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ORIGINAL ARTICLE

Role and significance of plasma citrulline in the early phase after small bowel transplantation in pigs

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Summary

A reliable serological marker of acute cellular rejection (ACR) after small bowel transplantation (SBTx) is still missing. Plasma citrulline level (PCL) reflects the functional integrity of intestinal mucosa which is partially lost during ACR. The aim of our study was to investigate the role of PCL as marker of ACR after SBTx. Eighteen German landrace pigs were used and divided into three groups. Group 1 (G1), autologous SBTx ($n = 4$) as control; group 2 (G2), allogeneic SBTx without immunosuppression (IS) ($n = 7$) and group 3 (G3), allogeneic SBTx with IS ($n = 7$). IS consisted of tacrolimus and steroids without induction treatment. Observation period was 14 days. Mucosal biopsies were obtained intraoperatively and daily using a Thiry–Vella loop. ACR was differentiated into indeterminate, mild, moderate and severe using a standardized grading schema. PCL was measured daily. An ACR onset occurred generally from postoperative day 4 both in G2 and G3 as mild form and developed differently in the two groups: moderate to severe in G2 and indeterminate to mild in G3. A significant decline of PCL occurred only in cases of moderate and severe ACR, but not in cases of indeterminate and mild ACR. The PCL failed as a marker in the early diagnosis of ACR and became reliable only when advanced mucosal damage was present.

Introduction

Acute cellular rejection (ACR) after small bowel transplantation (SBTx) still represents the most common cause of graft loss [1] and its diagnosis is often delayed [2,3].

Standard diagnostic tools of ACR after SBTx are represented by the combination of clinical assessment, endoscopy and most importantly, the histology of intestinal biopsies. Unfortunately, frequent endoscopies and biopsies are potentially hazardous because of procedure-related complications and delays in diagnosis [4,5].

Therefore, a noninvasive test that can assess mucosal allograft dysfunction (i.e. rejection) in a timely fashion is urgently needed for clinical and economical reasons [1,6,7]. Since the late 1980s, different methods, ranging from diagnostic tests to laboratory markers, have been suggested [8–11] but they either did not find acceptance or failed in their aim.

Pappas *et al.* [12] and Gondolesi *et al.* [13] recently proposed the use of plasma citrulline levels (PCL) as a new marker of mucosal injury after SBTx. Citrulline is a nonprotein amino acid synthesized from glutamine mainly in the intestine where it is released from the enterocyte into the circulation and converted to arginine by the kidneys to complete the urea cycle [14]. Because citrulline is neither present in enterally ingested foods nor in endogenous proteins, it can not be released by any cleavage processes. The sensitivity of PCL therefore is

specific to enterocyte function and not influenced by the amount of oral protein and calorie intake. As acute rejection is the major cause of mucosal injury following SBTx, we studied the role of PCL as a marker of ACR after SBTx in a large animal model.

Material and methods

Animals and experimental setting

Unrelated German landrace female pigs weighing 35–50 kg were used according the German Law on the Protection of Animals and the Principles of Laboratory Animal Care. All animals were kept in quarantine for 10 days and screened for Enterozoons, Salmonella, Shigella, Campylobacter, Yersinia enterocolica and Aeromonas by stool cultures.

Animals that passed the quarantine were divided randomly into three groups (G). G1, autologous SBTx ($n = 4$) served as our control; G2, allogeneic SBTx without immunosuppression (IS) ($n = 7$) and G3, allogeneic SBTx with IS ($n = 7$). Fourteen animals served as donors.

Anaesthesia

All animals were fasted 24 h before and 96 h after surgery. Animals were premedicated with ketamine 30 mg/kg, atropine 0.5 mg and azaperon 2 mg/kg, given i.m. After endotracheal intubation general anaesthesia was maintained with disoprivan and ketamine. Invasive monitoring of haemodynamics was ensured through central vein and arterial catheters inserted in the external jugular vein and carotid artery, respectively. The central venous catheter was maintained throughout the postoperative day (POD) to obtain blood samples and for parenteral nutrition. The arterial catheter was removed at the end of the operation.

Donor operation

Following a midline incision the small bowel was dissected proximally from 10 cm beyond the ligament of Treitz to a distal point almost 10 cm before the ileocaecal valve. The colonic vascular branches were ligated and divided. Systemic heparin in a dose of 100 IE/kg was administered and the small bowel was harvested after the dissection of the superior mesenteric artery (SMA) and the superior mesenteric vein (SMV) at the level of the inferior margin of the pancreas. At the back table, the graft was perfused with 1000 ml of University of Wisconsin Solution (UW) (Bristol Myers Squibb, Munich, Germany) at a pressure of 80–100 mmHg through the stump of the SMA and finally stored at 4 °C according to standard procedure.

Recipient operation

In all groups, including G1, the recipient's own small bowel was harvested after the dissection of SMA and SMV at the level of the inferior margin of colonic branches to preserve the colonic circulation. The small bowel graft was then implanted orthotopically. SMA and SMV were anastomosed end-to-end using a running 7/0 polydioxanone (PDS) suture (Johnson & Johnson Medical k. k., Hamburg, Germany). UW solution within the graft was washed out with blood through the SMV during reperfusion. The continuity of the small bowel was restored by a one-layer extramucosal jejuno-jejunostomy and ileo-ileostomy using a running 4/0 PDS suture. In order to obtain daily biopsies, 40 cm of the proximal part of jejunum was externalized as a Thiry–Vella loop [15]. A percutaneous gastrostomy was placed to prevent gastric distension secondary to postoperative gastroparesis.

Postoperative monitoring

The observation period lasted 14 days. The postoperative care included daily monitoring of general condition, appearance of the jejunal stoma, stool and body temperature. All recipients were given 2-l lactate Ringer's and 500-ml 20% glucose solution daily until they resumed a normal diet generally on POD 5. Perioperative antibiotic treatment consisted of i.v. metronidazol 500-mg twice daily and mezlocilline 4-g three times a day. As prophylactic treatment of peptic ulcer, all animals were given i.v. proton pump inhibitors (pantoprazol 40 mg/day).

Sepsis was defined as a combination of systemic inflammatory response syndrome (SIRS) and reduced alertness in the presence of ACR or infection. SIRS was assumed if hypothermia (<36 °C), tachycardia (>90/min) and tachypnea (>20/min) were present.

Plasma citrulline level, serum creatinine, lactate, blood count, liver function tests, haemoglobin and electrolytes were measured by daily blood samples starting preoperatively.

Immunosuppression (IS)

Double immunosuppressive therapy with tacrolimus and steroids, without any induction was administered only in the G3 animals. The IS started with 500 mg of i.v. methylprednisolone given intraoperatively during the anintestinal phase. Tacrolimus was given i.m. in a dose of 0.1 mg/kg/day from the day of surgery until POD 4 and then continued orally to maintain a serum level of 12–15 ng/ml. Methylprednisolone was given i.v. from POD 1 until death in a daily dose of 20 mg.

Histopathological examination

Mucosal biopsies were obtained intraoperatively (full-thickness biopsies from jejunum and ileum before harvesting, then at 20 min and 2 h after reperfusion), postoperatively (performed daily starting on POD 2, from the Thiry–Vella loop with biopsy forceps) and postmortem (at necropsy). Specimens were fixed in 10% buffered formalin, embedded in paraffin and cut at 4 μm and stained with haematoxylin–eosin. Two main parameters were investigated: ischemia reperfusion injury (IRI) and ACR.

Assessment of histological changes related to IRI was based upon a modified Park–Chiu classification and included degree of epithelial lifting and subepithelial space expansion in relation to villi [16].

Features of ACR included predominant infiltration by mononuclear inflammatory cells, crypt injury and increased number of crypt apoptotic bodies. The extent of crypt injury was estimated by mucin depletion, cytoplasmic basophilia, decreased cell height, nuclear enlargement and hyperchromasia, and inflammatory infiltrates of predominantly mononuclear cells. Crypt apoptotic bodies were identified as clear spaces inside crypt epithelium containing fragmented nuclear material. When detected, ACR was differentiated into indeterminate, mild, moderate and severe according to a standardized grading schema reported in Table 2 [17].

Citrulline analysis

Immediately after sampling, plasma was obtained by whole blood centrifugation. After precipitation, 200 μl of a 10% 5-sulfosalicylic acid solution were added to 800 μl of plasma. This compound was then vortexed and re-centrifuged. Supernatant was filtrated and adjusted to pH 2.2 by addition of 600 μl of 2% thioglycol dilution buffer. PCL was measured by using liquid ion-exchange chromatography equipped with a 440 and 570 nm photometric detector and EZChrom Chromatography Data System 6.7 (Scientific Software Inc. Dr Owens, Pleasanton, CA 94588 USA).

Based on G1 data and confirmed afterward in our other groups, the PCL cut off could be considered to be a value of 30 μmol/l, as also reported in human studies [14,18].

Statistics

All data, except for survival time in Table 1, are expressed as mean ± SE. Survival time in Table 1 is expressed as median. The statistical analysis was performed by one-way ANOVA analysis followed by the Tukey test using SPSS version 13.0 for Windows XP. *P*-values <0.05 were considered to be significant.

Table 1. Experimental setting, median survival time and cause of death.

Animals no.	Survival (day)	Cause of death
Group I: autologous SBTx		
1	≥14	–
2	≥14	–
3	≥14	–
4	≥14	–
Median	≥14	
Group II: allogeneic SBTx without IS		
5	8	Rejection
6	12	Rejection
7	7	Rejection
8	≥14	Rejection
9	5	Rejection
10	7	Rejection
11	9	Rejection
Median	8	
Group III: allogeneic SBTx with IS		
12	≥14	–
13	≥14	–
14	9	Rejection
15	≥14	–
16	≥14	–
17	13	Rejection
18	11	Rejection
Median	≥14	

IS, immunosuppression; SBTx, small bowel transplant.

Results

General observation

Overall 18 SBTx were performed. The overall mean cold and warm ischaemic time was 284 ± 20 min and 31 ± 1.5 min, respectively.

Survival

Data regarding survival time and cause of death are listed in Table 1. The mean survival rate in G1, G2 and G3 was 100%, 14% and 57%, respectively, the difference between G1 and G2 being statistically significant ($P < 0.05$). In G2, all animals developed clinical signs of sepsis associated with ACR starting on POD 5–6. Only one animal survived until POD 14. In G3, three out of seven animals developed sepsis associated with rejection and died on POD 9, 11 and 13, respectively.

Laboratory tests

All animals demonstrated normal kidney function with serum creatinine within the normal range. No significant differences were observed in blood count, lactate, electrolytes, coagulation parameters and liver function test

Table 2. ACR-scores according to the Pathology Workshop of the 8th International Small Bowel Transplant Symposium, Miami, USA September 2003 (17).

Histological criteria of ACR	Grading
No evidence of acute rejection	ACR-score 0
Indeterminate for acute rejection	ACR-score 1
Minor epithelial cell injury, less than six apoptotic bodies per 10 crypts, overlaying mucosa intact, minimal mononuclear inflammatory infiltrates.	
ACR, mild	ACR-score 2
Crypt injury, cytoplasmic basophilia, cuboid shape of epithelial cells, hyperchromasia, increased mitotic activity, six or more apoptotic bodies per 10 crypts, blunting and architectural distortion of the villus, mixed but primarily mononuclear inflammatory population involving the lamina propria or below.	
ACR, moderate	ACR-score 3
Six or more apoptotic bodies per 10 crypt cross sections accompanied by foci of confluent apoptosis, focal crypt loss, inflammatory infiltrate is often at moderate to severe intensity.	
ACR, severe	ACR-score 4
Marked degree of crypt damage and destruction, marked diffuse inflammatory infiltrate, complete loss of the bowel morphological architecture.	

ACR, acute cellular rejection.

between the various groups. All animals developed leucocytosis, elevated amylase and slight anaemia after transplantation without any significant differences between the groups.

Histopathology

Ischemia reperfusion injury (IRI)

Epithelial lifting as typical expression of IRI was already present 20 min after graft reperfusion, peaking to a maximal degree 2 h later in all three groups without any difference among them. On POD 2 histological changes related to IRI were no longer evident.

Acute cellular rejection (see Table 2 and Fig. 1)

In all G1 animals, histological signs of ACR were absent at each time point. Only sporadic, nonspecific changes of the mucosa in terms of submucosal haemorrhage, focal erosions of mucosa and granulocyte infiltration were seen during the observation period. Full-thickness biopsies of the graft at the time of the autopsy revealed normal mucosal appearance in all control grafts.

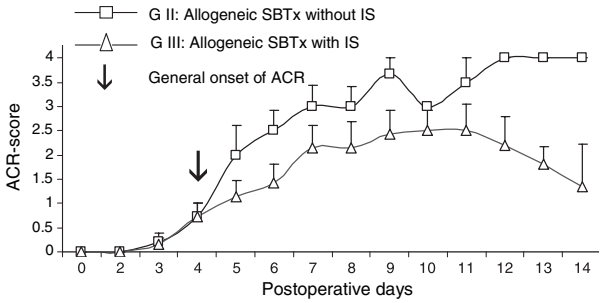

Figure 1 Progression of ACR (mean ACR-values ± SE) in immunosuppressed (G3) and nonimmunosuppressed animals (G2). After the general onset of ACR on POD 4 (arrow) the progression of ACR was more distinctive in nonimmunosuppressed animals. Due to presence of identical (POD 0, 2, 10 and 12) scores or single values (POD 13 and 14) the SE of mean citrulline value of nonimmunosuppressed animals was zero. Legend: IS, immunosuppression; ACR, acute cellular rejection; POD, postoperative day.

Almost all animals in G2 and G3 showed slight signs of ACR (score 1) starting on POD 4 (Fig. 1). Subsequently, the progression of ACR score was more distinct in G2 (moderate–severe) compared with G3 animals (indeterminate–mild) (Fig. 1). Early ACR (on POD 3) was observed in one animal from G2 and one from G3 (both score 1), with rapid progression and early death on POD 5 and 9, respectively.

Plasma Citrulline Level (PCL)

The overall variation of mean PCL in all groups is depicted in Fig. 2. The mean preoperative PCL was 45.2 ± 11 μmol/l, without any significant difference among the three groups. Within the first four PODs, the course of PCL was similar in all groups and it was characterized by a strong decrease on POD 1 (23.2 ± 2.5 μmol/l in G1, 18.9 ± 1.9 μmol/l in G2 and 38.1 ± 3.5 μmol/l in G3) followed by a progressive increase until POD 4 (25.9 ± 4.7 μmol/l in G1, 28.1 ± 3.3 μmol/l in G2 and 43 ± 4.9 μmol/l in G3). During this period the PCL in G3 was higher compared with other groups. In relation to group 2, this difference was significant on POD 1, 2 and 3 ($P < 0.05$) (Fig. 2). After POD 4 the PCL had a different course in different groups. In G1, it further increased, even exceeding the preoperative values by POD 14 (72.8 μmol/l ± 10.1). In G2, PCL decreased gradually during the remaining observation period with constant mean values below 20 μmol/l. In G3 PCL increased until POD 5 and subsequently declined to a steady state level (around 40 μmol/l) between POD 7 and 13. After POD 13 it increased again, reaching the baseline preoperative values.

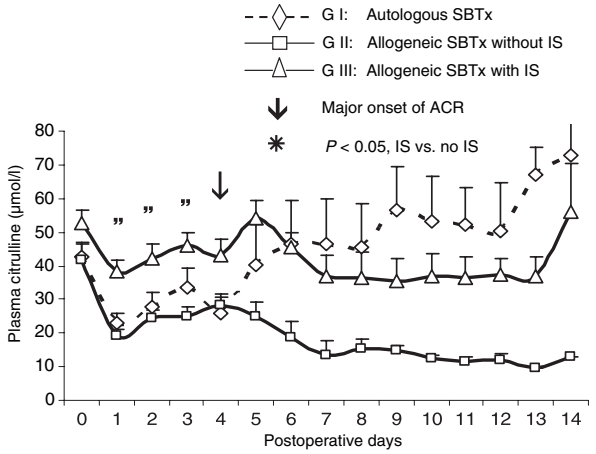

Figure 2 The overall course of plasma citrulline level after SBTx (mean values ± SE). On POD 1, 2 and 3 immunosuppressed animals presented significant higher plasma citrulline values compared with no immunosuppressed animals ($P < 0.05$). Beyond POD 4 no further significance was calculated because of heterogenic appearance of mucosal alterations and outcome of the animals in groups. The arrow marks the general onset of ACR. Legend: IS, immunosuppression; ACR, acute cellular rejection; POD, postoperative day; SBTx, small bowel transplantation.

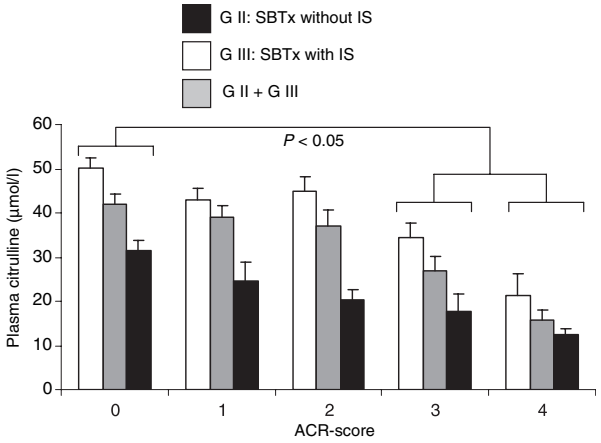

Figure 3 Mean plasma citrulline level (± SE) at different scores of ACR. The decline of PCL was only significant during moderate (score 3) and severe (score 4) ACR. This association between rejection score and citrulline decrease was seen when analysing both groups together as well as each group separately. Data from control animals (G1) were not included to avoid an overestimation of citrulline level at ACR-score 0. Legend: PCL, plasma citrulline level; IS, immunosuppression; ACR, acute cellular rejection.

When correlating the decrease of PCL with corresponding ACR-scores using the Tukey test (Fig. 3), we observed a significant decline of PCL only in case of moderate and severe ACR (score 3 and 4) but not in case of indeterminate and mild ACR (score 1 and 2).

Discussion

Many progresses have been recently performed in different aspects of SBTx (i.e. surgical, immunological, medical and even political) [19]. Notwithstanding, ACR still represents a major problem and a reliable noninvasive serological marker of ACR is still missing [1].

In clinical practice, PCL is already a reliable and established marker of intestinal failure in patients with short bowel syndrome, reflecting absorptive bowel mass [14,20,21].

It is still unclear if PCL may play a role as marker of ACR after SBTx, eventually representing a valid alternative to histological findings of mucosal biopsy. In SBTx, different events like IRI and ACR contribute in separate ways to mucosal damages. Similarly, PCL correlates differently to each of these events.

Our data confirm that PCL reflects the functional integrity of the intestinal mucosa. However, in our experimental setting, plasma citrulline failed to detect ACR in its milder forms (score 1 and 2) during the early post-transplant phases. One explanation for these results can be the nature of intestinal ACR. It is well accepted that rejection episodes after SBTx initially appear in a patchy manner, by infiltration of inflammatory cells in submucosa and muscularis [4,5,22]. As citrulline is produced and released by the enterocytes located in the mucosa, we may assume that in the early period of ACR the damage to the mucosa is not sufficient to compromise the functional capacity of enterocytes to release citrulline. Only a stronger progression of ACR, causing more significant damage to the mucosa, is accompanied by significant changes in PCL.

Mucosal alterations ascribed to IRI were noted to begin intraoperatively and were present invariably in all animals. Histological signs of IRI were temporary, and vanished completely by POD 2. Corresponding to the IRI, the course of PCL was similar in all animals and characterized by a strong decline on POD 1 with a gradual increase afterwards through POD 4. In G1 animals (autologous transplantation), without further significant damages to the intestinal mucosa, the PCL continued to increase gradually over the entire follow-up period and eventually even exceeded the baseline preoperative level.

Another possible confounding effect on graft histology may have been represented by intestinal atrophy because of missing enteral nutrition during the first 5–7 PODs. At this regard discrepant results have been reported in the literature. Enteral nutrition is believed to be beneficial because it protects the gut from mucosal atrophy. This concept was developed from the results of animal studies

showing that the administration of total parenteral nutrition (TPN) resulted in significant intestinal villus atrophy within a few days [23]. In experimental settings it seems that TPN may induce atrophy and hypofunction of the small intestine of Lewis rats even within 14 days after SBTx [20]. About the speed of onset of TPN-induced mucosal atrophy Niinikoski *et al.* [24] demonstrated that the transition from enteral to parenteral nutrition in neonatal piglets induced a rapid (<8 h) decrease in intestinal blood flow, and this likely precedes villous atrophy and the suppression of protein synthesis at 24 h, and of cell proliferation and survival at 48 h.

On the contrary Oste *et al.* [25] demonstrated that a short period of TPN does not induce mucosal atrophy in preterm pigs. Interestingly, a critical review of the data in the literature suggests that in the human subject TPN does not cause mucosal atrophy or increase translocation of bacteria through the small intestine. Atrophy was observed only when TPN was given to children for several months, during which time the children did not receive any food by mouth [23].

Indeterminate grade ACR was usually observed by histology starting on POD 4 in both G2 and G3. After POD 4, however, the ACR progressed differently in each study arm over time (moderate up to severe grade in G2 and remaining generally mild in G3) (Fig. 1). After a gradual increase between POD 1 and 4, PCL in G2 animals declined again starting around POD 4, whereas in G3 recipients it continued to increase until POD 5 (Fig. 2). During the first 4 PODs a statistical significant difference of PCL between G2 and G3 animals was observed (Fig. 2). The persistent difference between the two groups after POD 4 has no statistical value as these levels reflect only those few animals that were still alive. Additionally, after POD 4, PCL decreased differently according to the grade/score of ACR demonstrated by the subjects: no significant changes of PCL during indeterminate or mild ACR episodes, but significant decreases in PCL during moderate and severe ACR (Fig. 3). These data suggest that a decline in PCL correlates more to the degree of ACR than to its onset *per se*.

This association between rejection score and citrulline decrease was seen when both groups were analysed either together or individually and despite the fact that during the first PODs the PCL was significantly higher in G3 (with IS) than in G2 (without IS). It is unclear if the administration of steroids may justify these results. In fact, in weaning pigs, elevated plasma concentrations of cortisol have been shown to be responsible for increased activities of arginase, argininosuccinate synthase, argininosuccinate lyase and pyrroline-5-carboxylate synthase (as all of them have a cortisol receptor). Consequently, this leads to increased intestinal metabolism of glutamine and arginine which can lead to increased synthesis of citrulline [26–30]. During period of development (i.e. weaning), cortisol surges play an essential role in enhancing intestinal polyamine synthesis, which may be of physiological importance for intestinal adaptation and remodelling [29].

A decrease in the activities of phosphate-dependent glutaminase, ornithine aminotransferase, ornithine carbamyltransferase and carbamoyl-phosphate synthase were observed as the age of the pigs increased [30]. No data have been reported about cortisol levels in the postweaning period. We can only speculate that a decrease of arginase activity may be secondary to a down regulation of cortisol receptors, as arginine is a nutritionally essential amino acid for suckling piglets, but not for adult pigs [30]. Based on these observations we can only say that higher PCL can not be explained solely by the administration of steroids.

In summary our data clearly demonstrate that PCL is affected by several circumstances which may influence mucosal functionality. Changes in PCL did not detect indeterminate or mild ACR whereas a correlation was established during moderate to severe rejection episodes.

References

1. Fryer JP. Intestinal transplantation: an update. *Curr Opin Gastroenterol* 2005; **21**: 162.
2. Ishii T, Mazariegos GV, Bueno J, Ohwada S, Reyes J. Exfoliative rejection after intestinal transplantation in children. *Pediatr Transplant* 2003; **7**: 185.
3. Sudan DL, Kaufman SS, Shaw BW Jr, *et al.* Isolated intestinal transplantation for intestinal failure. *Am J Gastroenterol* 2000; **95**: 1506.
4. Yamataka A, Miyano T, Fukunaga K, Kobayashi H, Nozawa M, Sasaki K. Patchy distribution of rejection changes in small intestinal transplantation. *J Pediatr Surg* 1992; **27**: 602.
5. Kuusanmaki P, Halttunen J, Paavonen T, Pakarinen M, Luukkonen P, Hayry P. Acute rejection of porcine small bowel allograft. An extended histological scoring system. *Transplantation* 1994; **58**: 757.
6. Abu-Elmagd KM, Reyes J, Fung JJ, *et al.* Evolution of clinical intestinal transplantation: improved outcome and cost effectiveness. *Transplant Proc* 1999; **31**: 582.
7. Rovera GM, Sileri P, Rastellini C, Knight P, Benedetti E, Cicalese L. Quality of life after living related small bowel transplantation. *Transplant Proc* 2002; **34**: 967.
8. Grant D, Lamont D, Zhong R, *et al.* 51Cr-EDTA: a marker of early intestinal rejection in the rat. *J Surg Res* 1989; **46**: 507.
9. Farmer DG, McDiarmid SV, Kuniyoshi J, Robert ME, Shaked A, Busuttil RW. Intragraft expression of messenger

RNA for interleukin-6 and tumor necrosis factor-alpha is a predictor of rat small intestine transplant rejection. *J Surg Res* 1994; **57**: 138.
10. Teitelbaum DH, Dunaway DJ, Sonnino RE, Stellin G, Berend ME, Harmel RP Jr. Leakage of intraluminal low molecular weight polyethylene glycol as a marker of small bowel transplant rejection. *J Pediatr Surg* 1989; **24**: 64.
11. Maeda K, Schwartz MZ, Bamberger MH, Daniller A. A possible serum marker for rejection after small intestine transplantation. *Am J Surg* 1987; **153**: 68.
12. Pappas PA, Saudubray JM, Tzakis AG, *et al.* Serum citrulline as a marker of acute cellular rejection for intestinal transplantation. *Transplant Proc* 2002; **34**: 915.
13. Gondolesi G, Fishbein T, Chehade M, *et al.* Serum citrulline is a potential marker for rejection of intestinal allografts. *Transplant Proc* 2002; **34**: 918.
14. Crenn P, Coudray-Lucas C, Thuillier F, Cynober L, Messing B. Postabsorptive plasma citrulline concentration is a marker of absorptive enterocyte mass and intestinal failure in humans. *Gastroenterology* 2000; **119**: 1496.
15. Nishida S, Komokata T, Ogata S, *et al.* Allograft rejection of small bowel transplantation in pigs. *Surg Today* 1998; **28**: 1138.
16. Mueller AR, Nalesnik MA, Langrehr JM, *et al.* Evidence that small bowel preservation causes primarily basement membrane and endothelial rather than epithelial cell injury. *Transplantation* 1993; **56**: 1499.
17. Ruiz P, Bagni A, Brown R, *et al.* Histological criteria for the identification of acute cellular rejection in human small bowel allografts: results of the pathology workshop at the VIII International Small Bowel Transplant Symposium. *Transplant Proc* 2004; **36**: 335.
18. Gondolesi GE, Kaufman SS, Sansaricq C, *et al.* Defining normal plasma citrulline in intestinal transplant recipients. *Am J Transplant* 2004; **4**: 414.
19. Fishbein TM. The current state of intestinal transplantation. *Transplantation* 2004; **78**: 175.
20. Jianfeng G, Weiming Z, Ning L, *et al.* Serum citrulline is a simple quantitative marker for small intestinal enterocytes mass and absorption function in short bowel patients. *J Surg Res* 2005; **127**: 177.
21. Crenn P, Vahedi K, Lavergne-Slove A, Cynober L, Matuchansky C, Messing B. Plasma citrulline: a marker of enterocyte mass in villous atrophy-associated small bowel disease. *Gastroenterology* 2003; **124**: 1210.
22. Sigurdsson L, Reyes J, Todo S, Putnam PE, Kocoshis SA. Anatomic variability of rejection in intestinal allografts after pediatric intestinal transplantation. *J Pediatr Gastroenterol Nutr* 1998; **27**: 403.
23. Jeejeebhoy KN. Total parenteral nutrition: potion or poison? *Am J Clin Nutr* 2001; **74**: 160.
24. Niinikoski H, Stoll B, Guan X, *et al.* Onset of small intestinal atrophy is associated with reduced intestinal blood flow in TPN-fed neonatal piglets. *J Nutr* 2004; **134**: 1467.
25. Oste M, Van Ginneken CJ, Van Haver ER, Bjornvad CR, Thymann T, Sangild PT. The intestinal trophic response to enteral food is reduced in parenterally fed preterm pigs and is associated with more nitrergic neurons. *J Nutr* 2005; **135**: 2657.
26. Flynn NE, Wu G. Enhanced metabolism of arginine and glutamine in enterocytes of cortisol-treated pigs. G474. *Am J Physiol* 1997; **272**(3 Pt 1): G474.
27. Flynn NE, Wu G. Glucocorticoids play an important role in mediating the enhanced metabolism of arginine and glutamine in enterocytes of postweaning pigs. *J Nutr* 1997; **127**: 732.
28. Flynn NE, Meininger CJ, Kelly K, Ing NH, Morris SM Jr, Wu G. Glucocorticoids mediate the enhanced expression of intestinal type II arginase and argininosuccinate lyase in postweaning pigs. *J Nutr* 1999; **129**: 799.
29. Wu G, Meininger CJ, Kelly K, Watford M, Morris SM Jr. A cortisol surge mediates the enhanced expression of pig intestinal pyrroline-5-carboxylate synthase during weaning. *J Nutr* 2000; **130**: 1914.
30. Wu G, Knabe DA, Flynn NE. Synthesis of citrulline from glutamine in pig enterocytes. *Biochem J* 1994; **299**(Pt 1): 115.

