Studies of Experimental Bacterial Translocation

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Abstract

One of the main obstacles to maintaining patients with short bowel syndrome on parenteral nutrition, or successfully transplanting these patients with a small bowel graft, is the many severe infections that occur. Evidence is accumulating that translocating bacteria from the patient’s bowel causes a significant part of these infections. In this thesis bacterial translocation is studied in a Thiry-Vella loop of defunctionalised small bowel in the rat.

Bacterial translocation to the mesenteric lymph nodes (MLNs) occurs in almost 100% of the rats after three days. No systemic spread of bacteria is observed unless there is additional immunosuppression with depletion of Kupffer cells in the liver. However, blocking the function of α/β T cells does not increase the translocation. Removal of MLNs does not either aggravate bacterial translocation in the Thiry-Vella loop model. Conversely, after small bowel transplantation translocating bacteria spread systemically if the MLNs are removed.

The Thiry-Vella loop should also be a suitable model for the testing of potentially translocation-inhibiting substances. Reinforcement of the intestinal barrier with glutamine or phosphatidylcholine proved insufficient in decreasing bacterial translocation. Even selective bowel decontamination with tobramycin failed to abolish bacterial translocation. Thus, it seems that the driving force for translocation in this model is strong regardless of the relatively small trauma of intestinal defunctionalisation.

Flow cytometric studies of the immune cells in the spleen MLNs showed a decrease in MHC class II positive T cells in the MLNs of the Thiry-Vella loop. Concurrently the number of macrophages increased with time as observed by immunohistochemistry. The fraction of MHC class II negative macrophages increased in the spleens of rats treated with glutamine.

In conclusion, the Thiry-Vella loop model offers possibilities of immunological as well as mechanistic studies on bacterial translocation from small intestine.

Keywords: Intestinal barrier, short bowel syndrome, small bowel transplantation, mesenteric lymph nodes, gadolinium chloride, T cell inactivation, glutamine, phosphatidylcholine, rat, Thiry-Vella loop

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Nil sine magno labore vita
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This thesis is based on the following papers, which will be referred to in the text by their Roman numerals:

I  **Bacterial translocation from defunctionalized rat small bowel.**
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IV  **Effect of glutamine or phospholipid treatment on bacterial translocation from defunctionalised small bowel.**
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ABBREVIATIONS

AEC 3-Amino-9-ethyl-carbazole
BN Brown Norway
b.w. Body weight
CD Cluster designation (cluster of differentiation)
CFU Colony forming unit
CyA Cyclosporine A
EGF Epidermal growth hormone
FITC Fluorescein isothiocyanate
FSC Forward scatter
FW solution Frödin Wolgast solution
GALT Gut-associated lymphoid tissue
GdCl₃ Gadolinium chloride
GH Growth hormone
Gln Glutamine
GLP-2 Glucagon like peptide 2
IGF-1 Insulin like growth factor 1
Ig Immunoglobulin
i.v. Intravenously
M-cells Membrane cells
MHC Major histocompatibility complex
MLN Mesenteric lymph node
NK cells Natural killer cells
PAP Peroxidase-antiperoxidase
PBS Phosphate-buffered saline
PE Phycoerythrin
PhCh Phosphatidylcholine
PNALD Parenteral nutrition associated liver disease
p.o. Per orally
SBTx Small bowel transplantation
SSC Side scatter
SBD Selective bowel decontamination
T-V loop Thiry-Vella loop
TPN Total parenteral nutrition
INTRODUCTION

Bacterial translocation

The intestinal mucosa is a major barrier in preventing enteric bacteria from invading the body. However, under certain conditions viable indigenous bacteria from the gut can breach the mucosal barrier and migrate to mesenteric lymph nodes (MLNs) and other organ systems. This process has been termed bacterial translocation (Berg, and Garlington 1979) and was first described by Keller and Engley in 1958 (Keller and Engley 1958). The definition may be broadened to include transmucosal passage of nonviable microbes and their by-products (e.g., endotoxin) (Alexander et al. 1990). Three major factors promoting bacterial translocation have been identified: increased number of bacteria in the gut, impaired immune function and increased permeability of the mucosal barrier (Deitch and Berg 1987). Of these, bacterial overgrowth seems to be the most effective translocation promoting mechanism, at least as judged by cultures from MLNs. However, further systemic spread of bacteria often requires an immunodeficient state (Berg et al. 1988). Bacterial overgrowth is a common condition in patients receiving antibiotics. In addition, antibiotic resistant bacteria often colonize these patients’ intestinal tracts. An increased number of intestinal bacteria is also seen in patients receiving parenteral nutrition or that have dysmotility or obstruction of the gastrointestinal tract. Experiments have shown that there is a direct relationship between number of bacteria in the intestine and translocation to the MLNs (Steffen and Berg 1983).

Studies on impaired immune function have indicated that mucosal immunity (secretory immunoglobulin A; IgA), cell-mediated immunity (macrophages and T cells) and serum immunity (IgG and IgM) are probably all involved in the defence against bacterial translocation. However, the relative contribution of each of these has not been elucidated (Berg 1999).

Bacterial translocation also occurs in animal models where there is physical damage to the epithelium or increased intestinal permeability, such as in hemorrhagic shock (Baker et al. 1988), endotoxic shock (Deitch et al. 1989) and thermal injury (Maejima et al. 1984).
The phenomenon of bacterial translocation has obtained greater clinical attention during the last 30 years. The loss of intestinal barrier function and the subsequent passage of bacteria and endotoxins has been found to be a probable cause of systemic infection and multiple organ failure in certain patients (Caridis et al. 1972; Deitch 1990). More specifically, clinical conditions such as short bowel syndrome, bowel ischemia, hemorrhagic shock, thermal injury, trauma, malnutrition, bowel obstruction, liver cirrhosis, inflammatory bowel disease and small bowel transplantation have attracted attention. In addition, a vast number of studies has been made delineating endotoxin or lipopolysaccharide (LPS), a constituent of the outer membrane of gram-negative bacteria, as an important mediator in the sepsis syndrome after major surgery, trauma and hemorrhage (Morrison and Ryan 1987), (Glauser et al. 1994). Although the pathogenesis is not clear, gut barrier failure and bacterial translocation are considered to play a key role. Bacterial toxins, such as endotoxin, can induce a local activation of the inflammatory system and subsequent production of inflammatory cytokines, which leads to further deterioration of the gut barrier and increased bacterial translocation and eventually may develop into a septic shock (Caridis et al. 1972). In the last few years toll-like receptors on macrophages, dendritic cells, endothelial cells and cells in the gastrointestinal mucosa, are emerging as key mediators in the innate immune system (Medzhitov 2001), (Cario 2005). These receptors recognize defined molecular structures present in a variety of microorganisms, e.g. endotoxin, and trigger production of proinflammatory and immunoregulatory cytokines and chemokines (Kaisho and Akira 2002). The role of these structures in bacterial translocation remains to be clarified.

While bacterial translocation and its complications have clearly been shown to occur in animal models, its existence and importance in humans has been difficult to ascertain. However, it is becoming generally accepted that bacterial translocation is an important early step in the pathogenesis of opportunistic infections caused by endogenous intestinal flora (Brathwaite et al. 1993), (Rahman et al. 2003), (Vincent et al. 1988), (Cicalese et al. 2001).

Bacterial translocation may be a phenomenon that occurs in healthy individuals as well. In some experimental settings translocation of gastrointestinal bacteria to MLNs, spleen and liver has been confirmed in healthy animals in the neonatal period (Moy et al. 1999). Uptake of intestinal antigens and the display of these to the immune system is an important process that is administered via the M cells. These are specialised epithelial cells overlaying the lymphoid follicles of Peyer’s patches. M cells have a high capacity for transcytosis of microorganisms and macromolecules and are believed to act as an antigen sampling system. In that way they represent a weak point in the epithelial barrier and could be “hijacked” and used by bacteria to traverse the intestinal mucosa. There is increasing evidence that the increase of M cell
number and M cell apoptosis observed during chronic intestinal inflammation may be responsible for the enhanced uptake of microorganisms during these conditions (Kucharzik et al. 2000).

Short bowel syndrome

Short bowel syndrome is defined as the malabsorptive state that often follows massive resection of small intestine. Most cases originate in the neonatal period. Inborn malformations such as gastroschisis, volvulus and atresia as well as necrotising enterocolitis and intraabdominal tumours are often subjects of extensive surgery with resections of large lengths of bowel. Before the introduction of parenteral nutrition in the late 1960s most of these patients died from severe malnutrition and dehydration. Intravenous total parenteral nutrition (TPN) changed the outcome dramatically by providing nutritional support during the process of intestinal adaptation (Dudrick et al. 1968), (Broviac and Scribner 1974). Nowadays, the undisputed mainstay of therapy in short bowel patients is TPN through a central venous catheter. Excellent survival can generally be demonstrated, at least in the short term. The one-year survival of children on TPN is about 90% (Howard et al. 1995). However, some patients develop life-threatening complications such as catheter-related sepsis and parenteral nutrition associated liver disease (PNALD). Many of the septic episodes are caused by enteric organisms, suggesting that bacterial translocation from the remaining bowel could play a pivotal role in the genesis of these infections (Alverdy et al. 1988). The histopathological findings in PNALD are progressive intracellular and canalicular cholestasis, portal and lobular inflammation, macrophage hyperplasia, bile duct proliferation, and fibrosis (Dahms and Halpin 1981). Animal studies indicate that after massive intestinal resection, intestinal bacteria and their by-products can translocate to the liver via the portal venous and lymphatic systems (O’Brien et al. 2002). Bacterial byproducts inhibit hepatocellular bile acid transport and activate hepatic macrophages via locally produced cytokines (Reimund et al. 2001) (Kawaguchi et al. 2000). Hepatocellular cholestasis and necrosis, inflammation and fibrosis then supervene. The liver deterioration is probably worsened by multiple factors, including inadequate composition or delivery of the TPN solution, lack of enteral nutrition and disrupted enterohepatic recirculation of bile acids (Reimund et al. 2001), (Sondheimer et al. 1998). Once the PNALD is irreversible the only option left is combined liver and small bowel transplantation. This may permit survival of some patients but less drastic and more effective interventions are highly needed. One of those is the prevention of bacterial translocation.
Small bowel transplantation

Small bowel transplantation has lately become a therapeutic option for irreversible intestinal failure. However, the clinical progress has had some major setbacks, including high risks for infectious complications, rejection and development of lymphoproliferative disease (Grant 1999). These setbacks are primarily due to the great antigenicity caused by the large number of immunocompetent cells in the bowel in combination with the heavy immunosuppression thus needed. The effects of harvesting, preservation, ischemia, reperfusion and immunosuppression also damage the bowel mucosa and render the patient susceptible for bacterial translocation and sepsis. In addition, the permanent cessation of central autonomous innervation, temporary loss of normal lymphatic drainage, and disturbed peristalsis further enhances the risk of bacterial translocation. Consequently, infectious complications are the major contributors to mortality and morbidity after small bowel transplantation. From April 1, 1985 until May 31, 2003 a total of 989 intestinal transplants have been performed worldwide. The overall 1-year patient survival is approximately 65%. According to the intestinal transplant registry 46% of the mortality in intestinal recipients is primarily due to sepsis. According to Cicalese et al bacterial translocation from the gut plays an important role in the development of these infections (Cicalese et al. 2001). In contrast to what one might suspect from experimental studies (Grant et al. 1991), episodes of rejection did not aggravate the bacterial translocation in the clinical study. Measures to improve intestinal barrier function are important to make small bowel transplantation a true therapeutic alternative in cases of intestinal failure. This is particularly important in the light of increasing microbial resistance to conventional antibiotics.

The intestinal mucosal barrier

The microbial population of the small and large intestine is in a dynamic equilibrium with the normal physiologic and nutritional activities of the host. The mucosal surface is an environment of intimate interaction between microorganisms and eucaryotic mucosal cells. The total numbers, as well as the number of species, of microorganisms increase progressively down the gastrointestinal tract. In the healthy human, the stomach and proximal small intestine contain sparse bacterial flora that includes both aerobes and anaerobes (<10^4/ml aspirate). Acidity and mobility seem to be the major factors that inhibit the growth of bacteria within the stomach. The flora of the distal small bowel represent a transitional zone between the microflora of the upper and lower gastrointestinal tracts; the numbers of aerobic and anaerobic bacteria that are usually detected is about 10^5-10^6/ml. The concentration of bacteria is highest in the colon where up to 10^9 aerobic and 10^11 anaerobic
bacteria/ml of aspirate can be found (Langkamp-Henken et al. 1992). In
total, the human harbour more than 400 different species of bacteria. Of these species, few have been shown to translocate to the MLNs and other
glands with any frequency. Although anaerobic bacteria may outnumber aer-
obes by 10:1 to 1000:1, anaerobes rarely undergo bacterial translocation in
experiments. The mechanism responsible for anaerobes’ low rates of bacte-
rial translocation is unclear although their adhesive properties to epithelial
cells have been documented (Duffy et al. 1994), (Wells et al. 1987). Gram
negative, facultative anaerobic Enterobacteriaceae, e.g. Escherichia coli, Proteus mirabilis, Enterobacter cloacae and Klebsiella pneumoniae, are
frequent translocators and often found in cultures from MLNs in studies of
bacterial translocation (Steffen et al. 1988). The same bacteria are the ones
most frequently cause bacteraemia in hospitalised patients (Yinnon et al.
1997).

In conclusion, the large mucosal surface (~250 m²) of the gastrointestinal
tract is exposed to many potentially invasive microorganisms. The ecologic
stability of these endogenous organisms has been characterized as pivotal in
promoting resistance to mucosal colonization by exogenous microorganisms
(van der Waaij et al. 1972).

Immunological antibacterial factors
To handle the high microbial and antigenic load of the intestine, there is an
intricate and extensive defense system. The small intestinal immune system
contains the largest accumulation of immunocompetent cells in the body
(Trepel 1974). These cells are either localized in distinct aggregates (Peyer’s
patches and lymphoid nodules) or diffusely distributed in the lamina propria
of the mucosa. Lymphocytes are also present within the epithelial layer of
the mucosa. An important function of the mucosal immune system is to pre-
vent entry of microorganisms and their products (Janeway et al. 2001). Pri-
marily, this is achieved by the production of secretory IgA (Cebra et al.
1977) by the lamina propria plasma cells. Secretory IgA coats the lumen of
the small intestine and prevents any microbial pathogen or virus from pen-
etrating the epithelial layer and passing into other organs. By this blockage
the pathogens can be transported downstream and be eliminated (Spaeth et
al. 1994). The secretory IgA antibodies are directed against biologically ac-
tive antigens such as viruses, bacteria, enterotoxins and enzymes normally
not present in the intestinal tract. A reduction in the IgA level results in an
increased frequency of gastrointestinal infections and impaired reticuloendo-
theial or macrophage function that predispose the patient to systemic bac-
teremia (Alverdy 1992), (Mayer 2000). Microorganisms and antigens that
succeed in traversing the mucosal barrier and the basement membrane of the
epithelium are processed by dendritic cells and macrophages in the lamina
propria and presented to lymphocytes (James 1993). Activated macrophages and T cells start production of certain mediators such as proinflammatory cytokines, proteases and prostaglandins, which are responsible for the inflammatory systemic response that follows (Ayala and Chaudry 1996), (Brandtzaeg et al. 1989).

In order to delineate the role of the different parts of the immune system in bacterial translocation, scientists have used substances and antibodies that inhibit or impair certain steps in the immunological response. Especially when the researcher is involved in the field of transplantation focus has been set on the T cells. This is because T-cell immunosuppression has been the cornerstone in controlling organ rejection during the last 25 years. During this time it has become evident that viral infections, as well as bacterial infections, cause major morbidity in transplant recipients. Traditionally T cells have been considered to mainly be involved in the cellular defense against invading viruses and to have a central role in guiding the immune response. Depletion or inactivation of different subsets of T cells has been studied in a few different models of bacterial translocation (Maddaus et al. 1988), (Hoffmann et al. 2001), (Choudhry et al. 2001), (Choudhry et al. 2002). Most of these models have implicated a protective function of the T cell against bacterial translocation.

The effect of inactivation or activation of macrophages on bacterial translocation has been the subject of few studies up until now (Berg, R. 1999). Phagocytic cells, such as the macrophage, are likely the ultimate immune effector cells in the defense against bacterial translocation. However, the data are inconsistent. Most of the studies indicate a protective function of the macrophage. On the other hand, sometimes the activation of macrophages can induce an inflammatory response that actually increases the translocation of bacteria. This is the case for example in endotoxic shock (Deitch et al. 1991). Furthermore, other authors claim that the macrophage is responsible for the passage of bacteria to extra-intestinal sites (Wells et al. 1987). According to the upholders of this theory the macrophage phagocytizes bacteria in the bowel wall. Then, after being attracted to some other location in the body, the macrophage for some reason fails to degrade the ingested bacteria. After the death of the phagocyte the bacteria are released at an extra-intestinal site and the bacterial translocation is complete. Probably this mechanism occurs in some situations and some microorganisms. In summary, the macrophage in bacterial translocation seems to have a dual role. Since the macrophage population is so heterogeneous further studies to elucidate the role of different subpopulations of macrophages are relevant.
Non-immunological antibacterial factors

The constant peristalsis propels the intestinal contents in a distal direction towards the colon. This is important in preventing overgrowth of bacteria in the proximal bowel (Sherman and Lichtman 1987). Moreover, the ileocecal valve functions as a barrier to prevent reflux of colon contents into the small bowel and thus retrograde colonization of the ileum with colon flora (Schimpl et al. 1999).

An integral part of the intestinal mucosal barrier is the mucous gel layer that overlays the mucosal epithelial surface (Bengmark and Jeppsson 1995). It consists of mucin that is a network of high-molecular-weight glycoproteins. On top of this layer is a thin (bimolecular) layer of phospholipids forming a membrane with hydrophobic, non-wettable, acid-resistant properties. During the last 20 years, evidence has accumulated indicating that the hydrophobic characteristic of the mucus layer is important in the protection of the intestinal mucosa. The lipid bilayer is highly impermeable to ions and most polar molecules and forms a layer of defined viscosity and low friction (Lichtenberger 1995). The mucous gel layer also serves as an anchoring site for different indigenous bacteria and secretory immunoglobulins. In that way, providing receptor sites for probiotic flora as well as inhibiting the adherence of pathogenic bacteria to enterocytes. Taken together, these characteristics form a strong surface protection system against invading microbes and other potentially harmful substances in the luminal content.

Other intraluminal factors are probably also of great importance in upholding balance of bacterial ecology and integrity of the mucosal barrier. Gastric acidity acts as a defense against ingested bacteria. Gastric juice with a pH less than 4 is bactericidal for most organisms. Furthermore, enzymatic digestion significantly reduces bacterial overgrowth. Pancreatic juice is rich in digestive enzymes that have known antibacterial properties (Bassi et al. 1991). Lysozyme, produced by Paneth cells in intestinal glands in the small intestine, is another antibacterial peptide. In addition, osmolality, redox state, hormones and growth factors and the availability of certain nutrients are likely to affect microbial growth. These factors probably also influence the virulence of the indigenous bacteria. The genes that code for different virulence factors, for example proteases and different cell receptors, can be quickly turned on or off depending on the bacterium’s environment (Alverdy et al. 2003).

Furthermore, the intestinal tight junctions between enterocytes are thought to be an important part of the intestinal barrier. Tight junctions are dynamic structures and their function may be modulated by nutrients such as glucose and amino acids as well as bacterial toxins and chemotaxins. Glutamine is
considered to be the principal respiratory fuel for enterocytes. A lack of glutamine promotes mucosal atrophy and increases intestinal permeability to small molecules (Sasaki et al. 1997), (Wiren et al. 1995). If this increases bacterial translocation is not proven in the human. It was initially suggested that bacteria pass through the space between the enterocytes during the process of bacterial translocation. However, accumulating evidence indicate that most microbes instead pass through the enterocytes, and not between the cells, by a process that is different from phagocytosis (Alexander et al. 1990).

Beyond the intestinal mucosal barrier

If all intestinal defense systems fail and bacteria or other microorganisms succeed in traversing the bowel wall, other back-up systems go to work. These are primarily the MLNs that scan the draining lymph. MLNs belong to the gut associated lymphoid tissue. The MLNs drain lymph from the small and large intestine and take part in the elimination of antigens (Laissue et al. 1993). T cells are concentrated in the parafollicular region where dendritic cells present antigens, while B cells respond to antigens in the follicles of the cortex. Some B cells move to the medulla of the MLN and initiate antibody production. Both activated T and B cells leave the MLNs via the efferent lymph and migrate to the site of inflammation or infection. Adhesion molecules, such as CD11a/CD18, help in the migration of lymphocytes into mucosal sites by binding intercellular adhesion molecules (ICAMs) and are involved in various adhesive interactions in the inflammatory process (Bargatze et al. 1995). Selective localization is directed in part by the differential expression of homing receptors on the surface of circulating cells and endothelial cell adhesion molecules in the target tissues. The integrin \( \alpha_4\beta_7 \) that binds to mucosal adressin cell adhesion molecule (MAdCAM) is essential in lymphocyte homing to intestinal lamina propria and Peyer’s patches.

If the bacteria enter the portal venous blood instead of the lymph, they end up in another highly inhospitable environment. The blood is armed with macrophages, neutrophils, immunoglobulins and complement factors. Furthermore, upon entering the liver sinusoids they encounter the Kupffer cells of the liver that are highly efficient macrophages.

Some bacteria have adapted strategies to escape recognition by the immune system. Listeria for example can survive inside a macrophage and use it as a vessel to travel safely through the body (Vazquez-Boland et al. 2001). To what extent other bacteria can use similar approaches to survive is not entirely known.
When one or a few of these back-up mechanisms fail to clear bacteria, perhaps due to immunosuppression or too great a microbial load, systemic infection can ensue.

Experimental models
To get a better understanding of the processes involved in bacterial translocation, experimental studies have been performed in a number of animal models. These include models for short bowel (Aldazabal et al. 1998), small bowel transplantation (Grant et al. 1991), acute liver failure (Wang et al. 1994), acute pancreatitis (Marotta et al. 1996), peritonitis (Osterberg et al. 1997), enterocolitis (Fox et al. 1988), portal hypertension (Garcia-Tsao et al. 1993), thermal injury (Baron et al. 1994), haemorrhagic shock (Baker et al. 1988), malnutrition (Barber et al. 1991), and acute bowel ischemia (Hammer-Hodges et al. 1974).

Thiry-Vella loop model
To our knowledge, no model for bacterial translocation with indigenous flora from a disconnected and defunctionalised small bowel has been established, except the cumbersome small bowel transplantation setting. In Paper I we present such a model. Using a Thiry-Vella loop of small bowel we try to mimic some of the conditions that apply to both the short bowel syndrome and early after intestinal transplantation. Namely, the absence of passing food and proximal secretions, stagnation of intestinal content, transection of the bowel, the presence of stomas, probable overgrowth of bacteria and changes in the microenvironment.

Small bowel transplantation models
Lillehei et al did much of the pioneering work in the field of small bowel transplantation in the late 1950s. They used large animals such as the dog for their experiments. In 1971 Monchik and Russel presented an auxiliary, heterotopic technique for small bowel transplantation in the rat (Monchik and Russell 1971). The superior mesenteric artery and the portal vein of the graft were anastomosed to the recipient’s aorta and inferior caval vein, respectively. Wallander et al later modified this model by using a non-suture, collar and cuff method with the vessels anastomosed to the recipient’s left renal vessels (Wallander et al. 1988). The two ends of the graft can be exteriorized as stomas - thus producing a Thiry-Vella loop. This is the model used in this thesis. By choosing a syngeneic or allogeneic graft one can control the influence of rejection on the bacterial translocation. Earlier experimental studies have shown that when, as a result of rejection, the bowel loses its barrier
function, bacteria are easily translocated from the intestinal lumen to the MLNs of the graft and to the liver and spleen, thus increasing the risk of systemic infections (Grant et al. 1991). However, as mentioned above this might not be the case in clinical small bowel transplantation (Cicalese et al. 2001).

Treatment strategies in bacterial translocation

The ultimate goal of many scientists interested in the field of bacterial translocation has been to find a substance or treatment that can prevent bacterial translocation. So far no preventive measure or treatment has proven unequivocally effective. Many different candidate substances have been tested. Based on the knowledge of the predisposing factors these can largely be divided into three categories:

1. Substances that diminish the bacterial load in the intestine
2. Substances that reinforce the mechanical mucosal barrier
3. Substances that boost the immune system

Substances that diminish the bacterial load in the intestine

In the clinic, administration of classical antibiotics is probably still the most frequently used therapy in cases of suspected bacterial translocation. A course of broad-spectrum antibiotics is commenced and continued until the symptoms wean. This approach has several disadvantages: First, the broad-spectrum antibiotics affect all bacteria not only the ones responsible for the translocation. Second, many members of the so-called probiotic flora are more sensitive to antibiotics than the pathologic flora. Often one or a few species are more or less resistant against the antibiotics chosen and antibiotic treatment pave the way for their overgrowth. In accordance, studies have shown that treatment with antibiotics actually promote bacterial translocation (Berg 1981). Third, yeast and other fungi gain increased possibilities of growth within the intestinal tract.

An alternative strategy is treatment with probiotic bacteria. These are apathogenic bacteria that maintain a beneficial bacteriologic ecology inside the bowel – thus preventing the overgrowth and epithelial association of more pathogenic bacteria. This phenomenon has been termed colony resistance, where competition for receptor sites, substrates and trace elements such as iron limits bacterial growth. Typically these probiotics are members of lactobacillus or bifidobacter families. A number of experiments indicate a
decreased bacterial translocation using probiotics (Adawi et al. 2001; Eizaguirre et al. 2002; Seehofer et al. 2004).

Medications that decrease the intestinal transit time and support a normal secretion of saliva, gastric acid, pepsin, bile and pancreatic juice can also decrease the numbers of intraluminal bacteria (Erbil et al. 1998).

Substances that reinforce the mechanical mucosal barrier
Experiments have been made with growth factors such as growth hormone (GH; (Wang et al. 2001)), insulin like growth factor 1 (IGF-1; (Zhang et al. 1995), epidermal growth factor (EGF; (Xia et al. 2002)) and glucagone like peptide 2 (GLP-2; (Kouris et al. 2001)). In addition, enterocyte nutrients (e.g. glutamine and fibers), factors of the mucosal surface protection system (e.g. phospholipids and mucus), and other intraluminal protective substances (e.g. glutathione, endotoxin neutralizing antibodies) have been evaluated. Inhibition of bacterial association with the enterocyte and the subsequent transgression of the intestinal epithelium is the main goal.

Glutamine
The amino acid glutamine has been thoroughly studied in animal gastrointestinal research during the last 25 years. It is generally accepted that glutamine is used as a major substrate by enterocytes and immune cells. During critical illness the skeletal muscle exports a large amount of glutamine and the metabolism of glutamine utilizing tissues, such as the gut and the immune system, increases. When tissue glutamine utilization exceeds endogenous production, plasma glutamine levels drop. This relative glutamine deficiency has been proposed to compromise intestinal mucosal barrier function (Wilmore et al. 1988). A number of animal studies, in mainly rodents and pigs, support this theory and show that supplementation of glutamine enhances growth and function of the intestinal mucosa and decreases bacterial translocation and septic complications. The administration route of glutamine has varied in different studies, but it seems that both enterally and parenterally administered glutamine can have beneficial effects in rodents and in the pig (Li et al. 1999; Ding and Li 2003). Since many cells of the immune system use glutamine as a major substrate, it has been proposed that the positive effect seen in some of the models after treatment with glutamine is accounted for by an increased activity of the immune cells (Salvalaggio et al. 2002; Kuru et al. 2004; Wu et al. 2004).

Phospholipids
An integral part of the mucosal barrier is the mucus gel layer that lines the luminal surface of the bowel. This layer is thought to prevent pathogenic bacteria from adhering to the enterocyte, which is the initiating step in bacte-
rial translocation (Beachey et al. 1988). It also protects the bowel mucosa from acids and other noxious agents inside the bowel and it functions as an anchoring site for secretory immunoglobulins (McSweegan et al. 1987). The layer consists of water and electrolytes, cell debris, secreted immunoglobulins, mucin, and different kinds of phospholipids. The most abundant phospholipid, at least in the rat gastric mucus and rabbit small intestine, is phosphatidylcholine (Bernhard et al. 1995), (Okuyama et al. 1998). In certain experimental settings it has been shown that the addition of exogenous phospholipids decreases mucosal injury and bacterial translocation (Lichtenberger et al. 1983), (Anand et al. 1999), (Wang et al. 1994), (Guo et al. 1994). However, the effect of phospholipids on intestinal permeability is still controversial (Tagesson et al. 1985).

Substances that boost the immune system

As discussed above, the host immune defenses are integral parts of the pathogenesis of translocation. If this defense is efficient in killing translocating bacteria on their way to the MLNs or inside the MLNs no positive bacterial culture would be obtained and by definition no translocation has occurred. Many of the substances described above also affect the immune system. Different strategies to directly boost the immune system have been adopted. These include vaccination and the subsequent production of specific antibodies (Berg 1995), non-specific activation of macrophages (Fuller and Berg 1985), treatment with immune cell nutrients (e.g. glutamine; (Salvalaggio et al. 2002)), and priming of the immune system with intestinal microflora (e.g. probiotics (de Vrese and Schrezenmeir 2002)). Furthermore, addition of IgA may prevent bacterial translocation (Diebel et al. 1997). Evidence suggests that the host immune system is especially important in limiting translocation beyond the MLNs (Berg et al. 1988).
AIMS OF THE INVESTIGATION

The general purpose of this study was to further characterize mechanisms and immunological reactions involved in bacterial translocation from small bowel.

Additionally, specific aims were to:

- develop a model for bacterial translocation from small bowel in the rat that mimics some of the conditions that exist in the short bowel syndrome and small bowel transplantation
- study the effect of Kupffer cell depletion
- study the effect of mesenteric lymphadenectomy
- study the impact of T cell inactivation
- evaluate measures to improve the mucosal barrier with glutamine and phosphatidylcholine
- characterize the cell mediated immunological response to bacterial translocation

All studies were made in the Thiry-Vella loop model for bacterial translocation.
MATERIALS AND METHODS

Animals, operation and drug treatment

Animals and anaesthesia (Paper I-IV)
Male outbred Sprague Dawley rats and inbred Lewis (RT1\(^1\)) and BN (RT1\(^n\)) rats with conventional bacterial status were used. The animals were acquired from M&B (Ry, Denmark) and were allowed to settle for one week before any experiment was started. They were kept at 22\(^\circ\)C, with 12-h light/dark cycles, fed standard pellet chow and water ad libitum. At the time of operation their weight was 230-400 g (Sprague Dawley) and 200-270 g (Lewis, BN). Anaesthesia was induced by intraperitoneal injections with a mixture of chloral hydrate (180 mg/kg b.w.) and pentobarbital (40 mg/kg b.w.). Short anaesthesia during intravenous injections was induced using 4.5% halothane in oxygen. After that the rats were maintained on mask anaesthesia with a mixture of nitrous oxide, oxygen and isoflurane (30/70/1.2-1.5%; Paper III). All operations were performed under aseptic conditions. The experiments were approved by the regional ethical committee and performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Research Council in 1996.

Thiry-Vella loop operation (Paper I-IV)
After induction of anaesthesia the abdomen of the Sprague Dawley rat was opened by a midline incision. The small bowel and caecum were exposed and the ileum transected 10 cm and 55 cm proximal to the ileocaecal junction. The distal and proximal ends of the created 45-cm-long segment were exteriorised as two stomas on the left side of the abdomen - resulting in a Thiry-Vella loop (Figure 1). The stomas were sutured with 5-0 Prolene\(^®\) (Ethicon GmbH, Norderstedt, Germany); four sutures in the abdominal muscles and four sutures in the skin. The remaining small bowel was anastomosed end-to-end using 7-0 Vicryl\(^®\) (Ethicon GmbH) sutured continuously through all layers of the intestinal wall, to restore gastrointestinal continuity. This resulted in an approximately 50% reduction of functional small bowel
length. Finally, the abdominal muscles were closed with continuous 4-0 Vicryl®, as was the skin. Before closure, the abdomen was filled with sterile saline to compensate for some of the fluid losses.

Figure 1  Schematic drawing of the Thiry-Vella loop.

Small bowel resection (Paper I-III)
Operative controls underwent a similar procedure as above, but did not obtain a Thiry-Vella loop. Instead the 45-cm-long intestinal segment was resected.

Small bowel transplantation (Paper I and II)
Allogeneic (Paper I) or syngeneic (Paper II) small bowel transplantation was performed using the nonsuture cuff technique described in detail elsewhere (Wallander et al. 1988). When performing allogeneic transplantation, BN rats were used as donors and Lewis rats as recipients. In syngeneic transplantations Lewis rats were used as both donors and recipients. The entire small bowel of the donor was dissected free on a vascular pedicle consisting of the superior mesenteric artery and the portal vein. The vascular system was perfused with FW solution (Jacobsson et al. 1989) at low pressure (35 cm H₂O). Meanwhile, the lumen was irrigated with 20 ml FW solution. The bowel was then transplanted heterotopically to the left renal vessels of the recipient. After restoring the blood flow, the proximal and distal ends of the graft were exteriorised as stomas. A single dose of cefuroxim (Zinacef®, GlaxoWell-
come, Greenford, U.K.), 20 mg/rat, was given intramuscularly (Paper I). The transplanted rats were not given any immunosuppressive treatment.

Immunosuppressive treatment (Paper I and III)
Rats were treated with cyclosporine A (CyA; Novartis, Basel, Switzerland), 15 mg/kg b.w. per day, with start three days preoperatively. CyA was administered in an Intralipid® (Pharmacia & Upjohn, Stockholm, Sweden) emulsion by oral gavage using a nasogastric gavage sized 5 CH.

Mesenteric lymphadenectomy (Paper II)
Removal of the MLNs of the Thiery-Vella loop or small bowel graft was performed at the end of the operation, just prior to closing the abdomen. The peritoneum overlying the MLNs was opened and the MLN vessels were ligated with 7-0 Vicryl® and the lymph nodes were then removed with a small forceps. The MLNs of the rest of the bowel were left intact.

Kupffer cell depletion (Paper II)
On the first postoperative day the rats were reanaesthesised and 30 mg/kg b.w. of gadolinium chloride (Sigma-Aldrich Co., St. Louis, MO, USA) was injected, using a 27G needle, in the left femoral vein. The gadolinium chloride was diluted in sterile saline to a final concentration of 7.5 mg/ml before injection.
To control that gadolinium chloride treatment per se did not result in bacterial translocation, non-operated rats also received the same dose of gadolinium chloride.

T-cell inhibition (Paper III)
The monoclonal antibody R73 directed against the α/β subset of the T cell receptor was used to inhibit α/β T-cell function. Azide-free R73 was purchased from Serotec Ltd Scandinavia (Oslo, Norway) and diluted in saline to a final concentration of 1 mg/ml immediately before administration. Next, 0.5 mg of the antibody was injected intravenously immediately postoperatively and also one day postoperatively during short anesthesia. At harvest, R73 binding to T cells was confirmed using flow cytometry of cell suspensions from MLNs and spleen. A double staining protocol with the antibody R73 labelled with red phycoerythrin (PE) and a goat-anti-mouse-Ig (GAMIG) labelled with green fluorescein isothiocyanate (FITC) was used. In that way we were certain to label all T cells, both those with free antigenic sites for R73 on their T cell receptors and those that hypothetically have all
antigenic sites occupied with R73 given intravenously. The latter cells will be labeled by the binding of GAMIG to the R73 antibodies.

Enhancement of the mucosal barrier (Paper IV)

**Glutamine treatment**

L-glutamine (Sigma-Aldrich Sweden AB, Stockholm, Sweden) was diluted in sterile water to a concentration of 50 mg/ml. The animals were administered 1.0 g/kg/day by oral gavage 6 times daily. Treatment was started 7 days preoperatively and continued by giving the same amount in the proximal stoma postoperatively until the time for sacrifice.

**Phosphatidylcholine treatment**

One hundred milligrams of lyophilized 1,2-diacyl-sn-glycero-3-phosphocholine (Sigma-Aldrich Sweden AB, Stockholm, Sweden) was dissolved in 0.2 ml of 70% ethanol and then mixed in sterile water to a final concentration of 10 mg/ml. This emulsion was sonicated for 5 min to produce small liposomes (closed spherical bilayers) of phosphatidylcholine in an aqueous milieu. This emulsion was then administered to the animals at a dose of 130 mg/kg/day 4 times daily with start 3 days preoperatively. The fatty acid content of this phosphatidylcholine was 33% palmitic, 31% oleic, 15% linoleic, and 13% stearic (other fatty acids being minor contributors). The emulsion was freshly made before each administration.

**Intestinal decontamination (Paper IV)**

A group of animals received 24 mg/kg/day of polymyxin E (Sigma-Aldrich, Stockholm, Sweden) and 20 mg/kg/day of tobramycin (Nebcina®, Lilly, Indianapolis, USA) twice daily by oral gavage. Selective bowel decontamination (SBD) was started 3 days prior to the Thiry-Vella loop operation and continued in the proximal stoma until sacrifice of the animal.

**Bacteriology**

**Bacteriological culturing and analysis (Paper I-IV)**

After 1, 3 or 7 days tissue biopsies from MLNs of the Thiry-Vella loop or the intestinal transplant were collected aseptically and transferred into empty test tubes containing CO₂ to ensure survival of anaerobic bacteria. In Paper II, III and IV biopsies were also taken from MLNs of the normal bowel,
liver, spleen, and lung. In these papers biopsies were only taken after 3 days. After weighing, the samples were homogenised in 0.5 ml serum broth, and 0.1 ml of the homogenate were spread onto blood-, hematin- (CO$_2$ incubation) and CLED-agar, incubated aerobically and J-agar incubated anaerobically using Dickinson anaerobic GasPack system (Becton Dickinson Microbiology Systems, Cockneyville, USA). In paper II to IV only aerobic incubation was made on blood and CLED agar. The plates were incubated at 35°C for two to three days. Bacteria were counted (colony forming units, CFU) and classified according to standard methods. The numbers of CFUs were expressed per gram of tissue.

One milliliter of blood was injected into biphasic aerobic and anaerobic blood culture media. The bottles were incubated for 7 days at 35°C and visually inspected. Bottles with turbidity were spread onto agar plates and the bacteria were classified using standard methods. In Paper II, III and IV, instead 2 ml of blood were injected into BacT/ALERT®PF blood culture media (Biomérieux Inc, Durham, NC, USA). The bottles were incubated at 35°C and continuously monitored for growth. Positive cultures were subcultured onto blood agar and CLED agar and classified using standard methods. Bacterial growth in blood is expressed as positive or negative.

Swab samples from the peritoneal cavity were spread onto agar plates as described above. Bacterial growth is expressed as number of CFUs per swab sample.

Endotoxin determination (Paper I)

Blood from the portal vein and suprahepatic caval vein were collected in EndotubeET® vacutainer tubes (Chromogenix AB, Mölndal, Sweden) and kept on ice for approximately 20 min before centrifugation (2500 g, 10 min, 4°C). The plasma was immediately frozen at -70°C. Endotoxinfree glass tubes and vials, preheated at 180°C for 4 h were used for all endotoxin assays. Sterile pyrogen-free syringes and pipettes were utilised.

Analysis of endotoxin was performed in duplicate with the chromogenic LAL assay (Endosafe Inc, Charleston, USA). Samples were diluted 1:10 with endotoxinfree water and heated to 75°C for 5 minutes. Standard curves ranging from 0.06 to 0.6 EU/ml were obtained using pyrogen-free plasma. Samples with higher endotoxin values were diluted in endotoxin-free water. The concentration (EU/ml) of the samples was determined using standard regression analysis. Endotoxin levels above 0.1 EU/ml were considered positive.
Morphology and cell analysis

Flow cytometry (FACS; Paper III-IV)

Harvested MLNs from the Thiry-Vella loop and from normal small bowel as well as the spleen were placed in sterile phosphate buffered saline (PBS), then minced through a fine steel wire net and washed with PBS. To remove erythrocytes from the cell suspension, 1 ml of Red Cell Lysing Buffer (Sigma-Aldrich Chemie GmbH, Schnelldorf, Germany) was used for 5 min incubation followed by washing with PBS and diluted to a single cell suspension of approximately 100x10⁶ cells/ml. These cells were then incubated with antibodies (Table 1) and analysed by flow cytometry (Holmes and Fowlkes 1995). Briefly, cells from MLNs were diluted in 2 ml of PBS (containing 0.1% of NaN₃) and cells from the spleen in 10 ml. Then, 100 μl from each organ was distributed into each of seven tubes. The CD4/CD8 ratio and the percentage of T lymphocytes expressing major histocompatibility complex (MHC) class II antigens or CD25 (IL-2 receptor α chain; IL-2R) were determined by double staining using monoclonal antibodies conjugated with either fluorescein isothiocyanate or phycoerythrin (Table 1). The antibodies were added and the tubes were incubated at 4°C for 10 min. The cells were washed once with PBS (or saponin) and were then fixed in a 1% paraformaldehyde-PBS solution. Cells in one tube from each organ was washed with saponin instead of PBS in order to shatter the cell membrane to be able to label intracellular antigens in macrophages with the ED1 antibody. To determine possible autofluorescence of the analysed cells, antibodies were omitted from one of the tubes. Two tubes were used for single staining with each of the two fluorophores for compensation procedures.

Analysis was performed on an FACS Calibur® flow cytometer (Beckton Dickinson, San José, CA, USA) using the Cellquest® software. Leukocytes were identified and gated according to a forward scatter/side scatter dot plot with linear scales. A quadrant gate was used with FL1 on the x-axis and FL2 on the y-axis, to be able to separate unstained, single stained and double stained cells from each other according to fluorescence intensity. A total of 10,000 gated cells were analysed in each sample.
Table 1 Details for the antibodies used for flow cytometry

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Clone</th>
<th>Fluorophore</th>
<th>Specificity/Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>TcRαβ</td>
<td>R73</td>
<td>PE</td>
<td>α/β chain of T-cell receptor (97% of peripheral T cells)</td>
</tr>
<tr>
<td>CD8</td>
<td>OX8</td>
<td>FITC</td>
<td>Cytotoxic/suppressor T cells and most NK cells</td>
</tr>
<tr>
<td>ED1</td>
<td>ED1</td>
<td>FITC</td>
<td>Monocytes and most macrophages</td>
</tr>
<tr>
<td>MHC class II</td>
<td>OX6</td>
<td>FITC and PE</td>
<td>MHC class II antigens</td>
</tr>
<tr>
<td>IL-2R</td>
<td>OX39</td>
<td>FITC</td>
<td>Stimulated rat T-cells</td>
</tr>
</tbody>
</table>

Antibodies were acquired from Serotec (Oxford, U.K.).

Immunohistochemistry (Paper I-IV)

Tissue biopsies were obtained at the various time points and stained immunohistochemically. As primary antibodies the monoclonal mouse-anti rat antibodies R73 (Paper I-IV), ED1 (Paper I-IV), OX8 (Paper III-IV), ED2 (Paper II), and W3/25 (Paper III-IV) were used. For the specificity of the first three antibodies see Table 1. ED2 is directed against tissue macrophages and W3/25 is directed against the CD4 domain of helper T cells and macrophages.

Acetone-fixed cryostat sections, 6 μm thick, were stained using a peroxidase-antiperoxidase staining method PAP; (Sternberger 1979). In brief, the sections were incubated with 0.3% H₂O₂ in PBS to inhibit endogenous peroxidase activity and, thereafter, with normal goat sera to prevent non-specific background staining before incubation with the primary antibody. A secondary antibody, goat anti-mouse IgG (Jackson ImmunoResearch Laboratories, West Grove, PA, USA) was used and added in excess. In the next step, the sections were incubated with horseradish peroxidase-mouse antiperoxidase (Dakopatts, Glostrup, Denmark). Finally, H₂O₂ as substrate and 3-amino-9-etyl-carbazol as electron donor were added to react with the horseradish peroxidase to produce a brown colour. Counterstaining was performed using Mayer’s haematoxylin. In controls, the primary antibody was excluded. All slides were evaluated blindly. The follicular, cortical and marrow regions of the MLNs were scored separately as were the villi, crypts and mucularis of the small bowel, red and white pulp of the spleen and periportal and non-periportal zones in the liver.
Histology (Paper I)
In order to confirm rejection, biopsies were taken from the grafted small bowel at the time of sacrifice. The biopsies were fixed in 4% formalin and stored in room temperature until embedded in paraffin. Sections of 4 µm thickness were stained with haematoxylin and eosin and evaluated in a light microscope.

Statistical analysis (Paper I-IV)
For comparisons between groups the Mann-Whitney U-test was used. A p-value less than 0.05 was considered statistically significant. The levels of significance considered were p<0.05 (●), p<0.01 (★★) and p<0.001 (★★★).
EXPERIMENTS AND RESULTS

Paper I

In these experiments we studied whether bacterial translocation occurred from a defunctionalised segment of small bowel to MLNs of the segment or blood. An ileal segment from untreated or CyA-treated rats was exteriorised as a Thiry-Vella loop. After 1, 3 or 7 days, bacterial translocation and distribution of immunocompetent cells were assessed. The data obtained were compared with data from animals subjected to allogeneic intestinal transplantation. As operative controls we used rats that had the 45-cm segment resected. An additional control group was non-operated rats.

Translocation of bacteria to the MLNs was detected in 60% of the Thiry-Vella loop-operated rats day 1, in 100% day 3 and in 83% day 7. There was a tendency toward a greater number of translocating bacteria to the MLNs in the CyA-treated group than in the non-CyA-treated group, which was statistically significant on day 3 (p<0.01, Figure 2). Positive bacterial cultures from sampled blood were only seen in a few stray animals. None of the samples, MLNs or blood, from the control groups showed any bacterial growth at any time, except one animal in the non-operated group where a few coagulase-negative staphylococci, probably related to contamination, were found in the MLNs.

Rejecting small bowel grafts displayed a bacterial translocation to the MLNs of the graft in 100% of the animals 7 days after transplantation. The number of bacteria did not differ from either immunosuppressed Thiry-Vella loop animals at 1, 3 and 7 days or from non-immunosuppressed Thiry-Vella loop animals at 3 and 7 days.
Figure 2  Bacterial translocation to the mesenteric lymph nodes 1, 3 and 7 days after surgery in animals with a 45-cm-long Thiry-Vella loop. The animals were either non-immunosuppressed or treated with CyA (15mg/kg b.w. per day) with start three days preoperatively. The results are expressed as the number of CFUs per gram of MLN tissue.

![Graph showing bacterial translocation](image)

* p<0.05 vs Non-CyA treated

The most common bacterial species cultured from the MLN homogenates was *Escherichia coli*. Simultaneous translocation of other species of bacteria such as *Proteus mirabilis*, enterococci, staphylococci, and α-streptococci were encountered in less than one third of the animals with a Thiry-Vella loop. In the transplanted group there was growth of a wider variety of species including *Enterobacter cloacae*, providensiae species, and *Xantomonas maltophilia*.

Evaluation of the immunohistochemistry revealed that the number of ED1-positive macrophages and R73-positive T cells in the MLNs increased from day 1 until day 7 in non-immunosuppressed Thiry-Vella loop rats.
Endotoxin has been postulated to play a major role in bacterial translocation and in sepsis. Endotoxins were determined in blood sampled from the portal and suprahepatic caval vein. With one exception, positive endotoxins (>0.1 EU/ml) were only seen on day 1 in the operated rats. In the Thiry-Vella loop group 60% had positive endotoxins in the portal vein and 50% in the caval vein. The addition of CyA did not alter the results, as 66% were positive in the portal vein and 33% in the caval vein. In the resection group the results were 33% and 33%, respectively. Rats in the non-operated CyA-treated group (all times included) also displayed positive endotoxins in their blood samples (43% and 43% respectively) as well as the non-operated controls (17% and 17%, respectively). The endotoxin values were only once above 0.6 EU/ml.

It was concluded that bacterial translocation occurs from the small bowel to the MLNs in 100% of the animals after 3 days in the Thiry-Vella loop model presented in Paper I. The model offers possibilities to study mechanistic as well as immunological phenomena associated with microbial translocation.

**Paper II**

In this paper the Thiry-Vella loop model for bacterial translocation, described in Paper I, was further characterised and bacteriological sampling was enlarged to include not only MLNs of the Thiry-Vella loop and blood, but also MLNs of normal bowel, peritoneal cavity, liver, spleen and lung. In addition, bacterial translocation was studied not only in the original Thiry-Vella loop model, but also after depletion of Kupffer cells with gadolinium chloride, as well as after the removal of mesenteric lymph nodes of the disconnected segment. All experiments were conducted with day 3 as the endpoint. From Paper I we knew that the bacterial translocation to the MLNs was 100% at day 3.

First it was concluded that bacterial translocation occurred almost exclusively to the MLNs in the original Thiry-Vella loop setting. Only two rats showed growth of a few numbers of bacteria in the spleen and one rat in the peritoneal cavity. The incidence of translocation to the MLNs of the Thiry-Vella loop was still 100%.

When the MLNs of the Thiry-Vella loop were removed at the time of operation no changes in the distribution pattern or the number of translocating bacteria could be detected. Thus, no pronounced translocation to the liver, spleen, lung, peritoneum or blood was seen (Table 2). However, mesenteric lymphadenectomy of a syngeneic small bowel graft resulted in an increased
number of translocating bacteria to the spleen compared with a graft with intact mesenteric lymph nodes.

Treatment of Thiry-Vella loop operated animals with the Kupffer cell inhibitor gadolinium chloride resulted in a significantly increased translocation to the liver, spleen and lung compared to untreated animals with a Thiry-Vella loop and sham-operated controls treated with gadolinium chloride.

Table 2 Bacterial translocation to the different organs investigated (incidence).

<table>
<thead>
<tr>
<th>Group</th>
<th>MLN of loop</th>
<th>MLN of normal bowel</th>
<th>Liver</th>
<th>Spleen</th>
<th>Lung</th>
<th>Peritoneum</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-V</td>
<td>100%</td>
<td>50%</td>
<td>0%</td>
<td>33%</td>
<td>0%</td>
<td>17%</td>
</tr>
<tr>
<td>T-V-MLNs</td>
<td>63%</td>
<td>25%</td>
<td>25%</td>
<td>0%</td>
<td>25%</td>
<td></td>
</tr>
<tr>
<td>TV+GdCl₃</td>
<td>100%</td>
<td>83%</td>
<td>83%*</td>
<td>83%*</td>
<td>83%*</td>
<td>50%</td>
</tr>
<tr>
<td>Res+GdCl₃</td>
<td>50%</td>
<td>0%</td>
<td>67%</td>
<td>0%</td>
<td>17%</td>
<td></td>
</tr>
<tr>
<td>GdCl₃</td>
<td>17%</td>
<td>17%</td>
<td>17%</td>
<td>0%</td>
<td>17%</td>
<td></td>
</tr>
<tr>
<td>SBTx</td>
<td>50%</td>
<td>75%</td>
<td>75%</td>
<td>25%</td>
<td>50%</td>
<td></td>
</tr>
<tr>
<td>SBTx-MLNs</td>
<td>100%</td>
<td>80%</td>
<td>100%</td>
<td>0%</td>
<td>80%</td>
<td></td>
</tr>
</tbody>
</table>

* p<0.05 vs T-V and Res+GdCl₃

In conformity with Paper I, the most frequent bacteria to translocate were *Escherichia coli*. In addition enterococci, *Staphylococcus epidermidis*, *Proteus mirabilis*, *Enterobacter cloacae*, and *Klebsiella oxytoca* were sometimes cultured.

Immunohistochemical analysis of organs from the animals treated with gadolinium chloride showed a marked reduction of ED2 positive cells in the liver. There was also a profound effect on the ED2 positive macrophages within the spleen where this cell type had almost completely vanished. ED1 staining of the liver revealed a disappearance of the clusters of stained cells normally seen and the distribution of macrophages was scattered. In addition, “fragments” of positive cells were found between the hepatocytes. However, the effect on ED1-positive macrophages was not nearly as pronounced as that on ED2-positive macrophages, in either the liver or the spleen.

No effects were seen on the number of T cells in any of the organs examined.
Studies on rats without MLNs of the Thiry-Vella loop segment showed no difference in the numbers or distribution of T cells or macrophages in the MLNs of normal bowel, liver, spleen, or Thiry-Vella loop.

In conclusion, Kupffer cells seem to be important in controlling the systemic spread of translocating microbes. In addition, MLNs may limit the systemic spread of translocating bacteria after small bowel transplantation.

Paper III

This paper focuses on the influence of the T cell population in bacterial translocation from the Thiry-Vella loop by using the α/β T cell receptor antibody R73 or the immunosuppressant CyA to inhibit T cells. As in Paper II, all experiments were conducted with day 3 as the end-point.

The immunological cell make-up was characterized by flow cytometry using antibodies against macrophages and different T cell surface markers. Biopsies for this purpose were obtained from MLNs of the Thiry-Vella loop as well as from MLNs of normal bowel and from the spleen. In addition, immunohistochemistry was performed to determine whether the total number of cells changed.

Analysis of bacterial cultures revealed that the bacterial translocation did not differ between animals having their T cells inhibited by treatment with R73 and animals given saline. The R73 treated group tended to have a lower incidence of translocation, with two animals of six with no translocation to the MLNs. CyA-treated animals did not differ from those treated with R73 or saline. The most frequent bacteria to translocate were *Escherichia coli*. In addition, *Proteus mirabilis*, micrococci, and *Klebsiella oxytoca* were sometimes cultured.

Examination of the flow cytometry showed that there was a decrease in the proportions of MHC class II-positive and CD8-positive T cells in the spleen of saline-treated Thiry-Vella loop-operated animals compared with the spleens of non-operated controls. Moreover, there was also a decrease in the proportion of MHC class II positive T cells in the MLNs of the Thiry-Vella loop compared with the MLNs from non-operated controls (Figure 3). No difference could be seen in the macrophage population between the different groups in any of the organs studied. Neither could immunohistochemistry reveal any differences in the total cell populations studied in any of the organs. Animals treated with R73 exhibited a major binding of the antibody to T cells in the organs studied. The rate of T-cell depletion seemed to be low.
In conclusion, no importance of α/β T cell receptor bearing T cells in limiting bacterial translocation could be observed. However, there is evidence of a depressed activity of T cells in MLNs of the disconnected bowel and the spleen in the Thiry-Vella loop model.
Paper IV

The finding of substances that can inhibit bacterial translocation is the ultimate goal for many scientists involved in this field. In Paper IV, the role of orally administered phospholipid or glutamine, as potentially translocation-inhibiting substances, was studied. Furthermore, selective bowel decontamination with non-absorbable antibiotics was performed in one study group. Again bacterial translocation was studied in the Thiry-Vella loop model and measured in the same organs as in Paper II. Flow cytometry analysis and immunohistochemistry were performed in the same manner as in Paper III. All experiments were terminated on day 3 post-operatively.

Selective bowel decontamination resulted in a decreased number of translocating bacteria to the MLNs of the Thiry-Vella loop (p<0.05). Interestingly, the incidence of translocation to the MLNs was still 100%. No differences were found when comparing Thiry-Vella loop animals treated with glutamine or phosphatidylcholine with control animals given water in either the number of translocating bacteria or in the pattern of translocation. Thus, no pronounced translocation to the MLNs of the normal bowel, liver, spleen, lung, peritoneum or blood was detected in any of these three groups. The translocating bacteria were the same as those encountered in previous studies with the vast majority of isolates being *Escherichia coli*.

Flow cytometry analysis of the cell make-up in the MLNs and spleen demonstrated a marked increase in the proportion of MHC class II negative macrophages in the spleen of animals treated with glutamine (17.3 ± 7.2%) compared to animals given water (6.1 ± 1.2%; p<0.01). Such a difference could not be seen between animals treated with phosphatidylcholine and controls. Furthermore, there was an increase in the number of CD8-positive T cells in MLNs from the disconnected segment (30.2 ± 5.8%) compared to MLNs from normal bowel (24.1 ± 2.1%; p<0.05) in controls given water.

Analysis of the immunohistochemistry displayed an increase in the total number of T cells in the spleens of rats treated with phosphatidylcholine (Figure 4a) compared to controls given water (Figure 4b).
In conclusion, neither the enterocyte nutrient glutamine nor the mucous protective substance phosphatidylcholine could prevent bacterial translocation from defunctionalised small bowel. Selective bowel decontamination reduced the number of translocating bacteria but the incidence was still 100%.
DISCUSSION

Bacterial translocation

One of the main findings of this thesis is that bacterial translocation of indigenous bacteria occurs from a defunctionalised Thiry-Vella loop of small bowel. This Thiry-Vella loop model proved to be a simple and consistent method that displays bacterial translocation from small bowel to the MLNs in nearly 100% of the animals after three days. The development of the translocation seems to be fast, since a large proportion of the animals have positive bacterial cultures from their MLNs after only one day. These findings are observed without any further manipulations except the disconnection of the bowel. This implies an intricate and ever ongoing competition between the gut mucosal barrier and the microorganisms in the bowel and that even small changes in some of the predisposing factors can initiate bacterial translocation quickly. However, the bacteria seem to be more or less confined to the MLNs of the loop. Thus, the back-up mechanisms for a failure in the initial mucosal defense, i.e. the traversing of the epithelial layer of the small bowel and migration of live bacteria to the MLNs, seem capable of managing the situation. Sometimes bacteria were also found in the MLNs of the normal (not disconnected) bowel. Bacterial translocation to other sites was only seen in a few stray rats or after additional trauma (e.g. Kupffer cell depletion). Translocation to the blood was an extremely rare event. It is known from several earlier studies that the existence of bacteria in the blood stream is a rare occurrence even in patients with symptoms of septic shock. This is in accordance with the findings in this thesis. Even in animals that have positive cultures from distant organs such as the liver and the spleen one cannot find any bacteria in the blood except very few rats.

In the absence of bacteria in the circulation, translocation of endotoxins has been hypothesized to be involved in the systemic inflammation seen in septic patients. Endotoxin could translocate from the bowel, where gram-negative bacteria are abundant, to the blood and induce sepsis-like symptoms without displaying positive blood cultures. In this thesis bacterial translocation to the MLNs did not produce any pronounced endotoxemia in either the portal vein or in the systemic circulation and was primarily a perioperative phenome-
non. The mild endotoxemia did not persist and the endotoxin levels had returned to baseline on day 3 although translocation was still ongoing. Similar observations have previously been reported in models of small bowel transplantation (Browne et al. 1992; Kusne et al. 1994). Moreover we found no clinical signs that the rats were suffering from systemic inflammation. A transient down-regulation of the reticulo-endothelial system in the liver, seen initially after an endotoxin overload or anaesthesia, could be responsible for the mild perioperative endotoxemia (Oishi et al. 1994).

The most common bacteria to translocate were members of the gram-negative enteric flora and among these the *E.coli* were, by far the most frequently cultured species. This pattern was consistent in all experiments. The reason for this is not further studied in this thesis; neither did we perform any subtyping or analysis of the different bacterial virulence factors. However, it has been a common feature in many models of bacterial translocation that member of the family of *Enterobacteriaceae* are the bacteria most frequently found.

The number of translocating bacteria per gram of tissue varies slightly among the different papers. The most obvious reason for this is that they are performed at different times and there may be slight differences in methods. For example, the peritoneum and fat overlying the MLNs were resected prior to biopsy in Papers II-IV whereas they were included in the biopsy in Paper I.

In theory there are only a few limited ways in which the bacteria can migrate from the gastrointestinal tract to distant organ systems. The first step in all of these cases would be transgression of the mucous and phospholipid layer that covers the mucosal cells and then binding to an enterocyte or any other cell lining the luminal surface of the intestinal tract. Next the bacterium would have to either pass through the enterocyte or between two enterocytes and thus reach the lamina propria of the bowel wall. In the lamina propria there are four different possibilities:

1. The bacterium could continue directly through the muscular and serosal layers of the bowel and migrate into the peritoneal cavity. Once there it would probably be phagocytosed by peritoneal macrophages. If not it can spread per continuum to the intraperitoneal organs.

2. The bacterium could enter the lymphatic system through the lymphatic capillaries and then be transported to mesenteric and regional lymph nodes. This is proposed to be the main mechanism in situations with bacterial overgrowth (Berg 1999). If the bacterium then survives the passage of these lymph nodes it will reach the systemic circulation through the thoracic duct and the subclavian vein.
3. Instead of entering the lymphatic system one possibility is to enter the blood system directly. This can happen in models exhibiting increased intestinal permeability or actual mucosal damage.

4. Once in the lamina propria (or perhaps already in the intestinal epithelium) the microbe could be phagocytosed by a macrophage, transported to an extraintestinal site, and then liberated by a dead or dying macrophage (Wells et al. 1987).

Mesenteric lymphadenectomy

In the Thiry-Vella loop model presented in this thesis, bacterial translocation consistently occurs to the MLNs of the segment. The physical removal of these lymph nodes initiates no further spread of bacteria to other sites. In the syngeneic small bowel transplantation setting, however, the removal of MLNs lead to a systemic spread of bacteria. All animals with a syngeneic graft without MLNs had positive cultures in their spleens compared to 25% of Thiry-Vella loop-operated animals without MLNs. The differences between these two models are of course considerable. The small bowel graft is exposed to the ischemic trauma at harvesting and preservation. Furthermore, reperfusion injury at the time of restoration of the blood flow is known to produce many toxic substances, in particular free oxygen radicals.

Upon removal of the MLNs the lymph vessels are severed and most of them ligated. The lymph flow then either leaks into the abdominal cavity or is redirected to other lymph nodes via collaterals. If the lymph flow leaks into the abdominal cavity, the bacterial translocation to the peritoneal cavity should increase. This could not be verified by cultures (25% in rats without MLNs vs 17% in rats with MLNs), and thus did either not take place or the peritoneal immune system (mainly macrophages) is readily capable of clearing these bacteria. Moreover no increased translocation to the mesenteric lymph nodes of the normal bowel could be observed with certainty (63% vs 50%). We interpret this as though the back-up systems are capable of handling the bacterial translocation should the MLNs be failing. The same interpretation could not be made in small bowel transplantation setting and according to our results the removal of MLNs from a small bowel graft may increase the risk of bacterial translocation and infection. This is interesting because mesenteric lymphadenectomy has been discussed as a way of diminishing the immunogenic load of a small bowel graft. One fear has been that this measure would increase the risk of infection, which also the present results indicate.

Alterations of the immune system

If there is a physical transport of bacteria beyond the MLNs, or if some bacteria enter the portal venous system directly from the bowel wall, it is likely
that they end up in the reticulo-endothelial system of the liver. If this were true then the inhibition of Kupffer cells in the liver could result in positive cultures from at least the liver and possibly also from other organs. In Paper II we used the selective Kupffer cell inhibitor gadolinium chloride. In vivo depletion of Kupffer cells with gadolinium chloride forms a generally accepted approach to study the Kupffer cell functions and has been used extensively in hepatological research. However, few studies have been made using gadolinium chloride in models of bacterial translocation. One intravenous dose of gadolinium chloride inhibits Kupffer cell function for approximately four days (Hardonk et al. 1992). To allow the rats to handle any operative infection we administered the substance one day postoperatively. On the third postoperative day bacterial translocation had spread systemically to the liver, the spleen and the lungs. Seven days postoperatively the Kupffer cell pool was completely restored and there was no bacterial translocation beyond the MLNs (data not shown). Other authors have shown that there is an increased bacterial translocation after partial hepatectomy. They suggest that by removing a considerable part of the liver a significant number of Kupffer cells are also removed (Blair et al. 1998). This is in accordance with our findings in this study on Kupffer cell inactivation. Furthermore, in studies on obstructive jaundice depressed Kupffer cell function and bacterial translocation have been described (Tomioka et al. 2000). Since cholestasis is a common feature in the short bowel syndrome, it could be argued that one of the reasons for the many infectious complications is Kupffer cell dysfunction. However, this has to our knowledge not been investigated.

T cell immunosuppression is extensively used in organ transplantation and evidence is accumulating that bacterial translocation is a potential risk factor of sepsis in small bowel transplanted patients. Therefore, we decided to study the impact of T cell inactivation and immunosuppression on bacterial translocation from small bowel using the Thiry-Vella loop model. From earlier studies by others we had hypothesized that the translocation would increase when T cells were inactivated. Surprisingly, no increased translocation was seen when T cells were inactivated. On the contrary translocation tended to reduce. In this thesis the monoclonal antibody R73 was used to inactivate T cells. This antibody is directed against the α/β T cell receptors. These receptors account for antigen recognition by most of T cells in the body. The exception is in the gastrointestinal intraepithelial lymphocyte collection where lymphocytes with γδ T cell receptors are abundant. The γδ T cells are not affected by R73 treatment. Perhaps the γδ T cells that are preferentially seen in the intraepithelial lymphocyte compartment still have important functions in bacterial translocation. In a study by Gatreaux et al it was observed that depletion of CD4 and/or CD8 positive T cells (regardless of α/β or γδ) in-
crease the translocation to MLNs in *E.coli* C25 monoassociated mice (Gautreaux et al. 1994). The explanation given by those authors that CD8 depletion alone increased translocation was that CD8 positive γδ T cells were depleted from the intestinal intraepithelial zone. These cells are known not to require professional antigen-presenting cells to be activated. Probably intraepithelial columnar cells can present antigen in an MHC class I-like manner to these γδ T cells. Furthermore the γδ T cells have been described as having more helper properties than cytolytic properties and can secrete cytokines such as tumor necrosis factor α, granulocyte/macrophage stimulating factor, interleukin-3 and gamma interferon (Barth et al. 1991). In the same way one could explain the absence of increased translocation in the study presented here by the fact that the γδ T cells are still present and active, thus supporting the results seen in the previous studies. In support of the theory that the intraepithelial γδ T cells limit the translocation is the observation in all studies that there is no further translocation beyond the MLNs to other organ systems. Had the αβ T cells, which are abundant in the MLNs, been important then the translocation probably would have spread to other organs both in the T-cell-depletion study and after mesenteric lymphadenectomy. Probably the inactivation of γδ T cells is important in the first step of bacterial translocation. After this first cellular immunological line of defence has been broken probably other immunological defence mechanisms in the MLNs and other organs go to work. For example the Kupffer cells of the liver or macrophages of the spleen. However, unpublished studies were also made using the genetically T cell deficient nude rat. Establishing a Thiry-Vella loop in these animals induce a bacterial translocation to the MLNs after three days that is evident in 100% of the rats. No bacterial translocation is seen beyond the MLN complex. Neither is the number of translocating microbes in the MLNs increased compared to normal T cell competent rats with Thiry-Vella loops.

Moreover one cannot exclude the fact that CD4 positive monocytes and a subset of CD8 positive natural killer cells could be affected by the antibodies used in earlier experiments and thus be responsible for part of the effects seen.

In addition to the R73 antibody treatment in Paper IV, cyclosporin A was used to inhibit T-cell function. Paper I indicated that this treatment could enhance bacterial translocation because the median number of bacteria in the MLNs was increased at day three in animals treated with cyclosporin A. This could not be confirmed in Paper IV. Cyclosporin A did not increase the translocation to the MLNs and neither did we observe any significant translocation beyond the MLN complex. This is a finding for which we have no obvious explanation.
Treatment attempts

The prevention of bacterial translocation and gut derived septic shock is the ultimate goal in the clinical situation. This is especially true in light of increasing bacterial resistance against conventional antibiotics. A large number of studies have been performed where an extensive set of substances have been tried. No substance has been shown to be consistently applicable in cases of bacterial translocation. This probably is due to the heterogeneity in the pathogenesis of bacterial translocation. In addition, the studies have been performed in many different species and the results found in one species often cannot be extrapolated to others. The results in this thesis can therefore be regarded as an aid for delineating different aspects in the translocation process from a defunctionalised small bowel. Finding a working or non-working substance could help in defining weak points in the intestinal barrier. Potential translocation inhibiting substances have been tested in this thesis depending on their different abilities to support various parts of the barrier.

Intraluminally administered glutamine caused a tendency towards a decreased number of translocating bacteria to MLNs. However, the incidence of viable bacteria was still 100%. In a study by Salvalaggio et al. the effect of glutamine was examined in a rat model of bowel obstruction. In this model there was no decreased incidence of translocation to MLNs, but there was a decrease in translocation to blood and distant organs. The authors suggested that it was mainly the immune cells that were positively affected by the provision of abundant glutamine (Salvalaggio et al. 2002). If there were an effect in this Thiry-Vella loop model on the cells of the gut-associated immune tissue, this would be difficult to determine since there is a low incidence of translocation beyond the MLN complex in this model. However, the flow cytometric analysis did reveal a marked increase in the percentage of MHC class II negative macrophages in the spleens of glutamine-treated rats compared to control rats treated with water. These cells could constitute a population of macrophages that has not yet been activated. Either they are newly synthesized or recruited from the bloodstream or they are a resting population that resides inside the spleen. In a study on hypertensive damage to the brain, Abumiya et al found this population to have phagocytic properties without activating the immune system (Abumiya et al. 1996). In Paper II we show that the macrophages of the spleen and the Kupffer cells of the liver are important in limiting the spread of translocating bacteria to distant site. In conclusion, the treatment of glutamine marginally affects the bacterial translocation and immunological response in the parameters studied in this study. A more prominent effect could be expected in models of bacterial translocation where there is a known shortage of glutamine. In the present model there is no malnutrition of the animal and glutamine from the vascular
system should be available in almost normal quantities. However, there is no intraluminal source of glutamine and other nutrients in the Thiry-Vella loop. Furthermore, the animal has been subjected to surgery, which could transiently increase the need for glutamine.

It has been shown that the exogenous supply of phosphatidylcholine can protect the gastric and colonic mucosa against acid induced damage (Dunjic et al. 1993), (Fabia et al. 1992). Furthermore, it can prevent bacterial translocation after extensive hepatectomy (Wang et al. 1994). In the light of these findings we examined the effect of exogenous phosphatidylcholine in the Thiry-Vella loop model for bacterial translocation from small bowel. Phosphatidylcholine was administered in an aqueous suspension. When sonicated it forms liposomes of about 50nm in diameter (Ikegami et al. 1985). Once inside the bowel the phosphatidylcholine is disposed to the mucosal surface and hopefully reinforce the phospholipid layer. However, no difference in the incidence or the number of translocating bacteria to any of the organs studied could be demonstrated in the study presented here. On the contrary, there was a tendency towards greater translocation to the spleen and peritoneum. Thus, reinforcement of the phospholipid layer is not sufficient to diminish bacterial translocation in this model. This could be due to methodological problems – maybe the liposomes are not incorporated in the gel mucus layer. An additional effect to the mechanic protection of the phospholipid layer is that it constitutes a substrate for the production of arachidonic acid and prostaglandins by phospholipases. Prostaglandins are also known to have cytoprotective functions (Dawiskiba et al. 1984). However, the breakdown of phospholipids by phospholipase A2 also creates lysophospholipids that are powerful detergents and could disrupt cellular membranes (Tagesson et al. 1985). Perhaps there is a strong phospholipase activity in the Thiry-Vella loop producing lyso-phosphatidylcholine that could be toxic to cell membranes of the enterocyte. The lack of bile salts could also be influencing the process. An additional explanation is that the driving force for bacterial translocation is so strong that the marginal effect of reinforcing the gel mucus layer is negligible. Most studies that indicate a protective effect by exogenous phospholipids are performed in models of mucosal ulceration or inflammation. This is not the case in our model where the probable promoter of translocation is bacterial overgrowth with an almost normal mucosal appearance.

In an attempt to diminish the load of bacterial overgrowth, we used selective bowel decontamination with non-absorbable antibiotics pre- and postoperatively in this study on bacterial translocation. This caused a markedly reduced, but not completely abolished, bacterial translocation. This indicates that additional factors have a role in the translocation process from the Thiry-Vella loop other than an increased number of intraluminal bacteria.
The relevance of bacterial translocation

Alternative explanations that the symptoms associated with bacterial translocation and sepsis (e.g. malaise, pyrexia, increased inflammatory parameters etc) are not related to either translocating bacteria or endotoxin have lately been emerging. Possibly much of the pathophysiology seen may be orchestrated from within the bowel wall itself with the local production of proinflammatory cytokines being responsible for many of the symptoms seen. This would explain many of the inconsistent findings in research on bacterial translocation and is an area that needs further clarification. It is a fact that even in experiments where bacteria are injected in high concentrations directly into the caval or portal vein of an animal, these are rapidly cleared from the circulation (Koh et al. 1998). In addition, the systemic inflammatory response in such experiments is usually moderate and seldom elicits any organ failure (Gorby et al. 1988). Moreover, early studies showed that the lethality was higher when injecting the same amount of pathogenic bacteria into the caecum than intravenously (Schook et al. 1976). This raises the question if bacteremia per se is a dangerous situation in an individual with an otherwise normal immune status and no co-existing disease.

Furthermore, one might argue that bacterial translocation from the intestine, where nutrition for the bacteria is normally abundant and a balanced ecology occurs, is a suicide mission on the bacteria’s part. The probability of surviving in the lymph or blood stream any prolonged time is extremely low. It is hard to understand what triggers the bacteria to leave the intestinal lumen and decide to translocate. Most likely it is due to local factors in the gut such as the lack of nutrients or the presence of local factors indicative of a very ill host. The bacterial translocation from the Thiry-Vella loop is probably also elicited by local factors inside the intestine. The contents of a defunctionalised bowel, as in patients on total parenteral nutrition, are abolished from the trophic effect of passing food and nutrients (e.g. glutamine). The impairment of normal peristalsis of food that propels intestinal contents, including bacteria, along the gastrointestinal tract promotes the association of bacteria and enterocytes and alterations in the bowel microflora. Therefore, selective bowel decontamination to decrease the number of bacteria has been widely used to try to prevent bacterial translocation. In addition, the lack of proximal secretions from the stomach and proximal bowel as well as pancreas and liver further alter the microenvironment. This probably leads to an environment that favors some bacteria and promotes their growth. Furthermore, a more hostile environment may induce transition to a more virulent state of the bacteria, by switching on certain virulence genes. For example, these genes could code for adhesion factors, proteases and toxins that make the bacteria more invasive. In that case all efforts to reinforce the mucosal barrier for example with phosphatidylcholine could be beneficial. An additional
impact of factors of a more systemic nature, e.g., increase in stress hormones post-operatively, cannot be ruled out.

The process of recovery from bacterial translocation is not studied in this thesis. Although we have had a few rats for several weeks after the creation of the Thiry-Vella loop and they are healthy and the bacterial translocation has subsided. Thus homeostasis in the intestinal mucosal barrier has probably returned.

Based on the findings in this thesis it is difficult to conclude that bacterial translocation per se is vital for the health of the individual. It might be regarded as a pathological finding without any serious side-effects in an otherwise healthy and non-immunosuppressed individual. However, this is rarely the case in a patient with ongoing bacterial translocation often as a direct consequence of a severe coexisting disease.

Future perspectives

Despite intense research in the area of bacterial translocation during the last 30 years much remains to be understood. Whether bacterial translocation is actually a cause of disease or just an epiphenomenon seen in a number of different conditions (or both) is still debated. The contradictions discussed above, dealing with the disparity between the patient’s actual condition and the simultaneous existence or non-existence of translocating bacteria are interesting. The field is definitely heading in the direction of determining local processes inside the bowel wall such as the interactions between the bacteria and the immune system. Questions to be answered include: What makes bacteria suddenly become virulent? Which cytokines are produced? Can local reactions be transformed to systemic inflammation and sepsis syndrome without bacterial translocation? What part do the so-called toll-like receptors play in the process of bacterial translocation and microbial recognition inside the gastrointestinal tract?

The relationship between bacterial translocation and and/or recurrent septic episodes in patients with short bowel syndrome and the development of liver failure is also still to be elucidated - for example which inflammatory pathways that are involved.

The Thiry-Vella loop model may serve as a useful tool in clarifying some of the questions outlined above.
CONCLUSIONS

Bacterial translocation occurs from a defunctionalised Thiry-Vella loop of small bowel to MLNs of the loop after only a few days. Systemic spread of translocating bacteria beyond the MLNs is a rare occurrence. The bacterial translocation does not lead to any impaired vitality.

After small bowel transplantation bacterial translocation to the MLNs occurs in almost 100% of the rats. In contrast, in this model there is also a systemic spread of translocating bacteria in nearly all individuals.

The number of macrophages in the MLNs of the disconnected segment increases with time. Concurrently the fraction of active T cells in the MLNs decreased as measured by the expression of MHC class II.

Removal of the MLNs increases bacterial translocation from a small bowel graft but not from defunctionalised normal bowel. Depletion of Kupffer cells and spleen macrophages induces the systemic spread of translocating bacteria.

The bacterial translocation is refractory to treatment with mucosal protection enhancing substances. Glutamine treatment increases the fraction of inactive macrophages in the spleen as judged from MHC class II expression.
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