10.1 Summary

Sepsis is a major cause of multiple organ failure and death in the ICU. Despite modern anti-microbial therapy and intensive care facilities the mortality rate of sepsis remains high. First, basic treatment in the ICU should be done right according to the scientific evidence. In **chapter 1** the international literature concerning these aspects of sepsis is reviewed. Attempts to improve survival by interfering in the immune system have failed for various reasons. On the one hand we do not know when to intervene or in which part of the system; on the other hand we lack sophisticated tools[266]. Reasons for failure of immuno-modulating therapies are discussed. We need to develop more insight in the disease process for a better understanding of the molecular mechanisms involved before we can think of novel therapeutic approaches. Problems to define sepsis, but also the immune state during sepsis, and the pathophysiology of sepsis are discussed.

To develop more insight in the disease process of sepsis, we investigated in **chapter 2** the normal inflammatory response at a clinical, a cellular as well as a cytokine level during a prolonged period (24 h) after endotoxin challenge in human volunteers. An initial pro-inflammatory response is successfully compensated by an anti-inflammatory response, leading to homeostasis and recovery. This is in contrast to what happens in septic patients with compensatory anti-inflammatory response syndrome. The inflammatory balance, expressed as the cytokine pro/ anti-inflammatory ratio, is reflected at a cellular level.

In literature of septic patients, there is little information available on the variations of soluble L-selectin levels. Experimentally, neutrophil priming and activation can be best identified by a rise in the expression of integrins like CD11b/CD18 and a fall in L-selectin expression. L-selectin is shed upon neutrophil activation. We examined, in **chapter 3**, the role of soluble L-selectin in our model to study sepsis: experimental human endotoxemia. Endotoxin infusion induced shedding of L-selectin. The amount of shedding, however, had no statistically significant impact on the soluble L-selectin. We hypothesized that in the early stages of sepsis or SIRS no marked change in L-selectin levels occurs that has a role in diminishing leukocyte endothelial cell interactions.

The inflammatory response in humans is influenced by the genotype. A polymorphism of the TNF-α gene at position -308 has been associated with conflicting effects on TNF-α production or clinical outcome. We evaluated in **chapter 4** the influence of TNF-α and IL-10 gene promotor polymorphism on the production of these cytokines in experimental human endotoxaemia. There was no association between TNF polymorphism and TNF-α level, nor between IL-10 levels and IL-10 polymorphism. Other explanations has to be sought for the relationship between TNF2 polymorphism and clinical outcome like linkage of other disadvantageous genes on the same chromosome. Furthermore, the TNF2 allele was associated with higher inducible levels of IL-10 in vivo after single endotoxin challenge. This might be an other explanation for the TNF2 related mortality.

In **chapter 5**, the international literature concerning the role of p38MAPK activation in the inflammatory response is reviewed. Recent studies suggest that inhibition of the intracellular p38 mitogen-activated protein kinase (p38MAPK), a new target, may have a substantial effect on inflammation. p38MAPK is involved in cytokine production and cellular adhesion. In vitro and animal studies have demonstrated that blocking p38MAPK could mitigate the pro-inflammatory response and improve survival after endotoxaemia. Its possible relevance to systemic inflammation in sepsis and acute respiratory distress syndrome (ARDS) is discussed.

In **chapter 6**, we evaluated the attenuation of clinical and cytokine response to endotoxin after inhibition of p38MAPK by an single oral dose of RWJ-67657, a synthetic pyridinyl imidazole. Inhibitors of the p38MAPK have been used to study the signalling pathway of the immune response. After a single dose of RWJ-67657, the temperature and blood pressure response remained at the basal level. The inhibition of TNF-α, IL-6 and IL-8 response was dose dependent. With the maximum dosage, reduction in peak serum levels of the pro-inflammatory cytokines was greater than 90%. There was no drug-related toxicity. We concluded that inhibition of p38MAPK by RWJ-67657 might be a tool to intervene in the deranged immune response in sepsis and other inflammatory diseases.

In the next **chapter 7**, we studied the activity of a single oral dose of RWJ-67657, a synthetic p38MAPK inhibitor, in preventing dual leukocyte/endothelial activation after endotoxin infusion in healthy volunteers. Three dose levels were placebo-controlled, double-blinded tested. The endotoxin induced shedding of L-selectin was diminished in a dose dependent manner. High dose RWJ-67657 prevented upregulation of the integrin CD11b and CD66b on neutrophils. The endotoxin induced increse in cICAM-1 and cE-selectin was almost completely prevented by high dose RWJ-67657. Therefore, a single oral dose of RWJ-67657, prevented neutrophil and endothelial activation after endotoxin infusion.

In **chapter 8**, we focussed on the effect of RWJ-67657 upon in vivo endotoxin induced monocyte cytokine production and on endotoxin tolerance of monocytes. Endotoxin tolerance is a state of immunological hyporesponsiveness to endotoxin, a phenomenon in which monocytes/macrophages play a central role. This tolerance is characterised by decreased monocyte production of cytokines, such as for instance TNF-α and IL-1β upon a second endotoxin challenge. Although this phenomenon has been extensively studied, both in vitro and in vivo, in various animal species and cell types, the cellular and molecular changes that contribute to this phenomenon are not fully understood. RWJ-67657 treatment significantly, dose-dependently inhibited the percentage of circulating monocytes producing these cytokines. Tolerance to in vitro endotoxin challenge was most prominent at 3 and 6 hours after in vivo endotoxin infusion. This endotoxin tolerance could dose-dependently be inhibited by RWJ-67657 treatment. Thus, p38MAPK inhibition by RWJ-67657 inhibited the monocyte production of cytokines following in vivo endotoxin infusion. Moreover, treatment by RWJ-67657 also

reversed the endotoxin tolerance. Whether this result can be extended to the clinical situation remains to be elucidated.

In order to optimalise the current basic therapies as stated above, in **chapter 9**, we studied the pharmacokinetics of cefpirome in critically ill patients with sepsis and MODS who needed continuous veno-venous hemofiltration (CVVH) to replace renal function. Pharmacokinetics have been studied in healthy volunteers pre-registration and in patients with normal renal function, including those with multiple trauma admitted to the ICU. Cefpirome is one of the anti-microbial agents used to treat nosocomial infections in the ICU. To assess the optimal dosage schedule for administering cefpirome in critically ill patients with sepsis and MODS who needed CVVH, this study was performed. Cefpirome was administered intravenously at 2 g in 30 min, then continued 1 g intravenously b.i.d. During >90% of time, serum levels were maintained above killing concentrations for susceptible micro-organisms.The sieving coefficient (64%) indicates that a substantial fraction of the drug is not filtered; clearance by pathways other than CVVH mounted to 50% of total clearance and increased on day 2, indicating that the dosing schedule used is appropriate for this setting. Cefpirome appeared to be safe in these patients, and effective for most of the nosocomial microbial isolates.

10.2 Future directions

The disappointing results of the immuno-modulating therapies do not negate the validity of the concept of therapeutically blocking cytokines or modulating the inflammatory response to infection. Indeed, succesful outcomes have been achieved when the inflammatory reaction is triggered by a well-defined process. Succesful modulation of rheumatoid arthritis and Crohn's disease has been described in patients treated with TNF-blocking. However, in the complexity of sepsis, multiple cellular activation processes are involved and many humoral cascades are triggered. Therefore, it seems possible that the dysregulation of innate immunity that characterises sepsis, may be amenable to blockade of the noxious bacterial components, blockade of early inflammatory pathways by gene therapy, or to blockade the intracellular pathways triggered by these products.

p38MAPK pathway is one known and probably important intracellular pathway activated during inflammation. Recent studies suggest that inhibition of the intracellular p38MAPK may have a substantial effect on inflammation. p38MAPK is involved in cytokine production and cellular adhesion. Pyridinyl imidazoles are members of a new class of synthetic drugs that have been shown to attenuate cytokine synthesis by inhibiting p38MAPK and have extensively been tested *in vitro*. In this thesis, we described p38MAPK inhibition during experimental endotoxemia in human volunteers (e.g. *in vivo*) for the first time. These obtained data are encouraging and promising. Although to be proven, inhibition of p38MAPK may be of benefit before surgery to prevent

overwhelming SIRS. For instance in cardiac operations with cardiopulmonary bypass which cause a systemic inflammatory response, which can lead to organ failure and post-operative morbidity. To overcome this problem patients are treated pre-operatively with corticosteroids. This, however, results in susceptibility to infections post-operatively. RWJ-67657 may be useful in these situations: given pre-operative, it inhibits the inflammatory reaction peri-operatively. Moreover, since it also inhibits the endotoxin tolerance, it may render patients less sensitive to post-operative infections.

The role of p38MAPK inhibition in sepsis remained unclear. Possibly, it may prevent immunosuppression because recent data showed that p38MAPK activation plays a critical role in the induction of immune-suppressive macrophage phenotype[237], and inhibition of p38MAPK markedly improves survival in a murine model of polymicrobial sepsis. Further studies are needed to elucidate this issue in clinical sepsis.

New strategies for randomised controlled clinical trials evaluating new immunomodulating therapies in patients with sepsis and septic shock should be made and implementated[267]. First, all patients should have infection. Second, there should be evidence of a pathophysiologic process that represents a biologically plausible target. Third, patients should fall into an appropriate category of severity (severe sepsis). Fourth, mortality should be the primary endpoint. Fifth, power analysis should be performed. Even if investigators aim at detecting absolute differences of 5%, adequately powered studies require a total of 3000 patients. Furthermore, interim analysis should be few in number, while stopping rules are decisive. Subgroups should be few in number, and defined before randomisation.