and 0.75 mV as base-line PD, PD-theophylline and PD-glucose respectively).

Electrophysiological measurements in group II obtained at postoperative day 3 resulted in PD responses comparable to group Ia. At day 5, however, the base-line PD showed a positive value, the stimulated PD responses were significantly lower compared to the responses at day 3 ($p<0.05$) and in 3 dogs these responses even decreased to zero. At day 6 the stimulated PD responses showed positive values as in group Ib.

Table 6.2. Base-line transepithelial potential difference (base-line PD), theophylline-stimulated PD response (PD-theophylline), and glucose-stimulated PD response (PD-glucose)[a]

	base-line PD (mV)	PD-theophylline (mV)	PD-glucose (mV)
group Ia[b]			
day 3	−1.41 ± 0.87	−3.23 ± 0.50	−2.20 ± 0.38[f]
day 7	−2.79 ± 0.96	−3.56 ± 0.50	−4.02 ± 0.53
day 10	−1.61 ± 0.95	−2.47 ± 0.31	−3.16 ± 0.46
day 14	−0.67 ± 0.20	−2.41 ± 0.33	−3.10 ± 0.56
group Ib[b]			
day 2/3	1.25 ± 1.11	0.83 ± 0.35[c]	0.46 ± 0.31[c]
group II[b]			
day 3	−0.41 ± 0.70	−2.81 ± 0.71	−3.23 ± 0.52
day 5	0.83 ± 0.79	−0.62 ± 0.42[d]	−0.35 ± 0.32[e]
day 6	1.85 ± 0.72	0.28 ± 0.28[d]	0.28 ± 0.28[e]

[a]Results are expressed as means ± SEM; [b]group Ia: successful autotransplants (n=10); group Ib: non-successful autotransplants (n=4); group II: successful allotransplants (n=6); [c]$p<0.001$ compared with groups Ia and II at day 3; [d]$p<0.05$ compared with day 3; [e]$p<0.01$ compared with day 3; [f]$p<0.05$ compared with day 7.

Histology

In the biopsies of the non-successful auto- and allografts transmural necrosis consistent with haemorrhagic ischaemia was seen. These findings were confirmed at autopsy.

The biopsies of the successful autotransplants taken at postoperative day 3 were characterized by some villus shortening, lymph vessel dilatation and edema. During the remainder of test period the biopsies included no mucosal deformities. Biopsies taken simultaneously from both SB loops were histologically identical.

In the allotransplant biopsies, changes similar to those of the successful autotransplants were found at day 3. The biopsies showed changes indicating rejection at day 5: denudation and sloughing of the villi, increased numbers of mononuclear cells together with polymorphonuclear cells in the lamina propria and vasculitis with swelling of endothelial cells. However, even these severe changes were patchy in distribution. At

day 6 the changes progressed to complete destruction of the mucosa and submucosa and fibrinoid necrosis of vessel walls was present. At autopsy similar changes of allograft rejection were found. The serosal layer of the allografts and the host's own SB contained polymorphonuclear cells and fibrin exsudates indicating peritonitis. In the enlarged lymph nodes of the allograft mesentery follicular hyperplasia and sinus histiocytosis were seen. No signs of GVHD were found in all tissues examined.

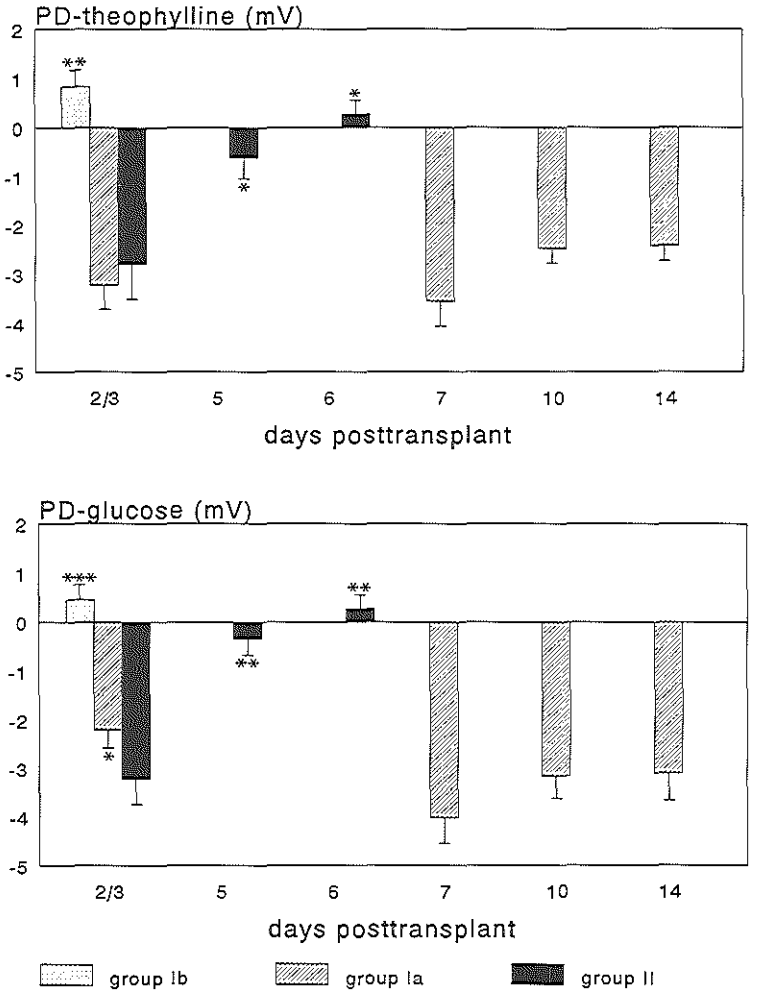

Fig.6.1. Theophylline-stimulated PD response (PD-theophylline) in groups Ia, Ib and II. Results are expressed in mV (mean ± SEM). *p<0.05 compared with the same group at day 3. **p<0.001 compared with groups Ia and II at day 3.

Fig.6.2. Glucose-stimulated PD response (PD-glucose) in groups Ia, Ib and II. Results are expressed in mV (mean ± SEM). *p<0.05 compared with the same group at day 7. **p<0.01 compared with the same group at day 3. ***p<0.001 compared with groups Ia and II at day 3.

6.5 Discussion

Since the introduction of cyclosporine much progress has been made in experimental transplantation of the SB (15-20), but its clinical application is still restricted because of the inability to prevent graft rejection and GVHD (1,2). Recently, however, an experimental study of rat SBT showed that FK-506 is a more effective single agent than cyclosporine in the prevention of acute SB rejection and lethal GVHD (21). Moreover, the successful clinical SB/liver transplantation by Grant clearly demonstrated that SBT has important potential for the treatment of the short bowel syndrome (22).

If SBT is to become a clinical reality, monitoring of the SB allograft to detect rejection is of the utmost importance. It would allow for timely initiation of appropriate immunosuppressive therapy and if this would fail the SB allograft could be removed to salvage the recipient.

Since there is not yet a consensus of opinion about the diagnostic value of functional tests, histological evaluation is still the gold standard for confirmation of SB allograft rejection (1,2). Recently however, several shortcomings of a histological evaluation were demonstrated (3-6). Mucosal biopsies may not be diagnostic for a rejection episode, whereas full-thickness biopsies bear the risk of perforating the graft (3,5). Moreover, a considerable variability can be expected within one biopsy, because of the patchy distribution of early rejection (4,6).

The results of our in vivo experiments demonstrate that electrophysiological parameters correlate well with the condition of the SB graft. This is in accordance with the in vitro experiments of Madara and Kirkman, who found that diminished active ion transport of the enterocytes caused by rejection resulted in decreased transepithelial PD responses (7,8). They could differentiate between crypt cell function and villus cell function by using a theophylline and glucose solution respectively: challenging the SB mucosa with theophylline results in cyclic-AMP-mediated chloride secretion by enterocytes of the crypts, whereas glucose perfusion leads to sodium coupled glucose absorption by villus cells (13,14). In a physiological situation both active transport processes result in luminal negative transepithelial PD responses. In our study these physiologic responses were found in group Ia.

Remarkably, in group Ia the glucose stimulated response, assessing the sodium coupled glucose transport, was substantially lower at day 3 compared to day 7. This decreased absorptive response correlated well with histology, which showed a reduced height of the villi at day 3, probably as a result of the short ischaemic period during transplantation. It is known that both ischaemia and reperfusion injury generated by oxygen-derived free radicals have damaging effects on intestinal mucosa (23,24). Nevertheless, SB has an enormous regenerative potential after a short period of ischaemia (24). This regeneration starts in the crypts and regenerated enterocytes gradually migrate

from the crypts to the villus tips. This could explain that the glucose stimulated response, which is a villus function, was still reduced at day 3, whereas at the same time point the regenerated crypt cells caused a normal PD response after theophylline stimulation.

In our acute rejection model we found that stimulated peak PD responses decreased significantly with histologic alterations indicating rejection at day 5. At day 6 the PD responses of the allografts paralleled those of the non-successful, ischaemic SB grafts. This observation lends support to the hypothesis that the major target of acute rejection is the endothelial cell of the microvasculature (7). If the rejection episode continues, swelling of the endothelial cells will result in progressive enterocyte damage and, eventually, ischaemic necrosis of the entire mucosa of the graft. The luminal positive values of the stimulated PD responses are probably caused by passive effussion of positive potassium ions from necrotic enterocytes. Thus, by using electrophysiological measurements it is not possible to differentiate between ischaemic necrosis and end-stage graft rejection. However, during a rejection episode serial PD monitoring will result in gradually decreasing PD responses as could be demonstrated in group II. On the other hand ischaemia of the graft caused by vascular thrombosis will result in an abrupt cessation of stimulated PD responses.

The significance of the base-line PD responses, measuring the base-line active transport processes, remains uncertain. Even in group Ia these responses showed a considerable range suggesting a substantial variation in base-line ion transports. Failure to demonstrate a significant difference compared with groups Ib and II is almost certainly related to the experimental variation. However, a trend towards significance could be demonstrated in group II during the rejection episode.

The rapidity of the vigorous rejection process made it impossible to determine a possible sequence in crypt cell and villus cell damage. To study this phenomenon it is necessary to evaluate in vivo PD parameters in a dog model with chronic rejection. Additionally, in such a model a possible discrepancy in the sequence of electrophysiological and histological changes may be more evident.

In summary, in vivo electrophysiological parameters correlate with histological alterations of acute SB rejection. Our experiments demonstrate that serial monitoring of transepithelial PD responses is a non-invasive method for detecting SB allograft rejection, which circumvents the disadvantages of a histological evaluation.

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CHAPTER 7

ELECTROPHYSIOLOGICAL AND HISTOLOGICAL MONITORING OF DLA-MATCHED AND MISMATCHED CANINE INTESTINAL ALLOGRAFTS

7.1 Abstract

Small bowel transplantation (SBT) has potential applications for the treatment of short-bowel syndrome (SBS). A major obstacle to its clinical use is rejection, which may partly be overcome by major histocompatibility complex (MHC) matching. At present, it is impossible to predict accurately the early onset of rejection. The aim of this study was to assess the value of in vivo measurements of transepithelial potential difference (PD) for the determination of rejection in DLA-matched and mismatched canine small bowel (SB) allografts. Four groups of dogs were studied: group 1 (controls, n = 10) ileal autotransplants; group 2 (n = 6) ileal allotransplants, fully mismatched; group 3 (n = 3) ileal allotransplants, 1 haplotype identical; and group 4 (n = 5), ileal allotransplants fully matched. No immunosuppression was given. All dogs of group 1 survived indefinitely and both PD and histology showed no abnormalities. In group 2, 3, and 4 the onset of histological changes related to rejection corresponded well with decreased PD responses. The survival times in group 4 (9.6 $\pm$ 2.7 days) were significantly longer than those of group 2 (5.5 $\pm$ 0.2 days; $p<0.05$, Wilcoxon rank-sum test). The one haplotype-identical SB allografts of group 3 had an intermediate survival time (7.7 $\pm$ 0.7 days). It can be concluded that serial PD measurements in SBT are a potential marker for rejection and that DLA matching is of significant importance for SB allograft survival.

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7.2 Introduction

In children, small bowel atresias, malrotation with midgut volvulus, gastroschisis, necrotizing enterocolitis, and extensive aganglionosis may require resections leading to SBS (1,2). The development of total parenteral nutrition (TPN), early institution of enteral feeding, and overall improvements in neonatal intensive care have contributed to an increased survival for infants with the SBS (3). Although a proportion of this growing population of patients with SBS may undergo intestinal adaptation to maintain themselves by oral alimentation, many will require a long-term or indefinite program of home parenteral nutrition (4-6). Such nutritional therapy is associated with serious complications such as sepsis, thrombosis, and liver impairment. Furthermore, TPN limits the patients' lifestyle, causes physiological problems, and is extremely expensive (7-10). These problems are accentuated in the treatment of children with TPN. Their long-term outlook is less favourable (14 to 31% mortality rate) than that of adults because of the higher risk of liver impairment, the greater and more specific nutritional requirements associated with growth, and the limitations on adequate venous access (4,11-13). Thus, the best method of treating the SBS would be the transplantation of a functioning small intestine.

However, transplantation of the SB, an organ with immunological, nutritional, motor, and hormonal functions, presents a number of obstacles. Despite immunosuppression, long-term survival of experimental and clinical small bowel transplants is hampered by rejection, graft-versus-host disease (GVHD), and sepsis (14-16).

One of the dogmas of immunology dictates that the severity of allograft rejection is significantly influenced by the degree of histocompatibility between donor and recipient. Experimental and clinical experience has verified the dogma and corroborated the evidence. The importance of compatibility of donor and recipient for the major histocompatibility complex (MHC) antigens has been shown in organ grafts in various animal species. Transplantation experiments with heart and kidney allografts have demonstrated that matching for antigens of the canine MHC (DLA) resulted in more than twofold increased survival times compared with those of mismatched grafts (17,18). Using this approach in SBT, rejection could possibly be delayed. Furthermore, living relatives could potentially donate allogeneically MHC-identical segments of intestine to serve as grafts for family members with severe SBS.

Madara and Kirkman investigated the effect of rejection on the electrophysiological functions of small bowel allografts in a rat model by in vitro techniques (19,20). They found that decreased transepithelial potential differences (PD) caused by diminished active ion transport of the enterocytes correlate with mucosal changes resembling rejection of the graft. Lee reported about in vivo transepithelial PD as a marker of rejection in a rat small bowel transplant model (21). He found that changes in transepithelial PD often occurred before histological changes. However, because of the invasive nature of their

method, he did not recommend it as a practical clinical tool (21). Recently, we described a noninvasive method for in vivo measurement of transepithelial PD in canine SB autotransplants; it showed a distinct correlation between electrophysiological responses and the condition of SB autografts (22).

The aim of the present study was to assess the value of in vivo measurements of transepithelial PD for the determination of rejection in DLA-matched and mismatched canine SB allografts.

7.3 Materials and methods

Experimental Groups

In total, 34 healthy adult Beagle dogs (Harlan, Zeist, The Netherlands) weighing 10 to 20 kg were used. In all dogs a heterotopic SBT was performed as described in chapter 4. After exclusion of the technically nonsuccessful SBT, four groups of dogs could be studied: group 1 (controls, n = 10) ileal autotransplants; group 2 (n = 6), ileal allotransplants, fully mismatched; group 3 (n = 3), ileal allotransplants, one haplotype identical; group 4 (n = 5), ileal allotransplants, fully matched. Tissue typing for antigens of the DLA complex was achieved by serological methods (class I antigens) and mixed lymphocyte cultures (class II antigens) (23,24). The selection of donor-recipient pairs in group 2 was based on a two haplotype difference resulting in a model in which acute rejection of the small bowel allograft could be expected. The selection of donor-recipient pairs in groups 3 and 4 was based on a one haplotype and a two haplotype similarity, respectively, resulting in models in which the effect of matching for the DLA complex could be investigated. Immunosuppression was given in none of the experimental groups.

Electrophysiological measurements

The electrophysiological measurements and test solutions have been decribed in chapter 4. The responses to theophylline and glucose are widely used indices of cyclic-AMP-mediated chloride secretion by crypt cells and sodium-coupled glucose absorption by villus cells, respectively (25-28). In group 1 measurements of the transepithelial PD were performed on postoperative days 3, 7, 10, 14, and 21; in group 2 on postoperative days 3, 5, and 6; in group 3 on postoperative days 3, 5, 7, and 9; and in group 4 on postoperative days 3, 5, 7, 9, and 14. The postoperative day on which electrophysiology no longer showed any stimulated PD responses or showed reversed PD responses was evaluated as parameter for the end-stage of graft survival.

Morphology

At the day of electrophysiological measurements, biopsies were obtained from both small

bowel loops at approximately 5 to 7 cm from the cutaneous stomas. The biopsies were prepared as described in chapter 4. Autopsies were performed on all dogs of groups 2, 3, and 4 at the day of sacrifice. At autopsy, full-thickness biopsy specimens of the allografts were taken, as well as samples of the vascular anastomosis, the recipient's own small bowel, lymph nodes, spleen, liver, lung, and skin.

Statistics

All data are expressed as mean $\pm$ the standard error of the mean (SEM). Statistical analysis of the electrophysiological parameters was performed by using the Student's *t* test. Survival data were analyzed using Wilcoxon rank sum test; $p<0.05$ was considered significant.

7.4 Results

Postoperative course

Ischaemic times in the experimental groups were as follows: 36.2 $\pm$ 1.2 minutes in group 1; 37.2 $\pm$ 2.1 minutes in group 2; 31.7 $\pm$ 3.7 minutes in group 3; and 31.2 $\pm$ 1.8 minutes in group 4. The overall technical failure rate was 29.4% (10 of the 34 transplanted dogs). In 8 dogs autopsy revealed thrombus formation at either the arterial or venous anastomosis, causing hemorrhagic ischaemic necrosis of the graft. One dog died from intussusception of the native SB on day 3 and one dog succumbed to a wound abscess on day 8. Consequently, in group 1 ten autotransplants were available for functional evaluation, in group 2 six fully MHC-mismatched allografts, in group 3 three one-haplotype identical allografts and in group 4 five fully MHC-matched SB allografts (Table 7.1).

The survival times of the SB grafts are shown in Table 7.1. Ten dogs of the autotransplant group (group 1) survived the experimental period. During the first postoperative day the cutaneous ileostomies produced mucosal sludge, but throughout the test period both stomas had a pink appearance and secreted a small amount of clear fluid. Graft survival of the fully mismatched (group 2), one haplotype identical (group 3), and fully matched (group 4) allotransplants was 5.5 $\pm$ 0.2 days, 7.7 $\pm$ 0.7 days, and 9.6 $\pm$ 2.7 days, respectively. The survival times of the SB grafts in group 4 were significantly longer than those of group 2 ($p<0.05$). These dogs initially showed a postoperative course comparable to the dogs of the autotransplant group. However, in group 2 at day 5, the cutaneous stomas showed purple discolorations and the dogs developed fever. At day 6 the cutaneous stomas appeared brown and dry and the general condition of the dogs deteriorated. Autopsy showed peritonitis and necrosis of the grafts and lymph node enlargement in the allograft mesentery. This clinical course was delayed in groups 3 and

4, in which the donor-recipient combination was matched for one and two haplotypes, respectively.

Table 7.1. DLA typing, donor-recipient pairs and graft survival in allotransplant groups

	DLA-A/B/C (class I antigens)			
experimental group	recipient	donor	MLR[a]	survival[b]
group 2	2,7/4,5/11	3,9/6,10/12	+	5
	2,9/5,6/12	9/4,10/12	+	6
	3,7/5,10/–	9/6/12	+	5
	2,3/4,10/11	9,R20/5,6/11,12	+	6
	3,9/6,10/12	1,2/4,13/11	+	6
	1,2/4,13/11	3,9/6,10/12	+	5
group 3	3/10/–	2,3/5,10/11	+	7
	9/4/12	2,9/4/11,12	+	9
	2/4/11	2,9/4,6/11,12	+	7
group 4	3/10/–	3/10/–	–	8
	3/10/–	3/10/–	–	10
	2,3/5,10/11	3/10/–	–	14
	2,9/4/11,12	9/4/12	–	7
	2,9/4,6/11,12	2/4/11	–	9

[a]Identity for class II antigens was based on the absence (–) of significant stimulation in mixed lymphocyte reactions (MLR) as compared with autologous controls. [b]Survival in days.

Electrophysiology

Results are shown in Table 7.2. In group 1 stimulated active chloride secretion after exposure to theophylline (Figure 7.1) and sodium-coupled glucose absorption after exposure to glucose (Figure 7.2) showed luminal negative peak PD responses. Electrophysiological measurements in group 2 obtained at postoperative days 3, 5, and 6 showed significantly decreasing PD responses from day 5 on ($p<0.05$). At day 6 the stimulated PD responses showed positive values. In group 3 and 4 this process of diminishing PD responses was less acute. In group 3 the stimulated responses on day 5 posttransplant were not significantly different from the stimulated responses on day 3. From day 7 on, significantly lower PD responses after theophylline-stimulated chloride secretion were seen ($p<0.05$). PD responses after exposure to glucose were significantly lower at day 9 posttransplant ($p<0.05$). At day 9 the responses were zero. In group 4 the stimulated PD responses were significantly diminished from day 9 on compared with the values on day 3 posttransplant ($p<0.05$). The stimulated PD responses became zero at day 14.

Table 7.2. Theophylline-stimulated PD response (PD-theophylline) and glucose-stimulated PD response (PD-glucose)[a]

group	day	PD-theophylline (mV)	PD-glucose (mV)
group 1	day 3	-3.23 ± 0.50	-2.20 ± 0.38
	day 7	-3.56 ± 0.50	-4.02 ± 0.53
	day 10	-2.47 ± 0.31	-3.16 ± 0.46
	day 14	-2.41 ± 0.33	-3.10 ± 0.56
	day 21	-2.17 ± 0.25	-2.69 ± 0.35
group 2	day 3	-2.81 ± 0.71	-3.23 ± 0.52
	day 5	-0.62 ± 0.42[b]	-0.35 ± 0.32[b]
	day 6	0.28 ± 0.28[b]	0.28 ± 0.28[b]
group 3	day 3	-2.80 ± 0.56	-3.83 ± 0.78
	day 5	-3.53 ± 0.80	-3.75 ± 1.39
	day 7	-0.40 ± 0.40[b]	-0.80 ± 0.80
	day 9	0[b]	0[b]
group 4	day 3	-3.25 ± 0.76	-3.03 ± 0.55
	day 5	-2.54 ± 0.47	-2.28 ± 0.56
	day 7	-1.70 ± 1.04	-1.48 ± 1.48
	day 9	-0.48 ± 0.48[b]	-0.48 ± 0.48[b]
	day 14	0[b]	0[b]

[a]Results are expressed as means ± SEM. [b]$p < 0.05$ versus day 3.

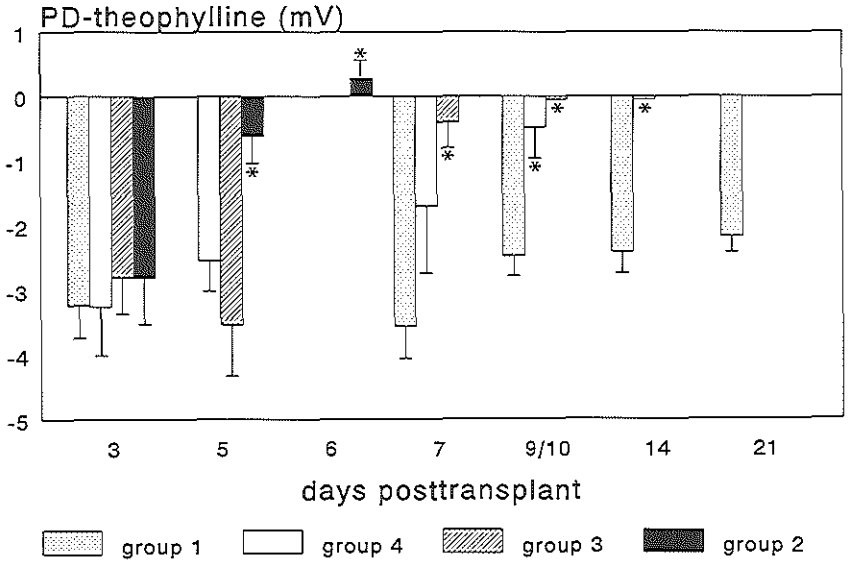

Fig. 7.1. Theophylline-stimulated PD response (PD-theophylline) expressed in mV (mean ± SEM). *$p < 0.05$ compared with the same group at day 3.

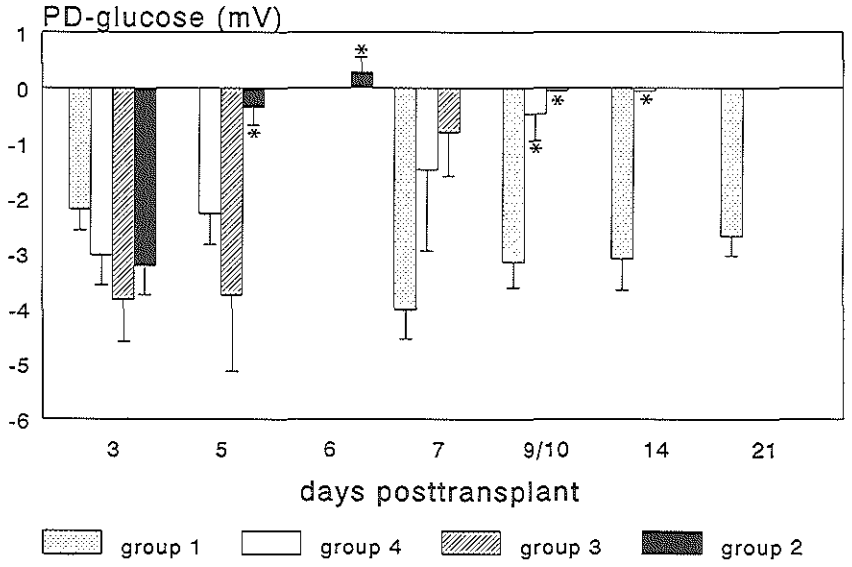

Fig. 7.2. Glucose-stimulated PD response (PD-glucose) expressed in mV (mean ± SEM). *$p<0.05$ compared with the same group at day 3.

Histology

The biopsies of the autotransplants (group 1) taken at postoperative day 3 were characterized by some villus shortening, lymph vessel dilatation, and edema. During the remainder of the 3-week test period the biopsies included no mucosal deformaties. Biopsy specimens taken simultaneously from both small bowel loops were histologically identical.

In the fully mismatched allotransplant biopsies (group 2), changes similar to those of the successful autotransplants (group 1) were found at day 3. The biopsy specimens showed changes indicating rejection at day 5: denudation and sloughing of the villi, increased numbers of mononuclear cells together with polymorphonuclear cells in the lamina propria, and vasculitis with swelling of endothelial cells. However, even these severe changes were patchy in distribution. At day 6 the changes progressed to complete destruction of the mucosa and submucosa and fibrinoid necrosis of vessel walls was present. At autopsy, similar changes of allografts and the host's own small bowel contained polymorphonuclear cells and fibrin exsudates indicating peritonitis. In the enlarged lymph nodes of the allograft mesentery follicular hyperplasia and sinus histiocytosis were seen. No signs of GVHD were found in all tissues examined. The histological observations in the one haplotype-identical allotransplant group (group 3) and the fully matched allotransplant group (group 4) were with a delay of 2 to 4 and 2 to 8 days,

respectively, entirely similar to those observed in the fully mismatched group. In the experimental groups 2, 3, and 4, the onset of histological changes related to rejection corresponded well with decreased stimulated PD responses.

7.5 Discussion

Significant progress has been made in SBT since the pioneering experiments of Lillehei (14,15,29). Especially since the introduction of cyclosporine, much progress has been made in experimental SBT (30-34). Recently developed immunosuppressive drugs such as FK-506 further enhance the expectations of treatment of the SBS with a SBT (35). Despite these improvements in immunosuppression, the clinical application of SBT is still restricted because of the inability to prevent rejection, GVHD, and sepsis (14-16).

Experimental and clinical data have clearly indicated that the ultimate fate of an organ graft is determined by several factors. Major factors are the modification of immune responsiveness and the degree of host/donor compatibility for MHC antigens. Modification of immune responsiveness can be obtained by immunosuppressive drugs, monoclonal antibodies, splenectomy, donor-specific transfusions, and minimizing graft antigenicity (eg, by irradiation of the graft). However, by using these approaches, the general immune status of the host may be affected. A second approach to prevent rejection and, thereby, improve graft survival is the selection of a donor-recipient combination by matching for the MHC antigens. This results in a selective suppression of the rejection reaction against tissue antigens without altering the immune status of the host. In canine kidney and heart transplantation and in human kidney transplantation, the effectiveness of this approach has been proven (17,18,36,37). The survival of MHC-identical sibling grafts is significantly better than that of one-haplotype mismatched grafts and unrelated grafts (36,37). Using this approach in small bowel transplantation rejection could possibly be delayed; furthermore, living relatives could potentially donate allogeneically MHC identical segments of intestine to serve as grafts for family members with severe SBS. The resemblance between the canine MHC and the human MHC justifies the use of the dog for this transplantation research (38).

The survival times in the three experimental groups increased with the degree of similarity in matching: fully mismatching, one haplotype similarity, and fully matching gave survival times of 5.5 $\pm$ 0.2, 7.7 $\pm$ 0.7, and 9.6 $\pm$ 1.2 days, respectively. The survival times of SB grafts in the fully matched group were significantly longer than those of the fully mismatched group. The one haplotype-identical grafts had an intermediate survival time. Thus, DLA matching prolongs SB graft survival in non-immunosuppressed dogs. However, it should be noted that this prolongation of graft survival seems to be of minor importance and contrasts unfavourably with earlier results obtained in beagle heart

and kidney allografts (17,18). In this respect the canine SB allograft behaves more like a skin allograft that has in the fully matched versus fully mismatched, nonimmunosuppressed donor-recipient combination in the dog a survival time of 12 and 18 days, respectively (39). To determine the usefulness of MHC matching for the survival of SB allotransplants, these experiments should be repeated in immunosuppressed recipients.

The results of these in vivo experiments demonstrate that electrophysiological parameters correlate well with the condition of the SB allograft. This is in accordance with the in vitro experiments of Madara and Kirkman, who found that diminished active ion transport of enterocytes caused by rejection results in decreased transepithelial basal-PD and stimulated-PD responses (19,20). In all three rejection models, stimulated peak PD responses decreased significantly with histological alterations indicating rejection at day 5 in the fully mismatched group, at day 7 to 9 in the one haplotype identical allotransplant group, and at day 9 in the fully matched group. In these acute rejection models we could not confirm the suggestion of Lee that in vivo measurement of the electrophysiological responses is an earlier parameter for rejection than histology (21). Finally, the rapidity of the vigorous rejection process in all experimental groups made it impossible to determine a possible sequence in crypt cell and villus cell damage by evaluating the PD after theophylline and glucose stimulation, respectively.

It can be concluded that serial PD measurements in small bowel transplantation provide a potential marker for rejection and that MHC matching is of significant importance for SBT. Supplementary research is currently underway to determine the significance of MHC matching in immunosuppressed dogs.

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CHAPTER 8

THE VALUE OF SERUM N-ACETYL HEXOSAMINIDASE IN DETECTING CANINE INTESTINAL ALLOGRAFT REJECTION

8.1 Abstract

Besides a morphological investigation it is impossible to diagnose small bowel (SB) allograft rejection at present. As a morphological evaluation has several disadvantages, we examined the validity of serum N-acetyl hexosaminidase (NAH) to monitor acute rejection of canine SB grafts. Earlier, it has been shown that elevated serum NAH activity may predict rejection in a rat model of SBT. Three groups were investigated: group 0 (n=12): ileal autotransplants; group I (n=6): MHC-mismatched ileal allotransplants; and group II (n=5): MHC-matched ileal allotransplants. None of the animals received immunosuppression. At day 3 after SBT all groups showed elevated serum NAH activity, probably as a result of the ischaemic period during transplantation. In the MHC-mismatched group an increasing trend of serum NAH activity occurred after histological signs of acute rejection, whereas in the MHC-matched group an elevation of NAH levels coincided with morphological signs of rejection. Therefore, we do not recommend serum NAH activity as a biochemical marker to monitor early SB allograft rejection in a dog model.

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8.2 Introduction

Small bowel transplantation (SBT) has potential applications for the treatment of the short bowel syndrome (SBS). However, major obstacles to clinical SBT are rejection of the SB allograft and secondary infectious complications, leading to a high rate of morbidity and mortality (1,2). Therefore, monitoring of the SB allograft to allow early recognition of rejection and subsequent treatment is a prerequisite to a clinical SBT program. At present, the standard to diagnose SB allograft rejection is a morphological examination of mucosal biopsies, although morphology may underestimate rejection because of the patchy character (3,4). In addition, an orthotopic SB graft may be relatively inaccessible for biopsy if no stoma is provided.

Ideally, a biochemical marker, such as creatinine for kidney allografts and creatine phosphokinase for heart allografts, could provide information about a rejection episode in sufficient time to initiate treatment before irreversible rejection occurs. Studies have demonstrated that N-acetyl hexosaminidase (NAH), a lysosomal acid hydrolase, is elevated in association with partial and complete intestinal ischaemia (5-7). Considering the fact that partial ischaemia may also occur in SB graft rejection, Maeda evaluated serum levels of NAH activity in a rat model of SBT (8). It was found that NAH levels to rise before histological signs of SB allograft rejection do occur. Maeda concluded that measurement of serum NAH activity is a simple, rapid test which may be useful as a serum marker to detect early rejection after SBT (8). The aim of the present study was to evaluate the validity of serum NAH as a marker to detect early rejection of MHC-matched and mismatched canine SB allografts.

8.3 Materials and methods

Animals and operative techniques

Adult Beagle dogs (Harlan, Zeist, the Netherlands) were used. Tissue typing for canine MHC antigens was achieved by serological methods (class I antigens) and mixed lymphocyte cultures (class II antigens) as earlier described in chapter 4. In each dog a heterotopic SBT was performed and 2 cutaneous stomas were created for monitoring purposes as described in chapter 4.

Experimental groups

Three groups of dogs were studied: Group 0 (n=12): ileal autotransplants; group I (n=6): ileal allotransplants in a MHC-mismatched donor-recipient combination; group II (n=5): ileal allotransplants in a MHC-matched donor-recipient combination. None of the animals received immunosuppressive treatment.

Serum NAH activity

At regular intervals serum samples were obtained. NAH activity was determined using a colorimetric assay (Boehringer Mannheim Biochemica). Principle: 3-cresolsulfonphtaleinyl -N-acetyl-β-D-glucosaminide, sodium salt, is hydrolyzed by N-acetyl-β-D glucosaminidase (NAH) with the release of 3-cresolsulfonphtalein, sodium salt (3-cresol purple), which is measured photometrically at 580 nm. The measured levels of NAH activity are expressed in IU/l.

Histology

At the day of serum NAH measurements histological specimens were obtained by forceps from the cutaneous stomas. The biopsies were prepared as described in chapter 4.

Statistics

All data are expressed as means $\pm$ the standard error of the mean (SEM). Statistical analysis of serum NAH levels was performed using Student's t test; $p<0.05$ was considered significant.

8.4 Results

Results are shown in Table 8.1. In all groups serum NAH activity showed an initial rise at day 3, probably as a result of the short ischaemic period during transplantation procedure. Also at day 3, all three groups showed an identical histological pattern with some villus shortening, lymph vessel dilatation and edema. In group 0 the NAH activity decreased from day 5 onwards and remained within normal limits during the remainder of the test period. After day 3 histology showed a normal pattern in group 0.

In group I, the MHC-mismatched group, the NAH activity showed increasing levels from day 5 onwards. Because of a large variation these results were not significantly different from NAH levels of group 0. As early as day 5 histology showed signs of rejection. In two dogs of this group biopsies were indicative for transmural necrosis of the SB allograft at day 5.

Serum NAH levels in group II, the MHC-matched group, never regained pretransplant levels. At day 3 serum NAH levels in group II were significantly higher compared with the corresponding levels in group 0. After this initial rise at day 3 the serum NAH levels in group II showed an increasing trend, but only from day 7 onwards NAH levels were significantly higher than those in group 0. From day 7 onwards histology showed gradually progressing morphological alterations indicating SB allograft rejection.

Table 8.1. Serum N-acetyl hexosaminidase activity after small bowel transplantation[a]

day	group 0	group I	group II
0[b]	3.48 ± 0.51	4.38 ± 0.88	4.90 ± 0.60
3	10.95 ± 2.65	23.34 ± 7.49	19.38 ± 1.42[c]
5	7.50 ± 2.30	9.98 ± 2.32	10.02 ± 0.44
7	7.05 ± 0.60	10.55 ± 2.15	10.30 ± 1.07[c]
9	3.77 ± 1.05		12.73 ± 2.71[c]
14	5.16 ± 0.75		21.60

[a]NAH activity expressed in IU/l (mean ± SEM). [b]Pretransplant NAH level. [c]$p < 0.05$ versus group 0.

8.5 Discussion

Monitoring of the SB graft for early recognition and treatment of rejection is mandatory in SBT. At present a histological investigation of the grafted tissue is the only reliable method to monitor a SB allograft. However, even histological monitoring has several shortcomings (3,4). Mucosal biopsies are not diagnostic, while full thickness biopsies bare the risk of sampling errors or perforation of the graft. Additionally, orthotopic SB grafts may be inaccessible to biopsy, whereas the SB mucosa of heterotopic grafts may atrophy and thus mimic a morphological pattern of rejection.

Several functional tests have been suggested to diagnose early rejection (1). However, none of these tests can be used as an early marker of rejection as histological alterations of rejection precede almost always changes of these functional tests.

Ideally, a biochemical marker could predict a rejection episode, after which treatment can be initiated to prevent irreversible damage to the SB graft. A clinically useful marker should be specifically released by the SB, easily monitored in the peripheral blood or urine, and rise early in the course of SB rejection. The magnitude of the rise should correlate with the degree of rejection.

Cohen introduced serum monocyte/macrophage procoagulant activity (PCA) to monitor SB graft rejection (9-11). PCA is a serological correlate of alloantigen-induced activation of the immune system and a measure of immune activation of mononuclear cells. Elevated levels of PCA correlated well with early SB graft rejection (10,11). However, besides allogeneic cells other stimuli, such as viruses and mitogens, may induce increased PCA levels. Moreover, a false positive rate of 33% has been reported using PCA in clinical renal transplantations (12).

Maeda evaluated if serum NAH levels are indicative for early rejection in a rat SBT model (8). The lysosomal acid hydolase NAH is known to exist in two iso-enzymic forms A and B. A deficiency of NAH has been discovered in two storage disorders: Tay-

Sachs disease (a deficiency of NAH A) and Sandhoff's disease (a deficiency of both NAH A and B)(13). NAH has been shown to rise in association with partial and complete intestinal ischaemia (5,6). In addition, Lobe suggested that serum NAH activity may provide an early biochemical indication of the presence of necrotizing enterocolitis in the preterm infant (7). In acute SB graft rejection partial intestinal ischaemia may occur, as fine vessels of the microvasculature are occluded by swollen endothelium (14). Indeed, Maeda found increased serum NAH activity before histological signs of SB graft rejection do occur (8). Therefore, Maeda proposed to use serum NAH as a marker to detect early SB graft rejection.

In our study we found elevated NAH levels in all three experimental groups at day 3 posttransplant. At day 3 histology showed similar morphology in all three groups with some villus shortening, lymph vessel dilatation and edema. This elevated serum NAH level and morphological pattern probably reflect the effects of the short ischaemic period during the transplantation. After day 3 morphology and serum NAH activity normalized during the remainder of the testperiod in group 0.

In the MHC-mismatched group histology showed signs of rejection from day 5 onwards. At the corresponding days serum NAH levels showed an increased trend, but the difference with the autotransplant group was not significant. Therefore, in this group with acute rejection alterations of serum NAH levels did not precede morphological alterations.

In the MHC-matched group the first histological signs of rejection coincided with a statistically increased serum NAH level. Thus, also in this group with less vigorous acute rejection as the mismatched group, serum NAH activity could not predict impending rejection. As a consequence, we can not recommend serum NAH activity as an early biochemical marker of canine SB allograft rejection. Probably, substantial ischaemic damage secondary to rejection may have occurred already, if serum NAH activity is elevated. This corroborates with the findings of Marks, who found elevated concentrations of porcine ileal peptide following one hour of mesenteric ischaemia, but normal serum NAH levels (15). It remains to be determined whether serum NAH activity with or without histology is useful to monitor chronic rejection of canine SB allografts.

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CHAPTER 9

DIMINISHED FUNCTIONAL CAPACITY AND COMPROMISED MUCOSAL INTEGRITY IN ACUTELY REJECTING CANINE SMALL BOWEL ALLOGRAFTS

9.1 Abstract

Clinical application of small bowel transplantation (SBT) to treat the short bowel syndrome is still restricted, because of the inability to prevent rejection and the inaccuracy to detect the early onset of rejection. The aim of the present study was to monitor rejection in canine intestinal allografts, which were matched or mismatched for antigens of the major histocompatibility complex (MHC). Monitoring was performed by serial determinations of brush border maltase and lactase activity in mucosal biopsies and by analysis of alkaline phosphatase activity and albumin contents in the graft effluent. Heterotopic SBTs were performed in four groups of dogs: group 1, ileal autotransplants (n=17); group 2, fully MHC-mismatched ileal allotransplants (n=8); group 3, one-haplotype identical ileal allotransplants (n=3); group 4, fully MHC-matched ileal allotransplants (n=6). No immunosuppressive therapy was given. Significantly reduced brush border maltase activities correlated with the first histological signs of rejection. Evaluation of lactase activities did not contribute to the detection of graft rejection. In group 4, decreased alkaline phosphatase activities in the graft effluent preceded morphological signs of rejection, but in groups 2 and 3 a decreasing trend only could be detected. During graft rejection an albumin-losing enteropathy was evident.

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9.2 Introduction

Despite improvements in immunosuppressive therapy the clinical application of SBT is still restricted, because of the inability to prevent graft rejection and sepsis (1,2). Another serious problem in SBT is the inaccuracy to detect the early onset of rejection. Early detection is of utmost importance to reverse a rejection process; mucosal biopsies alone are inadequate and the significance of functional tests remains debatable (3,4). Recently however, in a rat study of SBT it was shown that brush border enzyme activity declines simultaneously with histological signs of rejection (5).

The present study was undertaken to detect the onset of rejection in MHC-matched and mismatched canine intestinal transplants by serial determinations of brush border lactase and maltase activity in mucosal biopsies and alkaline phosphatase activity in the graft effluent. Additionally, we studied the integrity of the mucosal barrier during acute rejection and evaluated the significance of the mucosal integrity as a marker for rejection by determination of the albumin contents of the intestinal graft effluent.

9.3 Materials and methods

Experimental groups

In total 41 healthy adult Beagle dogs (Harlan, Zeist, The Netherlands) weighing 10 to 20 kg were used. All dogs were tissue-typed, after which a heterotopic SBT was performed as described in chapter 4. Four groups of dogs were created as follows: group 1 (controls, n=17), ileal autotransplants; group 2 (n=8), fully MHC-mismatched ileal allotransplants; group 3 (n=3), ileal allotransplants, one haplotype identical; group 4 (n=6), fully MHC-matched ileal allotransplants, two haplotypes identical. In none of the groups immunosuppressive therapy was given. In the allotransplant groups 7 dogs received a SB allograft from 7 donors, which were sacrificed at operation. In the remaining 10 dogs of the allotransplant groups transplantations were performed by exchanging a SB graft between dogs of selected donor-recipient pairs. Only non-littermate donor-recipient paire were selected.

Disaccharidase activity

Mucosal biopsies obtained during transplantation and at regular intervals posttransplant were snap frozen in liquid nitrogen and stored at -70°C. Brush border maltase and lactase activities were determined by a standard assay of intestinal disaccharidases (6). The mucosal biopsy was homogenized and weighed. The diluted homogenate (50 μl) was added to 50 μl of a maltose solution or 50 μl of a lactose solution (200 mg in 10 ml sodium maleate buffer 0.1M, pH=6.0) and incubated at 37°C for 60 minutes. The

enzymatic reaction was stopped by adding 1.5 ml of a Triton solution containing tris(hydroxymethyl)aminomethane buffer (61 g tris in 85 ml 5N HCl per 1,000 ml, pH=7.0), glucose oxidase, peroxidase and o-dianisidine. After incubating for an additional 60 minutes at 37°C extinction was read in a spectrophotometer (Gilford 3500 computer directed analyzer, Oberlin, Ohio USA) at 450 nm. A standard protein assay was performed on all mucosal biopsy samples (7). The disaccharidase activity was expressed in U/mg protein at 37°C.

Effluent analysis

Albumin and alkaline phosphatase contents were analyzed in the effluent of the blind-ending SB loop at regular intervals posttransplant. Using a double balloon catheter a graft segment of 6 ml could be isolated. The lumen of this segment was first flushed of debris with 60 ml of a 0.9% saline solution, after which 6 ml saline at 37°C was instilled in the graft. After 15 minutes the instilled saline was collected and the ileal segment was flushed with 18 ml saline. The collected effluent was stored at -70°C. After freeze-drying it was analyzed for albumin content and alkaline phosphatase activity by colorimetric methods using automated analysis kits (Cat.no. 263869 and 415278, Boehringer Mannheim GmbH, Mannheim, Germany). Albumin contents were expressed in mg/ml, alkaline phosphatase activities in U/l at 30°C. Additionally, at regular intervals serial blood samples were drawn, in which serum albumin levels (mg/ml) and serum total protein levels (mg/ml) were determined.

Morphology

Simultaneously with functional tests mucosal biopsies were obtained 5 to 7 cm from the cutaneous stoma. The biopsies were prepared as described in chapter 4. Autopsies were performed on all dogs of groups 2 to 4 after sacrifice and samples were taken from the graft, the vacular anastomosis and the host's SB, mesenteric lymph nodes, liver, spleen, lung and skin.

Statistics

All data are expressed as means $\pm$ SEM. Statistical comparisons between the experimental groups were performed using one-way analysis of variance followed by Student-Newman-Keuls test. Comparisons of data within experimental groups at different time points were performed using two-tailed paired Student's *t* test. For all tests $p<0.05$ was considered significant.

9.4 Results

Mucosal disaccharidase activity

In the autotransplant group, maltase and lactase activities showed a declining trend after transplantation ($p=0.09$ for maltase and $p=0.06$ for lactase, day 3 versus day 9)(Figure 9.1 and Table 9.1). After this initial decline both maltase and lactase activities increased from day 9 onwards and returned to pretransplant levels by day 21. During the remainder of the study period both enzyme levels remained constant.

In the allotransplant groups levels of maltase decreased after grafting as well, but the speed of decline was more pronounced in the allotransplant groups as compared to the autotransplant group (Figure 9.1). In group 2, the maltase activity was significantly lower on day 3 compared to day 0 ($p=0.030$). In groups 3 and 4, maltase levels were significantly smaller from day 6 ($p=0.010$) and 9 ($p=0.019$) respectively. These levels in the allotransplant groups diminished progressively during the remainder of the study. Comparisons with the maltase levels of group 1 revealed a significant difference at day 9 for group 3 ($p=0.025$) and group 4 ($p=0.014$).

Evaluation of the lactase activities showed no significant differences between the autografts and allografts (Table 9.1).

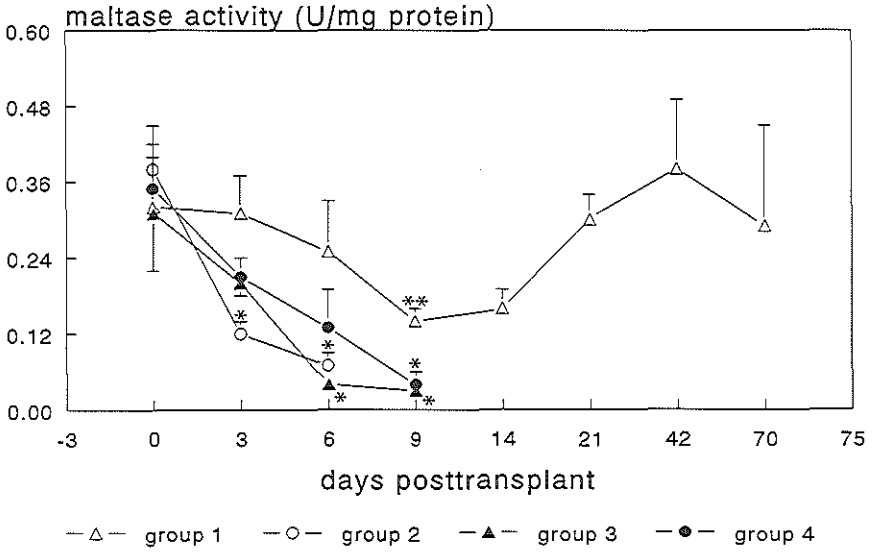

Fig. 9.1. Maltase activity in mucosal biopsies expressed in U/mg protein at 37°C (mean ± SEM). *$p<0.05$ compared with the same group at day 0. **$p<0.05$ group 1 versus groups 3 and 4.

Table 9.1. Lactase activities in mucosal biopsies and alkaline phosphatase activities in intestinal graft effluents (mean ± SEM)

	day	lactase[a]	alkaline phosphatase[b]
group 1	0	0.043 ± 0.018	n.a.[c]
	3	0.025 ± 0.012	1616.9 ± 594.7
	6	0.026 ± 0.010	1941.5 ± 507.5
	9	0.009 ± 0.004	2769.3 ±1164.4
	14	0.026 ± 0.011	1738.5 ± 391.7
	21	0.042 ± 0.016	2761.4 ± 757.7
	42	0.030 ± 0.010	2198.4 ± 531.2
	70	0.022 ± 0.012	2263.8 ± 760.6
group 2	0	0.063 ± 0.021	n.a.[c]
	3	0.011 ± 0.005	2208.0 ± 392.1
	6	0.006 ± 0.003	818.8 ± 246.0
group 3	0	0.032 ± 0.008	n.a.[c]
	3	0.019 ± 0.004	2117.7 ± 456.7
	6	0.024 ± 0.019	1881.0 ± 14.0
	9	0.022 ± 0.020	1339.5 ±1233.5
group 4	0	0.056 ± 0.010	n.a.[c]
	3	0.022 ± 0.007	1426.3 ± 103.2
	6	0.010 ± 0.004	1009.5 ± 166.0[d]
	9	0.004 ± 0.003	1067.3 ± 644.6

[a]Lactase activity expressed in U/mg protein at 37°C. [b]Alkaline phosphatase activity expressed in U/l at 30°C. [c]Effluent samples not available on day 0. [d]$p<0.05$ versus day 3.

Effluent analysis

In the autotransplant group, albumin concentrations in secretions of the SB grafts showed stable values up to day 21 (Figure 9.2). On days 21 and 70 posttransplant however, the albumin concentrations were higher than those on day 3 ($p=0.002$ day 3 versus day 21; $p=0.032$ day 3 versus day 70).

In the allotransplant groups albumin contents in the graft effluent increased after transplantation. On day 6, group 2 showed a significantly increased albumin content effluent compared with the other three experimental groups ($p=0.003$). By day 9 effluent albumin levels in groups 3 and 4 were significantly different from the autotransplant group ($p=0.006$).

Alkaline phosphatase activities in the graft effluent did not change in group 1 during the test period (Table 9.1). In groups 2 and 3, alkaline phosphatase levels showed a decreasing trend (group 2, $p=0.08$, day 3 versus day 6); in group 4, alkaline phosphatase activity was significantly lower on day 6 than on day 3 ($p=0.048$). Differences between allotransplants and autotransplants were not significant.

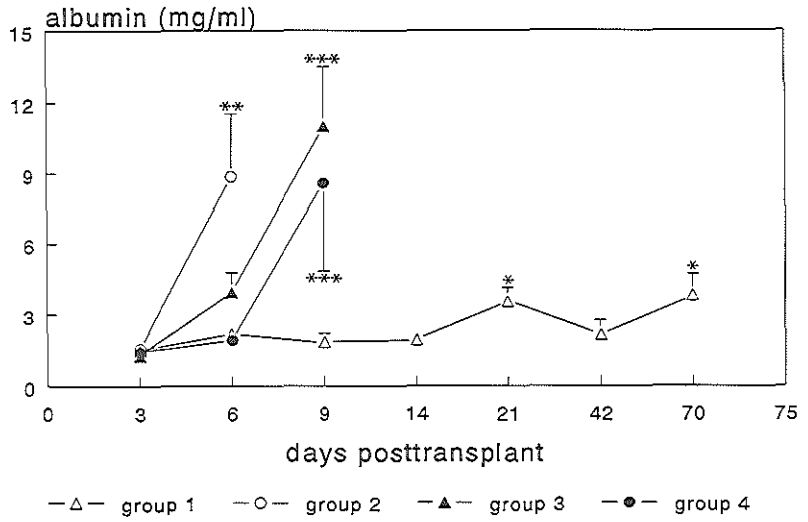

Fig. 9.2. Albumin concentration in intestinal graft effluents expressed in mg/ml (mean ± SEM). *$p<0.05$ compared with the same group at day 3. **$p<0.05$ group 2 versus groups 1, 3 and 4. ***$p<0.05$ group 1 versus groups 3 and 4.

Table 9.2. Serum albumin and serum total protein levels (mean ± SEM)

	day	albumin[a]	total protein[a]
group 1	0	37.06 ± 1.90	55.45 ± 2.63
	3	29.23 ± 0.56[b]	49.03 ± 0.73
	6	29.14 ± 1.22[b]	54.50 ± 1.39
	9	31.04 ± 2.23[b]	55.52 ± 3.27
	14	29.91 ± 1.27[b]	55.43 ± 1.50
	21	30.30 ± 1.22[b]	54.54 ± 1.89
	42	34.05 ± 2.57	56.20 ± 2.44
	70	32.46 ± 1.29	61.83 ± 2.74
group 2	0	32.05 ± 1.55	54.62 ± 1.77
	3	27.90 ± 0.75	55.85 ± 3.50
	6	27.63 ± 0.54	53.40 ± 6.26
group 3	0	38.00 ± 0.49	59.63 ± 1.88
	3	n.a.[c]	n.a.[c]
	6	n.a.[c]	n.a.[c]
	9	32.15 ± 5.85	62.85 ± 13.95
group 4	0	33.12 ± 1.15	60.06 ± 0.84
	3	n.a.[c]	n.a.[c]
	6	23.90 ± 3.46[b]	48.67 ± 4.81
	9	24.33 ± 3.34[b]	56.03 ± 4.98

[a]Results are expressed in mg/ml. [b]$p<0.05$ versus day 0. [c]Samples not available.

Serum albumin and total protein

In all groups serum albumin levels decreased after transplantation, with a significant decrease in group 1 ($p=0.013$, day 0 versus day 3) and group 4 ($p=0.034$, day 0 versus day 6)(Table 9.2). In group 1, pretransplant levels of serum albumin were reached as late as 42 days posttransplant.

Serum total protein levels remained constant in all experimental groups for the duration of the study (Table 9.2).

Morphology

Histological evaluation of the biopsies of the successful autotransplants demonstrated a reduced height of the villi with dilated lymphatics on day 3 posttransplant. After day 3 the height of the villi increased to reach a normal morphological pattern on day 10. From day 42 onwards a progressive reduction in villus height was seen concomitantly with irregular crypts suggesting mucosal atrophy. Biopsies taken simultaneously from both small bowel loops showed identical histological characteristics.

Changes comparable to those found in group 1 were present in the biopsies of the allotransplanted dogs on day 3. The histological alterations of superimposed graft rejection were found in all allotransplants of groups 2, 3 and 4, but the time of onset varied from 5 to 10 days. The earliest changes of rejection indicated by increased numbers of mononuclear cells were followed by denudation and blunting of the villi, polymorphonuclear cells in the lamina propria with cryptitis and vasculitis with endothelial swelling in the submucosa. Finally, necrosis of the mucosa and a diffuse polymorphonuclear cell infiltrate in the muscularis propria were found. The serosal layers of grafts and native intestines were covered by a dense infiltrate of polymorphonuclear cells and fibrin exsudates indicating a purulent peritonitis. The enlarged lymph nodes of the allograft mesentery showed follicular hyperplasia and sinus histiocytosis. None of the examined tissues showed signs of graft-versus-host disease (GVHD).

9.5 Discussion

Recently developed immunosuppressive agents like FK-506 and rapamycin enhance the expectations that SBT could be used to treat the short bowel syndrome (8,9). At present however, these improved immunosuppressants cannot reliably prevent rejection of experimental intestinal allografts. Clinical experience shows only a few successful intestinal transplants so far, with rejection and subsequent sepsis remaining the major reasons for most clinical failures (10,11). Moreover, in multivisceral allografting the intestinal component seems to be the 'Achilles heel' of the procedure (12).

If SBT is to become a safe solution for the short bowel syndrome, early detection

of rejection will be necessary to reverse a rejection episode. Histological monitoring alone being inadequate, several functional parameters have been studied to complement a histological evaluation (3,4). However, the significance of these functional assays is as yet not clear (1,4).

In this study we examined the usefulness of serial determinations of the brush border enzymes maltase and lactase to monitor the SB graft. The disaccharidases maltase and lactase are critical to the digestive process of the carbohydrates maltose (two molecules glucose) and lactose (one molecule galactose and one molecule glucose). Billiar showed a correlation between declined maltose absorption and the onset of intestinal rejection (13). Consequently, as maltose absorption is dependent upon brush border maltase activity, it may be expected that reduced maltase levels occur concomitantly with, or even earlier than, decreased maltose absorption.

In all allotransplant groups we found decreased maltase levels after transplantation, but the speed of decline was more pronounced in the allotransplant groups than in the autotransplant group. In the allotransplant groups 3 and 4 the onset of significantly reduced maltase levels correlated with the first histological signs of rejection. In group 2 maltase activities showed reduced values even before morphological signs of rejection were evident. In all allotransplant groups the decline in maltase activity was progressive, whereas in the autotransplant group maltase activity returned to pretransplant values after an initial, non-significant decline. The transplantation procedure itself might have been responsible for this transient decline in group 1, whereas the significant and progressive decline in the allotransplant groups may be due to a combination of operative ischaemia and immunological injury. Remarkably, in group 1 histology showed a return to normal appearance after day 10, at which time maltase activity had not reached the pretransplant level. This was probably caused by the immaturity of the mucosa, which was unable to produce normal maltase levels. Nevertheless, on day 9 maltase levels in the autotransplant group were significantly higher than those in groups 3 and 4.

Evaluation of lactase activities did not contribute to the detection of rejection. This is in contrast with the findings of Teitelbaum, who reported that in adult rats with orthotopic allogeneic jejunal grafts lactase activity might serve as a useful adjunct in the monitoring of SB transplant rejection (14). A possible explanation for these different findings is the fact that in our experiments the grafts were ileal and not jejunal in origin as in the study of Teitelbaum. There might also be a species difference; in rats recovery of the mucosa after an insult takes 36 to 72 hours, but clinical studies have shown that recovery of secondary lactase deficiency after intestinal injury takes weeks (15,16).

A positive correlation between mucosal brush border enzyme activity and enzyme activity in jejunal fluid has been described (17). In this study we analyzed the alkaline phosphatase activity in the graft effluent in an attempt to correlate this with the brush border condition. In group 4 diminished alkaline phosphatase activity in the graft effluent

preceded histological signs of rejection. However due to a considerable experimental variation a decreasing trend only could be detected in groups 2 and 3. Consequently, the significance of alkaline phosphatase levels in the effluent to monitor the intestinal graft remains uncertain.

Recently, the intestine has been demonstrated to be an important source of sepsis in case of bacterial overgrowth (18). Absorption of bacterial toxins and bacterial translocation may cause such sepsis during episodes of increased intestinal permeability. Grant found intestinal permeability to be increased during rejection of heterotopic SB transplants as well (19). Additionally, permeability testing with ^{51}Cr-EDTA was promoted as a sensitive method to monitor the intestinal graft for early rejection. We evaluated the integrity of the intestinal mucosal barrier after transplantation by determination of albumin contents in the graft effluent. Acutely raised albumin contents were detectable in group 2 on day 6 and in groups 3 and 4 on day 9, whereas in the meantime low and stable albumin contents were found in group 1. These findings emphasize that in our experimental model albumin-losing enteropathy is not useful to detect early phases of rejection. However, it underscores that the mucosal barrier is highly permeable during a rejection episode and may thus contribute to the development of sepsis. In this respect decontamination of the gut by antibiotics may possibly play an important role in preventing septic episodes.

Our findings are in accordance with the results of Collin, who also described a protein-losing enteropathy in heterotopic canine ileal allografts (20). However, they noted that plasma albumin levels in the allografts were lower than in the autografts and supposed that this hypalbuminaemia was caused by large volumes of protein contents lost from the allografts. In contrast, we found low plasma albumin levels in both allografted and autografted dogs. In our opinion the hypalbuminaemia is a consequence of reduced food intake rather than a result of protein-losing enteropathy. This explains the slowly recovering plasma albumin levels in the autotransplanted dogs by day 42, after they had regained their normal eating habits.

In summary, serial determinations of brush border maltase activity in conjunction with histological parameters can detect early phases of SB allograft rejection. During a rejection episode the mucosal barrier is compromised, which may possibly result in bacterial translocation and sepsis.

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CHAPTER 10

LONG-TERM SURVIVAL OF DLA-MATCHED SEGMENTAL SMALL BOWEL ALLOGRAFTS IN DOGS

10.1 Abstract

The aim of this study was to investigate the combined effect of DLA matching and immunosuppressive therapy on the survival of segmental small bowel transplantation (SBT) in dogs. Orthotopic segmental SBTs (25 to 30% of total small bowel (SB) length) were performed in two stages: first a heterotopic segmental SBT, followed after 5 to 8 weeks by a second stage operation during which the heterotopic graft was placed in an orthotopic position and the native SB was resected. All dogs received Cyclosporine A immunosuppression. Control dogs (n=4), subjected to total enterectomy, survived 37.3 $\pm$ 7.1 days (mean $\pm$ SEM). Recipients of DLA-mismatched small bowel grafts (n=6) survived 113.2 $\pm$ 37.0 days, which was significantly shorter than dogs with a DLA-matched graft (n=6, 211.5 $\pm$ 38.8 days, $p<0.05$). None of the matched allografts was rejected during CsA treatment, whereas four of six mismatched grafts were ($p<0.05$). The control dogs uniformly showed progressive weight loss, steatorrhoea and hypalbuminaemia. The dogs with DLA-mismatched grafts did not regain initial body weight, whereas animals with DLA-matched grafts recovered preoperative weight after 20 weeks. Both transplanted groups showed near normal faecal fat excretions and constant serum albumin, cholesterol and triglyceride levels, whereas serum total protein levels increased during follow-up. We conclude that segmental SBT between DLA-matched donor-recipient pairs results in long-term survivors with an adequate nutritional status. This may have important implications for future living-related SBT.

This chapter is an adaptation of an original article accepted for publication in Transplantation.

10.2 Introduction

Initial clinical experience with simultaneous SB/liver transplantation and the development of improved immunosuppressants such as FK-506 and rapamycin enhance the expectations that SBT could be used to treat the short bowel syndrome (SBS)(1-3). However, rejection with subsequent sepsis, and development of lymphoproliferative disorders remain the major reasons for most clinical failures (4,5). Moreover, in multivisceral allografting the intestinal transplant seems to be the most immunogenic component of the procedure (6).

These findings underline that SB allograft immunogenicity is a powerful stimulus for rejection (7,8). This means that methods combining reduction of intestinal graft antigenicity with improved immunosuppressive therapy may offer prospects for progress. This combination could possibly allow for reduction of immunosuppressive therapy and thus decrease the attendant risk of infectious complications and lymphoproliferative malignancies.

A strategy to minimize graft antigenicity is selecting a donor-recipient combination by matching for major histocompatibility complex (MHC) antigens (9-12). Recently, in a non-immunosuppressed heterotopic canine SB transplant model, we demonstrated that matching for canine MHC antigens (DLA) results in prolonged survival times of intestinal grafts in proportion to the degree of histocompatibility between donor and recipient (13).

The present study was undertaken to investigate the combined effect of DLA matching and immunosuppressive therapy on the survival of canine SB grafts. Regarding a potential role for living-related SB transplantations, in which only a partial resection of the donor SB is possible, we also studied functional aspects of segmental allografting.

10.3 Materials and methods

Animals and experimental groups

In total 24 healthy adult Beagle dogs (Harlan, Zeist, the Netherlands) weighing 10-20 kg were used. We created 3 experimental groups: group 1 (n=4), short bowel controls in which a total enterectomy was performed; group 2 (n=12), fully DLA-mismatched orthotopic segmental ileal allografts; group 3 (n=8), fully DLA-matched orthotopic segmental ileal allografts. Only non-littermate donor-recipient pairs were selected (Table 10.1). Transplantations were performed by exchanging a SB graft between dogs of selected donor-recipient pairs as described in chapter 4.

Postoperative treatment

Cyclosporine A (CsA, Sandimmune, Sandoz, Basel, Switzerland) was dissolved in olive

oil. From one day before operation until the end of the first postoperative week all animals received 15 mg/kg/day CsA intramuscularly, thereafter 30 mg/kg/day CsA orally. From postoperative day 200 onwards CsA treatment was gradually tapered off and stopped between days 217 and 267.

Table 10.1. DLA typing and donor-recipient pairs of successfully transplanted dogs

	DLA-A/B/C (class I antigens)		
group	recipient	donor	MLR[1]
group 2	1,2/5,13/11	2,9/4,6/11,12	+
	2,3/4/11	1,9/4,13/12	+
	1,9/4,13/12	2,3/4/11	+
	7,9/4/12	1,9/6,13/12	+
	2,3/5,10/11	2,9/4,6/11,12	+
	2,9/4,6/11,12	2,3/5,10/11,12	+
group 3	2,7/4/11	2,7/4/11	-
	2/4/11	2/4/11	-
	2,9/4/11,12	2,9/4/11,12	-
	2,9/4/11,12	2,9/4/11,12	-
	2,7/4/11	2,7/4/11	-
	2,7/4/11	2,7/4/11	-

[1]Identity for class II antigens was based on the absence (-) of significant stimulation in mixed lymphocyte reactions (MLR) as compared with autologous controls.

Postoperative monitoring

All animals were weighed once a week. At regular intervals blood samples were drawn, in which plasma trough levels of CsA were measured using the cyclo-Trac SP radioimmunoassay kit (Incstar Corporation, Stillwater, Minnesota, USA). Faecal fat excretion was determined using the method of Van de Kamer (17). Serum total protein and albumin levels were measured by means of the Biuret method and the bromcresol-green method (Boehringer Mannheim GmbH) respectively. Serum triglyceride levels were determined colorimetrically using the triglycerides GPO PAP system (Boehringer Mannheim GmbH). The Monotest cholesterol (Boehringer Mannheim GmbH) was used to measure serum cholesterol levels.

Morphology

During both operations full-thickness biopsies were taken from native and grafted SB and at regular intervals mucosal biopsies were obtained by forceps from a cutaneous stoma. At necropsy full-thickness biopsies were taken from the SB graft, the recipient's native SB, liver, lung, spleen, vascular anastomosis, mesenteric lymph nodes and skin were

taken. Biopsies were prepared as described in chapter 4.

Statistics

All data are expressed as means ± the standard error of the mean (SEM). Survival data were analysed using the Wilcoxon rank sum test. Statistical comparisons between the experimental groups of weights, faecal fat contents and biochemical parameters were performed using one-way analysis of variance followed by the Student-Newman-Keuls test. Comparisons within a group were performed using paired Student's *t* tests. For all data $p<0.05$ was considered significant.

10.4 Results

Postoperative course

Seven of the 24 dogs (none in group 1, six dogs in group 2 and one in group 3) died of technical failures prior to the sixth postoperative day: 5 dogs revealed a thrombus formation at either the arterial or venous anastomosis, one dog had an insufficient intestinal anastomosis and one dog showed a large hematoma in the abdomen. At day 14 posttransplant one dog of group 3 died of a perforation in the SB graft caused by taking a mucosal biopsy with the forceps. Histological examination showed no signs of rejection or graft-versus-host disease (GVHD) in the former dogs. These 8 animals were excluded from further analysis.

Survival

Survival rates of the 16 successfully operated animals and causes of death are listed in Table 10.2.

All four dogs of group 1, in which a near total SB resection was performed, showed persistent diarrhoea and weight loss. Eventually all these animals were killed because of progressive weight loss of more than 30% between postoperative days 28 and 58 (survival 37.3 ± 7.1 days, mean ± SEM). At necropsy no abscesses, obstructions or perforations were found.

The mean survival in group 2 was 113.2 ± 37.0 days (range 28 to 272 days, $p<0.05$ vs group 1). Four of the six dogs had to be killed because of a deteriorating condition marked by loss of appetite, diarrhoea and weight loss. Autopsy showed intestinal grafts with a thickened and friable SB wall. Histopathological examination showed rejection of the SB grafts with a diffuse mono- and polymorphonuclear cell infiltrate, transmural intestinal necrosis and endovasculitis in the submucosa and muscularis propria. At posttransplant day 162 one dog had to be killed because of diffuse wart formation. Microscopic examination did not reveal signs of rejection of the SB graft.

Only one dog of group 2 survived the 200-day period after which CsA therapy was tapered off. After stopping CsA therapy at day 250 the general condition deteriorated and the dog was sacrificed at day 272. Histologic examination of the SB grafts showed chronic rejection of the SB graft characterized by a diminished villus length and a mononuclear cell infiltrate in the muscularis propria. No signs of GVHD were noted.

Dogs of group 3 survived 211.5 $\pm$ 38.8 days (range 99 to 361 days, $p<0.05$ vs groups 1 and 2). During CsA treatment none of the animals suffered from rejection ($p<0.05$ vs group 2, chi-square test). Three animals were killed at days 99, 145 and 161 because of herpes infection, perforation of the native terminal ileum and diffuse wart formation respectively. The SB grafts of these three dogs showed no rejection. Three dogs survived the 200-day CsA treatment period; after stopping CsA therapy at days 217, 217 and 267 these dogs showed progressive weight loss and were sacrificed at posttransplant days 248, 255 and 361 respectively. After autopsy microscopic examination showed evidence of chronic rejection in the SB transplants. No signs of GVHD were seen.

Table 10.2. Survival rates and causes of death

group	survival (days)	cause of death
group 1	28	weight loss > 30%
	28	weight loss > 30%
	35	weight loss > 30%
	58	weight loss > 30%
group 2	28	acute rejection
	56	acute rejection
	62	acute rejection
	99	acute rejection
	162	diffuse wart formation
	272[1]	chronic rejection
group 3	99	herpes infection
	145	perforation host's ileum
	161	diffuse wart formation
	248[1]	chronic rejection
	255[1]	chronic rejection
	361[1]	chronic rejection

[1]Cyclosporine A therapy was tapered off after day 200.

Weights

All control dogs progressively lost weight, significantly more than both transplanted groups (Figure 10.1). Eventually they lost more than 30% of their preoperative weight.

Dogs of groups 2 and 3 showed prolonged weight loss. Animals of group 2 did not regain their preoperative weight: 16 weeks after orthotopic placement of the graft body weight averaged 91.0 ± 3.0% (mean ± SEM) of initial body weight. The surviving animals of group 3 regained their preoperative body weights only from 20 weeks posttransplant onwards.

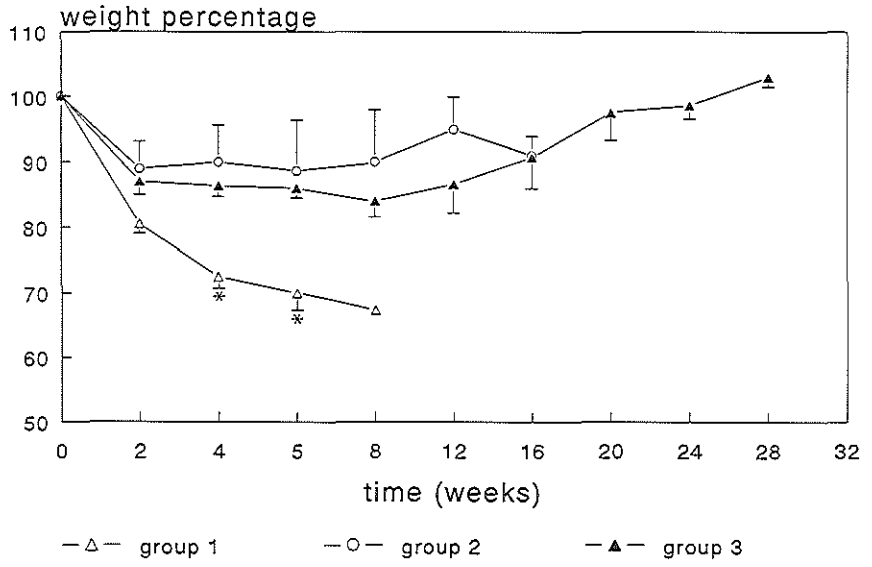

Fig.10.1. Body weight percentages after performing enterectomy or orthotopic small bowel transplantation at 0 weeks. *p<0.05 group 1 compared with groups 2 and 3.

Serum parameters

During the experimental period serum albumin levels significantly decreased in group 1 (p=0.001 week 5 versus week 0, Table 10.3). Serum albumin levels remained constant in group 3 during the experimental period. but in group 2 eventually decreased (p=0.019 week 15 versus week 0). At 5 weeks postoperatively serum albumin levels were significantly reduced in group 1 as compared with group 3 (p=0.001). In contrast, serum total protein levels showed increasing values in all groups during follow-up (group 2, p=0.044 week 15 versus week 0; group 3, p=0.021 week 15 versus week 0). Serum cholesterol and triglyceride levels were not different between or within the experimental groups during the experimental period.

Table 10.3. Serum total protein, albumin, cholesterol and triglyceride levels (mean ± SEM)

group	week	total protein (g/l)	albumin (g/l)	cholesterol (mmol/l)	triglyceride (mmol/l)
group 1	0	61.10 ± 2.46	33.25 ± 1.11	3.26 ± 0.30	0.31 ± 0.06
	5	73.48 ± 4.69	24.03 ± 0.95[1,2]	2.86 ± 0.78	0.38 ± 0.05
group 2	0	63.68 ± 3.11	32.00 ± 0.74	3.45 ± 0.23	0.33 ± 0.04
	5	67.30 ± 4.10	29.05 ± 4.05	3.28 ± 0.47	0.47 ± 0.22
	10	71.30 ± 5.30	28.40 ± 3.40	2.81 ± 0.22	0.48 ± 0.02
	15	81.25 ± 8.98[1]	21.90 ± 3.90[1]	2.34 ± 0.29	0.73 ± 0.24
group 3	0	59.32 ± 2.18	31.72 ± 0.65	3.10 ± 0.25	0.36 ± 0.10
	5	64.13 ± 3.16	32.43 ± 1.12[2]	3.11 ± 0.25	0.56 ± 0.07
	10	64.35 ± 1.36	31.92 ± 0.78	3.07 ± 0.17	0.66 ± 0.04
	15	72.15 ± 4.49[1]	28.50 ± 1.57	3.36 ± 0.21	0.61 ± 0.04

[1]$p<0.05$ versus preoperative values (week 0) in the same group. [2]$p<0.05$ group 1 versus group 3.

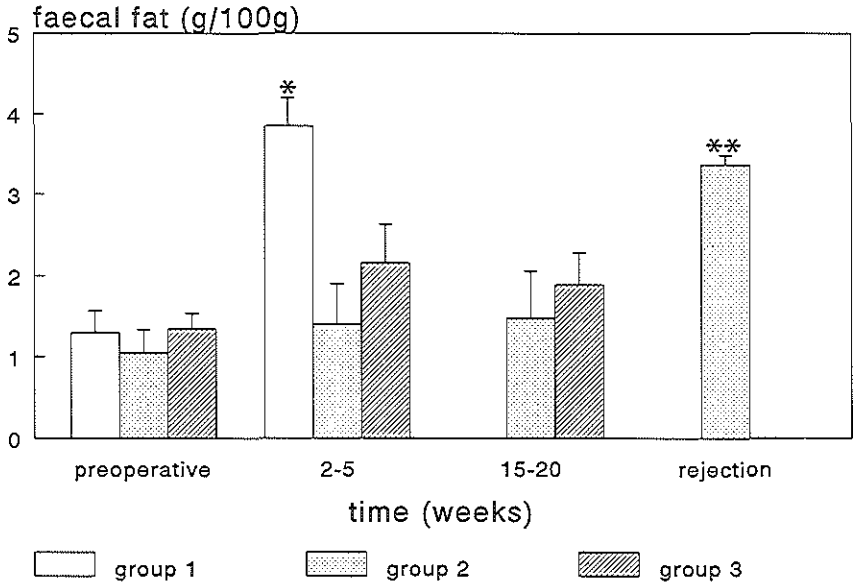

Fig.10.2. Faecal fat excretions before and after performing enterectomy or orthotopic small bowel transplantation at 0 weeks. *$p<0.05$ group 1 compared with groups 2 and 3. **$p<0.05$ rejecting grafts of group 2 compared with non-rejecting grafts of the same group.

Faecal fat excretion

Two to five weeks posttransplant faecal fat excretion in the control group was significantly higher than in groups 2 and 3 ($p<0.05$, Figure 10.2). Although both transplanted groups showed slightly increased faecal fat excretions, these differences were not statistically significant compared with preoperative values. Figure 10.2 also showes the elevated faecal fat excretions from three dogs of group 2 with a rejecting SB graft.

CsA levels

Plasma trough levels of CsA showed considerable variations (Figure 10.3). However, in the control group CsA levels showed decreasing values ($p=0.041$ week 5 versus week 2). At 4 weeks postoperatively CsA levels of group 3 were significantly higher as compared with CsA levels of groups 1 and 2. Thereafter groups 2 and 3 showed comparable CsA levels.

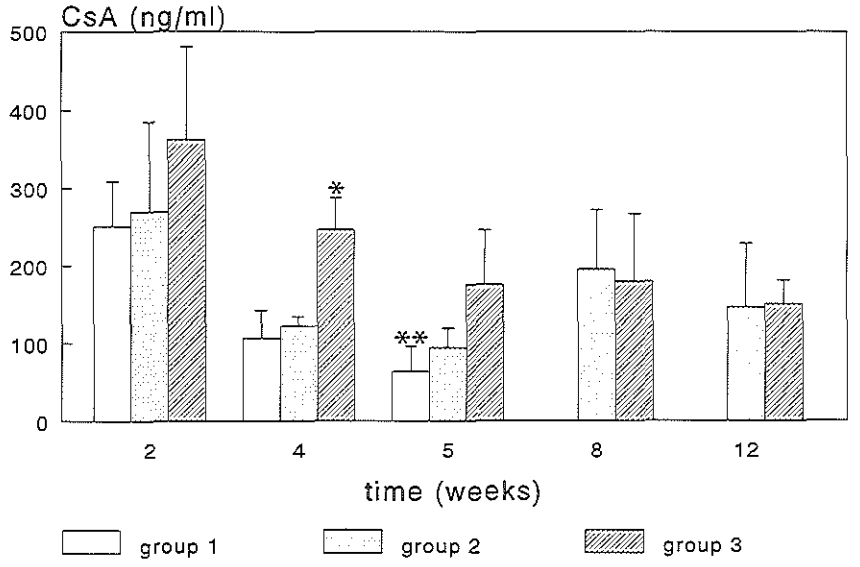

Fig.10.3. Cyclosporine A levels after performing enterectomy or orthotopic small bowel transplantation at 0 weeks. *$p<0.05$ group 3 compared with groups 1 and 2. **$p<0.05$ compared with the same group at 2 weeks after enterectomy.

10.5 Discussion

Intestinal transplantation is expected to be the ultimate therapeutic treatment for adult patients with irreversible intestinal failure (18,19). Indeed, recent clinical and

experimental experiences with SBT are promising (1-3,20). However, rejection, GVHD, infectious complications and lymphoproliferative disorders remain major problems to be solved (4-6).

Recently, we showed that DLA matching results in prolonged survival rates of intestinal grafts in a non-immunosuppressed heterotopic canine SB transplantation model: 5.5 versus 9.6 days, fully DLA-mismatched versus fully DLA-matched respectively (13). In the present study we demonstrated that long-term survival of canine SB transplants can de achieved using fully DLA-matched donor-recipient combinations and CsA immunosuppression. Although CsA considerably prolonged graft survival, DLA matching resulted in significantly longer survival rates compared to the DLA-mismatched donor-recipient pairs. In addition, during the CsA treatment period none of the matched allografts was rejected, whereas in the mismatched group four of six dogs suffered from graft rejection. Interestingly, after stopping CsA the matched animals survived 31, 38 and 94 days respectively, whereas the only surviving mismatched dog survived 22 days. Although not statistically proven, this again suggests a positive influence of DLA matching on SB allograft survival. These observations confirm that class I and class II MHC antigens strongly influence SB graft immunogenicity and immune response (21). Moreover, our study corroborates recent clinical studies in which a beneficial effect of HLA matching on renal and cardiac allograft survival is proven (9-12). Our findings are also in accordance with the observation of Deltz et al, who reported about a successful clinical case of SBT using a HLA-matched donor-recipient combination (22).

From an immunological point of view living-related SBT using HLA-identical siblings would be the ideal situation, in which major as well as minor histocompatibility complex matching can be accomplished. However, living-related SB grafting is only meaningful if segmental transplants are functionally competent to maintain a normal nutritional status of the recipient.

In this study we show that segmental transplantation of 25 to 30% of total SB length is able to overcome the symptoms of a surgically created lethal short bowel syndrome in dogs. The transplanted animals showed reversible weight loss, whereas all control dogs progressively lost weight. This has also been reported in rat studies using a short bowel control group (23,24). In our study, however, the surviving dogs of the matched group regained their initial body weight only 20 weeks after orthotopically positioning of the SB graft. Prolonged weight loss was not seen by Diliz-Perez et al in their long-term surviving dogs after transplantation of the entire SB (25). This suggests that the segmental grafts in our study initially had a limited absorptive surface, structurally as well as functionally. Probably an adaptation process took place as documented in a pig study by Kimura et al, in which the recipients of segmental jejunal allografts regained their preoperative weight by day 50 posttransplant (26). The longer period of weight loss in our study may reflect the less pronounced adaptive capacity of

adult canine SB compared to the intestine of growing pigs.

Segmental SBT resulted in normal serum albumin levels as has been reported by others in rat studies (27,28). However, 15 weeks after transplantation the mismatched group showed decreased albumin levels, which could be attributed to chronic rejection. Unlike the transplanted groups, the controls developed hypalbuminaemia within 5 weeks, again showing intestinal failure after enterectomy. Remarkably, the transplanted groups showed increasing serum total protein levels. As serum albumin remained constant this rise of total protein is probably caused by an increase in acute phase proteins or serum globulins. This phenomenon has also been reported by Raju, who demonstrated increased serum globulin after total SB autotransplantation in dogs (29).

The control dogs developed steatorrhoea, whereas the transplanted dogs showed near-normal faecal fat excretions. This is in parallel with the normal serum cholesterol and triglyceride levels in our transplanted animals. This means that even short segments of transplanted ileum can result in physiological fat absorption. This is in contrast with the results of Diliz-Perez, who found increased faecal fat excretion after total SB transplantation compared to faecal fat excretion in normal control dogs (25). However, in the latter study the control dogs did not receive CsA, which by itself or as a result of the oily vehicle can induce decreased fat absorption (30). The dogs of the mismatched group with a rejecting graft showed steatorrhoea and developed a trend towards decreased serum cholesterol levels, demonstrating the deleterious effect of rejection on the fat absorptive capacity, and thus CsA absorption, of SB grafts.

Oral CsA is absorbed through the small bowel (31). However, at present no consensus exists whether oral CsA treatment will result in predictable CsA tissue levels in the early posttransplant period (28,31-33). To circumvent inadequate CsA levels we treated all animals with CsA intramuscularly from one day preoperative until the end of the first postoperative week. This regimen resulted in comparable plasma CsA levels in all three experimental groups. When oral CsA treatment was instituted plasma levels in the short bowel control group diminished uniformly, which underlines that the SB is the site of CsA absorption (31). At 4 weeks posttransplant reduced CsA levels were found in the mismatched group, probably caused by graft rejection in several dogs. However, in the matched group, and from 6 weeks onwards in the mismatched group, the segmental SB grafts were sufficiently capable of absorbing CsA to maintain plasma CsA levels of about 200 ng/ml.

The local and systemic side-effects of CsA treatment remain points of concern (34,35). In the present study four dogs probably suffered from CsA side-effects: two dogs developed diffuse wart formation, one showed intracellular inclusion bodies suggesting herpes infection, and the fourth dog had a perforation of the native terminal ileum. Recent clinical SBT studies report frequent bacterial, fungal and CMV infections and a high rate of lymphoproliferative disorders caused by vigorous immunosuppression (4,5). It remains

to be determined whether reducing immunosuppressive treatment will result in less toxic effects without inducing graft rejection or GVHD.

In conclusion, these data suggest that it is possible to achieve long-term survival of canine SB grafts using DLA-matched donor-recipient pairs. Additionally, segmental ileal allografts can serve as effective substitutes for resected SB and maintain an adequate nutritional status of recipient dogs. This may have important implications for clinical SB transplantation in which the use of living-related segmental SB grafts leads to better HLA matching; this could reduce the need for potential toxic immunosuppression with improved long-term results.

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PART D

GENERAL DISCUSSION AND SUMMARY

CHAPTER 11

GENERAL DISCUSSION

11.1 Introduction

Several congenital disorders and extensive small bowel resection may cause a short bowel syndrome (SBS), a condition in which one is unable to adequately digest and absorb food, water, minerals, and vitamins (1,2, Chapter 1). At present the treatment of choice is total parenteral nutrition (TPN) during a short or longer period (3). Unfortunately, about one adult per million per year develops irreversible intestinal failure and remains permanently dependent on TPN (4). Long-term TPN has several complications which affect length and quality of life: catheter-related, related to inadequate or inappropriate nutrient provision, and metabolic (particularly in liver and bone). Obviously, small bowel transplantation would be the only definite treatment for patients with irreversible intestinal failure and those with intolerance to long-term TPN (5). Although the small bowel was one of the first organs to be transplanted experimentally, it is the last to be engrafted successfully in humans (6). Recent clinical experiences are promising, but several hurdles remain to be taken, the most demanding of which involve infectious complications, graft rejection, and lymphoproliferative disorders caused by immunosuppressive therapy (7-10, Chapter 2).

The studies described in this thesis were performed to elucidate various immunological and functional aspects of small bowel transplantation in a pre-clinical animal model.

11.2 Immunological obstacles

Of the vascularized organs the small bowel is the most difficult to transplant. This is partly due to the fact that the small intestine contains large amounts of lymphoid cells in mucosa, lamina propria, Peyer's patches and mesenteric lymph nodes. It is not surprising, therefore, that the small bowel is highly immunogenic, so that transplantation will lead to a vigorous rejection reaction. In addition, immunoreactive lymphoid cells of the graft may give rise to a graft-versus-host reaction. It has been suggested that in rats a certain immunological balance exists between rejection (host-versus-graft) and graft-versus-host-disease (GVHD)(11-15). In large animals and man the problem of GVHD and this immunological balance are less pronounced; it is believed that graft rejection predominates (16). Small bowel graft rejection could have a catastrophic outcome, compromising the mucosal barrier (17,18). Immunosuppressive treatment of the host also

compromises intestinal integrity. This underscores that the window for therapeutic immunosuppression may be narrow compared to that of other organs. On the one hand, too little immunosuppression will lead to rejection, with increased intestinal permeability and absorption of toxins. GVHD may also further compromise the host's defenses. On the other hand, excessive immunosuppression will obviously also compromise the host's immune function, with the attendant risk of sepsis. Moreover, the transplantation procedure itself will alter the bacterial flora and increase the risk of bacterial translocation and bacteraemia when immunosuppression is added (19-21). A final disadvantage of high-dose immunosuppressive therapy is the development of lymphoproliferative disorders (10,22,23).

In an attempt to reduce the degree of immunosuppression, we evaluated the effect of minimizing graft antigenicity by selecting donor-recipient combinations after tissue typing for antigens of the canine MHC, in other words DLA matching. This approach will selectively suppress the rejection reaction against tissue antigens without altering the immune status of the recipient. The effectiveness of tissue typing has been proven in experimental and clinical renal and cardiac transplantation (24-31). Remarkably, the effect of MHC matching has been investigated rarely in small bowel transplantation. Two clinical experiences provide indirect evidence for a positive effect of tissue typing (32,33). In the pre-CsA period the longest surviving human intestinal graft came from a HLA-identical donor (32). Moreover, the first successful human intestinal transplantation was performed in a HLA-matched donor-recipient combination (33). In the early 1970s Westbroek demonstrated the beneficial influence of DLA matching on the survival of heterotopic small bowel allografts in the neck without using immunosuppression (34). We also showed that DLA matching results in prolonged survival rates of non-immunosuppressed small bowel grafts; while DLA-mismatched grafts survived a mean of 5.5 days, DLA matching resulted in a mean survival of 9.6 days (Chapter 7). However, these results contrast unfavourably with results obtained in canine renal and cardiac transplantation, which emphasizes the impact of the immunogenicity of the small bowel allograft (24,25).

Combining DLA matching with CsA immunosuppression resulted in long-term intestinal graft survival (Chapter 10). Although CsA on its own considerably prolonged graft survival, DLA matching resulted in significantly longer survival rates compared to the DLA-mismatched donor-recipient pairs; DLA-matched grafts survived a mean of 211.5 days, whereas DLA-mismatched grafts survived a mean of 113.2 days. In addition, during the CsA treatment period none of the matched allografts was rejected, whereas in the mismatched group four of six dogs suffered from graft rejection. These observations confirm that class I and class II MHC antigens strongly influence intestinal graft immunogenicity and immune response. Our findings suggest that it is possible to achieve long-term survival of small bowel grafts using MHC-matched donor-recipient pairs. From

an immunological point of view our findings may have important implications for future intestinal transplantations using living-relatives, which may enable better HLA matching.

Using a slight adaptation of the immunosuppressive regimen reported by Grant and Kaneko, we achieved long-term survival, but were confronted with CsA-related side-effects: two dogs developed diffuse wart formation, a third showed intracellular inclusion bodies in the liver suggestive of a viral infection (35,36, Chapter 10). In transplantation medicine in general, the local and systemic side-effects of CsA are points of concern (37-39). Therefore, it remains to be determined whether less CsA or other immuno-suppressants will have less toxic effects without inducing intestinal graft rejection or GVHD. A recent study demonstrated the immunosuppressive effect of low-dose CsA combined with prostaglandin E_2 without toxic side-effects (40).

11.3 Functional obstacles

The small bowel has the following functions: it provides intestinal motility, it governs hormonal, immunological, and nutritional aspects, and it forms an intestinal barrier. As a consequence of the transplantation procedure the small bowel graft faces several physiological obstacles: ischaemic and reperfusion injury, intrinsic and extrinsic denervation, and disruption of the lymphatic drainage. These factors may adversely influence the intestinal functions. Immunological reactions may further compromise small bowel functioning. Although it is known that lymphatics are re-established within several weeks, less is known about the effects of harvest injury on small bowel graft function, or whether regeneration of neuronal continuity or adaptation to chronic denervation takes place (41,42).

By means of electrophysiological parameters we found indirect evidence that extrinsic denervation leads to increased secretory activity of the crypts in a heterotopic autotransplant model without immunological phenomena (Chapter 5). The observed luminal negative base-line potential difference (PD) responses may have been due to net chloride secretion by crypts no longer controlled by the autonomic nervous system, as suggested by Watson (43). This increased secretory activity explains the watery diarrhoea found early after intestinal transplantations. In our experiments the base-line PD response was normalized after 70 days, which could be due to either an adaptation process or regeneration of neuronal continuity. However, it is more likely that this 'normalization' was the result of mucosal atrophy of the non-functioning heterotopic graft, because both theophylline- and glucose-stimulated PD responses were diminished from day 21 onwards. Mucosal atrophy was proven by histology. Interestingly, it was more advanced in the autotransplants than in the control dogs, which suggests that the degenerative changes of the intestinal mucosa are accelerated by extrinsic denervation. Therefore, the long-term

results of our heterotopic autotransplant model do not indicate whether regeneration of nerves or adaptation takes place. Unfortunately, this disuse atrophy shows a shortcoming of our heterotopic transplant model: it cannot be used to monitor functional or immunological aspects of long-term surviving small bowel grafts. To circumvent these problems in future experiments, an orthotopic small bowel transplantion with a Bishop-Koop anastomosis should be performed; this gives the advantage of an easily accessible, cutaneous stoma through which the orthotopic graft can be monitored.

In Chapter 9 we evaluated the mucosal integrity of acute rejecting intestinal grafts by analyzing albumin contents in the graft effluent. We found a protein-losing enteropathy corresponding with rejection, which underscores that the mucosal barrier is highly permeable during a rejection episode. Grant demonstrated that rejection of an intestinal graft leads to increased intestinal permeability to ^{51}Cr-EDTA and to increased bacterial translocation (18,45). It has also been shown that the transplantation procedure itself results in Gram-negative bacterial overgrowth, which may be even more critical after addition of CsA (19-21). These factors create an important source of infectious complications after small bowel transplantation, even more pronounced during a rejection episode (46). Therefore, strategies should be designed to enhance the mucosal barrier of the intestinal graft. Selective gut decontamination, early enteral nutrition, and modified TPN could be important to achieve this aim. In this context the amino acid glutamine supplemented to enteral or parenteral nutrition may prove its value (47,48).

From a functional point of view it only makes sense to perform living-related small bowel transplantations if a segmental intestinal graft is able to maintain a normal nutritional status of an adult host, or normal growth and development of a child. In addition, a segmental graft may be less immunogenic than a total small bowel graft. This has been shown in a rat study by Kimura, who suggested a direct relationship between the severity of rejection and the amount of transplanted small bowel (43). In Chapter 10 we showed that long-term surviving dogs with a DLA-matched, orthotopic segmental ileal graft (25 to 30% of total small bowel length) regained their initial body weight after 20 weeks. Dogs with a surgically created SBS showed progressive weight loss. Furthermore, dogs with a segmental graft had normal serum albumin levels, near-normal faecal fat excretions, and sufficient CsA absorption. In contrast, dogs with a SBS showed hypalbuminaemia, steatorrhoea, and decreased CsA absorption. These findings indicate that a small bowel graft comprising only a quarter to a third of the total small bowel may result in adequate nutritional parameters of the host. Our results can be partly explained by an adaptation process of the transplanted small bowel, as has been described in a rat and a pig study (36,44). It remains to be determined whether segmental intestinal grafting will allow for normal growth and development in immature dogs.

11.4 Graft monitoring

Monitoring a small bowel graft is of cardinal importance to prevent rejection and its complications. As histological monitoring alone has several disadvantages (sample error, risk of perforation of the graft, inaccessibility of an orthotopic graft), many investigators have sought for an ideal functional test or biochemical marker to detect a rejection episode.

Madara investigated the effect of rejection on the electrophysiological function of small bowel allografts in a rat model by in vitro techniques (49). He found that decreased electrophysiological responses to glucose and theophylline stimulation correlate with rejection. In Chapter 5 we describe an in vivo method to evaluate electrophysiological parameters of intestinal autotransplant mucosa. Serial monitoring of electrophysiological parameters proved to be a valid, non-invasive method to detect intestinal allograft rejection (Chapter 6). Unfortunately, we were not able to use this method to monitor the function of long-term surviving grafts, because disuse atrophy in the heterotopic grafts led to diminished electrophysiological responses.

In Chapter 8 we examined the value of the biochemical marker N-acetyl hexosaminidase (NAH) to detect canine intestinal graft rejection, a marker which had shown to be useful in a rat study (50). We cannot recommend serum NAH activity as an early marker of canine intestinal allograft rejection, because substantial ischaemic damage due to rejection had already occurred before NAH levels rose.

Billiar showed a correlation between declined maltose digestion by maltase (and subsequently reduced glucose absorption) and the onset of intestinal rejection (51). In Chapter 9 we demonstrated that diminished brush border maltase activity in mucosal biopsies coincides with the first histological signs of rejection.

In conclusion, using functional tests we were not able to predict a rejection episode, but monitoring of in vivo electrophysiological measurements and maltase activity in mucosal biopsies proved valuable adjuncts to histological monitoring.

11.5 Future directions

Future small bowel transplantation research should be directed to a better understanding and prevention of acute and chronic rejection. The induction of specific tolerance or improved (combinations of) immunosuppressive agents may be a conditio sine qua non for the success of clinical small bowel transplantation. MHC matching may be invaluable to reduce the vigorous immunosuppressive regimen. In addition, MHC matching may pave the way for living-related intestinal transplantation.

An important issue is the proposed protecting effect of a liver graft in conjunction

with a small bowel graft. Although experimental studies and one clinical case showed the superiority of combined small bowel/liver transplantation, this effect could not be confirmed in the first reported series of intestinal transplantation, either in composite visceral grafts or alone (7,52,53). The convalescence of the eight patients receiving an intestinal graft alone was more trouble free than after small bowel/liver or multivisceral transplantation, with no greater difficulty in control of rejection (54). This would reserve small bowel/liver transplantation for specific indications, such as coexisting liver failure from TPN or a hepatic inborn error of metabolism. However, it is too early to predict the function of the chronically tolerated intestinal graft, whether transplanted alone or as part of an organ complex.

Special attention should be focused on cell migration, chimerism, and small bowel graft acceptance. Starzl found replacement of donor lymphoid tissue in small bowel transplants (55). He hypothesized that donor lymphoid cells migrate to host lymphoid tissues, creating a state of mixed allogeneic chimerism (56). Migration of dendritic and lymphoid cells may be associated with graft acceptance rather than rejection, depending on the quality of immunosuppression, the immunological substrate of the organs, donor-recipient histocompatibility, and even other factors (56).

New avenues to prevent infectious complications and to enhance the mucosal barrier should be entered. In this context selective gut decontamination, early enteral feeding, and modified TPN may be important tools. Potential beneficial effects of glutamine, short-chain fatty acids, and polyamines added to enteral or parenteral nutrition should be investigated.

Recent clinical small bowel transplantations should be critically evaluated. Thus, extending our experimental knowledge with insights derived from clinical experiences will eventually result in safe clinical small bowel transplantations from both immunological and functional points of view.

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SUMMARY

Chapter 1

In this chapter the problem of the short bowel syndrome (SBS) is delineated. The SBS presents when the length of the small intestine remaining after massive small bowel resection is insufficient for adequate digestion and absorption of food, water, minerals, and vitamins. It is manifested by diarrhoea, steatorrhoea, malabsorption, and weight loss. In neonates and children, congenital disorders and necrotizing enterocolitis are mainly responsible for SBS. In adults, intestinal resections following intestinal vascular insults and inflammatory bowel disease may lead to SBS. The severity of SBS is influenced by the extent and site of resection, the presence or absence of an ileocaecal valve, and the degree of adaptation of the remaining small bowel and colon. Total parenteral nutrition (TPN) is the treatment of choice early and sometimes late in the course of SBS. Enteral nutrition is started as soon as possible to maintain the intestinal integrity, to stimulate intestinal adaptation, and to minimize bacterial translocation. Approximately one adult per million per year turns out to have irreversible intestinal failure and remains permanently dependent on TPN. Long-term use of TPN can cause complications, such as catheter-related bacteraemia and sepsis, metabolic complications, and hepatic abnormalities ranging from asymptomatic cholelithiasis to liver insufficiency.

Chapter 2

A review is given of the history and present state of experimental and clinical small bowel transplantation, which would be the only causal therapy for patients with irreversible intestinal failure. The intestine was one of the first organs to be transplanted experimentally, but the last to be engrafted successfully in humans. Two major obstacles still hamper clinical small bowel transplantation as an established therapy. First, the large amount of transplanted lymphoid tissue results in a highly immunogenic graft leading to rejection, and alloreactive cells in the graft may cause graft-versus-host disease (GVHD). Rat experiments suggest that an immunological balance exists between rejection and GVHD, but it remains obscure whether this balance will be of importance for clinical small bowel transplantation. Strategies to prevent rejection are host immunosuppression and reduction of graft immunogenicity. Passive and active enhancement have also been attempted. GVHD may be prevented by host immunosuppression and reduction of the number of immunocompetent T lymphocytes in the graft. A second obstacle may be the inevitable consequences of the surgical procedure: transection of the intestinal wall with disruption of the intrinsic neuronal continuity, extrinsic denervation, interruption of the lymphatic drainage, and harvest injury. These surgical insults may - at least temporarily - adversely influence intestinal motility, hormonal, immunological and nutritional functions,

and the physiological intestinal barrier function. Both immunological reactions in combination with immunosuppressive therapy may further compromise intestinal functioning. Recent clinical experiences are promising, but rejection, infectious complications, and lymphoproliferative disorders are still unsolved problems.

Chapter 3
Questions and objectives of investigation are formulated. What is the effect of the surgical procedure on the functions of the transplanted intestine? Is it possible to detect graft rejection at an early stage using non-invasive methods? What is the influence of MHC matching on the survival of the graft? What is the effect of a segmental graft on the nutritional status of the host?

Chapter 4
In Chapter 4 the materials and methods are described. Tissue typing and the operative techniques of heterotopic and orthotopic small bowel transplantation are outlined. The equipment used for electrophysiological measurements is described.

Chapter 5
Long-term functional sequelae of vascular, neural, and lymphatic division were studied in control dogs and heterotopic ileal autografts using morphometric analysis and electrophysiological parameters. In vivo evaluation of electrophysiological parameters was feasible and provided an elegant, practical tool in the functional assessment of ileal autotransplants. Indirect evidence was found that denervation leads to an enhanced secretory activity of the crypts. Unless reinnervation occurs, an abnormal absorptive transplant will be the result. In the autotransplants electrophysiological responses diminished with time. With some delay, morphometric parameters showed changes indicative of mucosal atrophy. This means that the mucosa of a heterotopic small bowel graft, which is deprived of intraluminal nutrition, will atrophy.

Chapter 6
Heterotopic ileal autotransplants were compared with allotransplants that were fully DLA-mismatched. No immunosuppressive treatment was given. The grafts were monitored by in vivo electrophysiological measurements and by biopsies. Diminished electrophysiological responses coincided with the onset of histological alterations of rejection. The rapidity of the rejection process made it impossible to determine a possible sequence in crypt cell and villus cell damage. The allografts survived a mean of 5.5 $\pm$ 0.2 days. Serial monitoring of electrophysiological parameters proved to be a valid, non-invasive method to detect intestinal allograft rejection, thereby circumventing the disadvantages of a morphological evaluation.

Chapter 7

In this chapter the effect of DLA matching in non-immunosuppressed dogs is evaluated. Four groups were studied: ileal autotransplants; ileal allotransplants, fully DLA-mismatched; allotransplants, one-haplotype-identical; and allotransplants fully DLA-matched. No immunosuppression was given. In all allografts the onset of histological changes related to rejection corresponded with decreased electrophysiological responses. The survival times in the DLA-matched group (mean 9.6 $\pm$ 2.7 days) were significantly longer than those in the DLA-mismatched group (mean 5.5 $\pm$ 0.2 days). Thus, DLA matching prolongs intestinal allograft survival in non-immunosuppressed dogs, but this prolongation contrasts unfavourably with other organ allografts.

Chapter 8

This chapter describes the value of serum N-acetyl hexosaminidase (NAH) to detect allograft rejection. NAH is a lysosomal acid hydrolase, which shows elevated activity in association with intestinal ischaemia. In rat studies it was shown that elevated serum NAH preceded histological changes of rejection. Ileal autografts, DLA-mismatched and DLA-matched allografts were investigated. In the mismatched group an increasing trend of serum NAH activity occurred after histological signs of rejection, whereas in the matched group elevated NAH levels coincided with morphological alterations of rejection. Therefore, serum NAH activity cannot be used as a marker to detect early intestinal allograft rejection in dogs.

Chapter 9

In this chapter are described the results of monitoring small bowel grafts by serial determinations of brush border lactase and maltase activity in mucosal biopsies and alkaline phosphatase activity in the graft effluent. Heterotopic intestinal transplantations were performed in four groups of dogs: ileal autotransplants; ileal allotransplants, fully DLA-mismatched; allotransplants, one-haplotype-identical; and allotransplants, fully DLA-matched. No immunosuppressive agents were given. In the mismatched group maltase activities showed reduced values even before morphological signs of rejection were evident. In both other allotransplant groups the onset of significantly reduced maltase levels corresponded with the first histological signs of rejection. Generally, lactase activity and alkaline phosphatase activity were not informative for the onset of rejection, although in the mismatched group diminished alkaline phosphatase activity preceded histological signs of rejection. A protein-losing enteropathy was evident in the rejecting allografts, which emphasizes that the mucosal barrier is highly permeable during a rejection episode and may thus contribute to the development of infectious complications and sepsis.

Chapter 10

In this chapter the combined effect of DLA matching and immunosuppressive therapy on the survival of orthotopic segmental intestinal allografts (25 to 30% of total small bowel length) is described. A heterotopic small bowel transplantation was followed by a second operation after five to eight weeks, during which the heterotopic graft was placed in an orthotopic position and the native intestine was resected. All dogs received cyclosporine A (CsA) immunosuppression during 200 days, after which CsA was tapered off. DLA-matched grafts survived a mean of 211.5 $\pm$ 38.8 days, which was significantly longer than survival in the DLA-mismatched group (mean 113.2 $\pm$ 37.0 days). Interestingly, none of the matched grafts was rejected during CsA treatment, whereas four of the six mismatched grafts were. This finding may have important implications for future living-related intestinal transplantations, which lead to closer HLA matching. However, living-related small bowel transplantation can only become reality if segmental grafts are functionally competent to maintain an adequate nutritional status of the host without parenteral supplements. In this study, segmental grafting resulted in normal serum albumin levels, near-normal faecal fat excretions, and sufficient CsA absorption. The dogs with DLA-matched grafts eventually regained their preoperative body weight. In contrast, a short bowel control group showed progressive weight loss, hypalbuminaemia, steatorrhoea, and decreased CsA absorption. Segmental intestinal transplantation between DLA-matched donor-recipient pairs results in long-term survivors with an adequate nutritional status.

SAMENVATTING

Hoofdstuk 1

Men spreekt van een 'korte darm syndroom' wanneer na een darmresectie het resterende deel van de dunne darm te kort schiet in de vertering en opname van voedselbestanddelen, water, mineralen en vitaminen. Het korte darm syndroom wordt gekenmerkt door diarree, steatorroe, malabsorptie en gewichtsverlies. Aangeboren afwijkingen en necrotiserende enterocolitis zijn de belangrijkste oorzaken van het korte darm syndroom bij neonaten en kinderen. Bij volwassenen kunnen intestinale vaataandoeningen en inflammatoire darmziekten leiden tot het korte darm syndroom. De ernst van het syndroom wordt bepaald door de mate en plaats van darmresectie, het al of niet aanwezig zijn van een ileocoecaal 'klep', en de mate van adaptatie van het resterende deel dunne darm en colon. Sinds het eind van de jaren zestig bestaat de behandeling van patiënten met een korte darm syndroom uit totale parenterale voeding, vooral in de eerste fase. Zo snel mogelijk na het ontstaan van het korte darm syndroom dient te worden gestart met enterale voeding om de integriteit van de mucosa (=slijmvlies) van de dunne darm te behouden, om adaptatie van de dunne darm te bevorderen, en om bacteriële translocatie tegen te gaan. Men schat dat bij één volwassene per miljoen per jaar het syndroom leidt tot een onomkeerbaar functieverlies van de dunne darm, waardoor men levenslang afhankelijk blijft van totale parenterale voeding. Langdurige parenterale voeding brengt echter de nodige complicaties met zich mee, zoals infecties en sepsis door kathetergebruik, metabole complicaties, en leveraandoeningen variërend van asymptomatisch galsteenlijden tot leverinsufficiëntie. Dunne darm transplantatie zou voor deze groep patiënten uitkomst kunnen bieden.

Hoofdstuk 2

In hoofdstuk 2 worden de ontwikkeling en de huidige stand van zaken van dunne darm transplantaties besproken. Alhoewel experimentele dunne darm transplantaties reeds aan het eind van de jaren vijftig werden verricht, zijn tot op heden slechts weinig succesvolle klinische toepassingen bekend. Twee belangrijke problemen staan klinische dunne darm transplantaties als standaardbehandeling voor het korte darm syndroom in de weg. In de eerste plaats zorgt de grote hoeveelheid lymfoid weefsel in de lamina propria, de Peyerse plaques, en de mesenteriale lymfklieren van de dunne darm voor een zeer immunogeen transplantaat; dit kan leiden tot zowel afstoting van het transplantaat als een graft-versus-host reactie (=transplantaatziekte) door alloreactieve cellen in het transplantaat. Bij experimenten met ratten lijkt er een immunologische evenwicht te zijn tussen afstoting en graft-versus-host reactie. Bij grote proefdieren echter treedt de graft-versus-host reactie minder op de voorgrond en het is de vraag of bovengenoemd immunologische balans van

klinische betekenis is. Een tweede probleem wordt gevormd door de onvermijdelijke gevolgen van de chirurgische procedure: intrinsieke en extrinsieke denervatie, onderbreking van de lymfedrainage, en aantasting van de kwaliteit van het transplantaat, welke ontstaat in de periode tussen uitnemen en implanteren. De verschillende functies van de dunne darm, zoals motorische, hormonale, immunologische en nutritionele functies, en de mucosale integriteit, kunnen hierdoor (tijdelijk) nadelig worden beïnvloed. Bovendien hebben afstoting en graft-versus-host reactie in combinatie met immunosuppressieve therapie eveneens een negatieve invloed op de functies van de dunne darm. Alhoewel recente ervaringen veelbelovend lijken, liggen er nog belangrijke obstakels op de weg naar klinische dunne darm transplantaties: afstoting van het transplantaat, infectieuze complicaties, en lymfoproliferatieve aandoeningen als gevolg van immunosuppressieve therapie.

Hoofdstuk 3
De vragen en doelstellingen van het experimentele onderzoek worden geformuleerd. Wat is de uitwerking van het operatieletsel op de functie van de getransplanteerde dunne darm? Is het mogelijk met non-invasieve methoden afstoting van een transplantaat in een vroeg stadium aan het licht te brengen? Wat is de invloed van weefseltypering op de overlevingsduur van het transplantaat? Wat is het effect van transplantatie van slechts een gedeelte van de dunne darm op de voedingstoestand van een ontvanger?

Hoofdstuk 4
In dit hoofdstuk worden de materialen en methoden van de experimenten beschreven, met name weefseltypering (DLA typering), de operatietechnieken voor heterotope en orthotope dunne darm transplantatie, en de apparatuur voor electrofysiologische metingen.

Hoofdstuk 5
In hoofdstuk 5 worden de gevolgen van de transplantatieprocedure op de functie van de dunne darm bestudeerd met behulp van een morfometrische analyse en electrofysiologische parameters. Bij dit experiment werden controlehonden vergeleken met honden met een heterotoop autotransplantaat, dus zonder immunologische verschijnselen. In vivo meting van electrofysiologische parameters bleek een praktisch hulpmiddel ter evaluatie van de functie van villus- en cryptcellen van de dunne darm mucosa. Denervatie van de dunne darm leidde tot een verhoogde secretoire activiteit van dunne darm cryptcellen. Na verloop van tijd namen de electrofysiologische responsen van de mucosa van de heterotope transplantaten af. Dit ging gepaard met een afname van de morfometrische parameters wijzend op atrofie van de dunne darm mucosa. Dit betekent dat de mucosa van een heterotoop dunne darm transplantaat, dat geen voeding en spijsverteringssappen ontvangt, atrofieert.

Hoofdstuk 6

In dit hoofdstuk wordt het aantonen van allograft afstoting aan de hand van electrofysiologische parameters beschreven. Heterotope autotransplantaten werden vergeleken met DLA-ongelijke heterotope allotransplantaten. De ontvangers werden niet behandeld met immunosuppressiva (=afweer onderdrukkende middelen), zodat een acute afstoting optrad in de allotransplantatie groep. De conditie van de transplantaten werd gevolgd middels electrofysiologische parameters en histologische biopten. Afname van de electrofysiologische responsen viel samen met de eerste histologische aanwijzingen voor een afstoting. De snelheid van het afstotingsproces maakte het echter onmogelijk een eventuele tijdsrelatie tussen villus- en cryptcel beschadiging aan te tonen. Met behulp van deze electrofysiologische methode is het mogelijk om op een non-invasieve manier acute afstoting van een dunne darm transplantaat te detecteren.

Hoofdstuk 7

In hoofdstuk 7 wordt het effect van DLA typering op de overlevingsduur van dunne darm transplantaten zonder CsA behandeling bestudeerd. Vier groepen honden werden samengesteld: 1. autotransplantaten; 2. allotransplantaten, volledig DLA-ongelijk; 3. allotransplantaten, gedeeltelijk DLA-gelijk; en 4. allotransplantaten, volledig DLA-gelijk. In geen van de groepen werden immunosuppressiva gegeven. De overleving van de dunne darm transplantaten in de DLA-gelijke groep bedroeg gemiddeld 9,6 dagen, en was hiermee significant langer dan het gemiddelde van 5,5 dagen in de DLA-ongelijke groep. Deze langere overleving van het dunne darm transplantaat staat echter in schril kontrast met betere resultaten van andere orgaantransplantaten, en vormt een aanwijzing voor de hoge immunogeniciteit van het dunne darm transplantaat.

Hoofdstuk 8

De waarde van serum N-acetyl hexosaminidase (NAH) voor het aantonen van transplantaat afstoting wordt onderzocht. NAH is een lysosomaal enzym, waarvan de activiteit in serum verhoogd is bij zuurstoftekort van de darm. In de literatuur is beschreven dat een verhoogde serum NAH activiteit tevens een voorspellende waarde heeft voor een afstotingsepisode van een dunne darm transplantaat bij de rat. Wij onderzochten autotransplantaten, DLA-ongelijke en DLA-gelijke allotransplantaten. In de DLA-gelijke groep viel een verhoging van serum NAH activiteit samen met afstoting, maar in de DLA-ongelijke groep trad een (statistisch niet significant) verhoogde NAH activiteit pas op na een histologisch bewezen afstoting. Bepaling van serum NAH activiteit is dus geen goede indicator voor het aantonen van afstoting van het dunne darm transplantaat bij de hond.

Hoofdstuk 9

In hoofdstuk 9 worden de activiteit van de borstelzoom enzymen lactase en maltase in mucosa biopten, en de alkalische fosfatase activiteit in het transplantaateffluent bestudeerd tijdens acute afstoting. Maltase activiteit in mucosa biopten kwam overeen met histologische aanwijzingen voor acute afstoting. In het algemeen gaven lactase en alkalische fosfatase activiteiten geen aanwijzingen voor een op handen zijnde afstoting. Tijdens afstoting van een dunne darm transplantaat werd een sterk verhoogde eiwit uitscheiding in het transplantaat effluent aangetoond, wat een aanwijzing kan zijn voor een verhoogde doorlaatbaarheid van de darmmucosa tijdens een afstotingsepisode.

Hoofdstuk 10

In dit hoofdstuk wordt het gecombineerde effect van DLA typering en CsA behandeling op de overleving van een partieel dunne darm transplantaat beschreven. In twee sessies werd een orthotope allotransplantatie verricht van 25 tot 30% van de totale dunne darm. Gedurende 200 dagen werden de ontvangers behandeld met CsA, waarna de immunosuppressie werd afgebouwd. Controlehonden met een korte darm syndroom overleefden gemiddeld 37,3 dagen. Honden met DLA-gelijke transplantaten overleefden gemiddeld 211,5 dagen, wat significant langer was dan het gemiddelde van 113,2 dagen voor honden uit de DLA-ongelijke groep. Bovendien werd in de DLA-gelijke groep tijdens CsA behandeling geen enkele afstotingsepisode waargenomen, terwijl in dezelfde periode in de DLA-ongelijke groep vier van de zes honden een afstoting doormaakten. Partiële dunne darm transplantatie leidde tot een normaal serum albumine niveau, nagenoeg normaal vetgehalte van de ontlasting, en adequate CsA absorptie. Bovendien bereikten de honden met een DLA-gelijk, partieel dunne darm transplantaat uiteindelijk weer hun preoperatieve gewicht. In de controlegroep met het korte darm syndroom was er sprake van progressief gewichtsverlies, verlaagd serum albumine niveau, steatorroe, en verlaagde CsA absorptie. Partiële dunne darm transplantatie tussen DLA-gelijke donor-ontvanger paren in combinatie met CsA behandeling leidt tot lange overlevers met een adequate voedingstoestand.

APPENDICES

LIST OF ABBREVIATIONS

ALS	anti-lymphocyte serum
ATG	anti-thymocyte serum
ATP	adenosine triphosphate
BN	Brown Norway
CCK	cholecystokinin
c-AMP	cyclic adenosine monophosphate
CsA	cyclosporine A
CT	cholera toxin
DLA	dog leukocyte antigen
DST	donor-specific blood transfusion
GVHD	graft-versus-host disease
HLA	human leukocyte antigen
Ig	immunoglobulin
IL	interleukin
l	litre
Lew	Lewis
mg	milligram
MHC	major histocompatibility complex
ml	milliliter
MMC	migrating motor complex
NAH	N-acetyl hexosaminidase
PCA	procoagulant activity
PD	potential difference
PEG	polyethylene glycol
SB	small bowel
SBS	short bowel syndrome
SBT	small bowel transplantation
SEM	standard error of the mean
TNF	tumor necrosis factor
TPN	total parenteral nutrition
U	unit
VIP	vasoactive intestinal peptide

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