Chapter 1

In chapter 1 an overview is given about the pathogenesis of infections which can threaten patients with burns. The role of endotoxin, a compound of the outer membrane of Gram-negative rods, in relation to cytokines and the development of septic complications is described in connection with immunosuppression in severely burned patients. The phenomenon of bacterial translocation (BT) is described. BT is the passage of microorganisms and parts of microorganisms, like endotoxin, from the lumen of the gut through the gut mucosa into the lamina propria and from there into the mesenterial lymphnodes as well as other organs like the liver and the spleen. Translocation is mainly studied in experimental animals. The results of this research, largely in small rodents, could contribute to a better insight in the pathogenesis of infections in the clinical situation. In the chapters 2, 3, 4 and 5 experiments in rodents are described which have a relation to bacterial translocation in severe burns.

Endotoxin can be bound chemically in the blood by specific lipo- and glycoproteins. These specific proteins provide the blood with a certain endotoxin-inactivating capacity (EIC). The value of the EIC could play a predictive role as factor in the pathogenesis of Gram-negative sepsis. In chapter 6, a study based on this hypothesis is described in severely burned patients and in a group of healthy persons.

Chapter 2

In earlier studies it has been shown that wound colonization with *Pseudomonas aeruginosa* resulted in an increased incidence of BT. It is postulated that endotoxin released by *Pseudomonas* causes the increased BT. Polymyxin B (PB) binds and neutralizes endotoxin in subtherapeutic concentrations. In Swiss mice, burns of approximately 30% 'total body surface area' were contaminated with *Pseudomonas aeruginosa*. In half of the mice 12.5 μg PB was administered i.m. immediately after thermal injury. Two days later the mice were sacrificed and the organs were cultured. There was a significant lower incidence of BT in the PB treated group in the spleen, the liver, the lungs and the heart compared to the untreated group. Besides, in plasma of the PB treated mice, endotoxin was detectable in a significantly lower incidence in comparison with the control group. Based on this experiment it is assumed that endotoxin of Gram-negative microorganisms may have BT-enhancing effect.

Chapter 3

Because the cell wall of Gram-positive microorganisms has only very little endotoxin, the influence of wound colonization by these microorganisms was also studied in a comparable model as mentioned in chapter 2. Wound colonization with *Staphylococcus aureus* was not associated with increased BT. However, wound colonization with *Streptococcus pyogenes* resulted in increased BT to the spleen and the liver. In Gram-positive bacteria other factors may play a role in the enhancement of BT than in *Pseudomonas aeruginosa*.
Chapter 4

It is well known that the gastro-intestinal tract of severely ill patients such as thermally injured patients, can be stronger colonized by potentially pathogenic microorganisms than in healthy individuals. The absence of sufficient antibodies directed against these microorganisms may facilitate BT in these (immunocompromised) patients. This hypothesis, concerning the importance of antibodies in the control of translocating bacteria was tested in a burn model. One group of mice was subjected to a short period of 'intestinal overgrowth' and an other group to a longterm 'overgrowth' by Escherichia coli before a thermal injury was applied. Two days postburn BT to various organs was determined. Plasma antibody titers of IgA, IgM and IgG against E. coli were measured by indirect immunofluorescence with a camera and an image processing system. It was shown that despite increased IgG titers against the E. coli detected in the group with the longterm overgrowth, this was not associated with decreased BT.

Chapter 5

To determine the possible role of endotoxin related cytokines as tumor necrosis factor (TNF) and interleukin-6 (IL-6) in the pathogenesis of burn-induced bacterial translocation a Wistar rat model was used in which enhanced sensitivity to TNF/endotoxin reactions was achieved by Galactosamine (GalN) "treatment". Rats "treated" with GalN showed a significantly increased BT. The combination of GalN with a 30% total body surface area scald led to mortality and increased BT while IL-6 and alanine-amino-transferase (ALAT) values were strongly elevated. Although TNF was only sporadically detectable, the results of this experiment suggests a role of TNF and much less of IL-6 as mediators in the process of BT. Also, this study indicates the important clearing function of the liver.

Chapter 6

A low endotoxin inactivating capacity (EIC) in serum could indicate a risk for development of an endotoxemia/ bacteremia in severely burned patients. In a clinical study performed in four burn-units, the EIC in serum of 20 patients was determined in sequence and related to the development of bacteremia. Also TNF and IL-6 concentrations were determined. Both are related to infections and the effects of endotoxin. Seven of the 20 patients developed a bacteremia: four patients with a Gram-negative bacteria. Compared to healthy volunteers the EIC in these burn patients was decreased and more fluctuating. No significant differences were found in EIC in patients with a bacteremia and those without. TNF and IL-6 concentrations were significantly increased in all patients with bacteremia. This study in severely burned patients does not support the hypothesis that MOF due Gram-negative bacteremia is due to insufficient buffering capacity; i.e. with a low initial EIC or due to an increased EIC consumption.
The conclusions of this thesis are:

- The endotoxin in *Pseudomonas aeruginosa* colonized burn wounds seems to play a role in bacterial translocation (BT) from the gut.

- Wound colonization with *Streptococcus pyogenes* is in mice associated with an increased BT. This phenomenon is not found after wound colonization with *Staphylococcus aureus*. To clarify this difference found between the effect on BT is difficult. Streptococcal pyrogen exotoxine is capable to increase the susceptibility to the lethal effects of endotoxin. In this way, BT could be increased. However, endotoxin inactivation by polymyxin B can not decrease BT when burnwounds are colonized with *Streptococcus pyogenes*. Also, it is not clear in which way an increased endotoxin susceptibility is created. A possibility could be that TNF production is increased by which gut mucosa is more damaged and BT is enhanced. In *in vitro* studies it has been found that lipoteichoid acid of streptococci increases the TNF production by mononuclear monocytes. On the other hand staphylococci also have lipoteichoid acid in their cell wall and are capable to stimulate TNF production.

- IgG antibodies against (potential) translocating *Escherichia coli* bacteria in burns does not lower BT in mice, although the antibiotic polymyxin B, which can inactivate endotoxin, does decrease BT. An explanation could be that the lipid A part, which seems responsible for many effects of endotoxin, is inactivated by polymyxin B whereas the specific IgG detected is not directed against this part of the endotoxin molecule. In this case the toxic part of the endotoxin is not inactivated by IgG.

- The interleukins, TNF and to a lesser extend IL-6, may play a role as mediators in BT in burns and BT is increased when the important clearing function of the liver is decreased.

- The endotoxin-inactivating capacity (EIC) in serum is decreased in all severely burned patients. In patients who developed Gram-negative bacteremia no evidence was seen of increased "EIC consumption".