CHAPTER 1

INTRODUCTION AND SCOPE OF THE THESIS
History

The classic signs of inflammation (rubor, tumour, calor, dolor) were first described by Celsus in the 1st century A.D. About two centuries later Galen added functio laesa of the affected area as the fifth symptom. These signs of inflammation are still recognised in trauma patients with skin wounds, but also appear in a more generalised pattern following major surgery and severe infections. This is known as the systemic inflammatory response syndrome (SIRS). Rubor results from the increased blood flow caused by vasodilatation. In systemic inflammation, vasodilatation presents as a decrease in systemic blood pressure with compensatory tachycardia and an increased cardiac output. Tumour develops because of (generalised) oedema resulting from increased capillary permeability and fluid leak. Calor is a local manifestation of inflammation presenting as warm skin. Systemically, calor may present with fever, caused by interleukins and toll-like receptor triggering, which elevates the set point of the thermoregulatory center in the hypothalamus (1). Dolor is manifested as local or generalised pain. Functio laesa presents locally as the inability to use the affected body part. With systemic inflammation, functio laesa presents as multi-organ failure.

Surgery dates back to prehistoric times, and the first successful therapeutic arm amputation was likely performed in ancient Egypt (2). During the middle ages, surgical interventions were usually performed by barber-surgeons. Although most of the surgical procedures they performed were straightforward, complication rates were high (3). Furthermore, patients suffered considerable pain as anaesthesia was not yet available. These two factors contributed to the negative image and low social status of the non-academic barber-surgeons.

Important changes in medicine and in surgery as a profession were required before major surgery would be feasible. Physicians became increasingly interested in the field of surgery during the 18th century due in part to the increased knowledge of pathological anatomy. At the same time, the former barber-surgeons became more interested in medical research and began to separate themselves from the barber trade.
As a result, their social standing rose (3). Ultimately, a confrontation resulted at the University of Paris between the physicians and the surgeons, who demanded the right to teach the science and art of surgery instead of limiting themselves to wound treatment, setting bones, letting blood, and trimming beards. This marked the beginning of the acceptance of surgery as an academic degree by universities.

Figure 1. View of a right foot of a mummy from ancient Egypt. The left panel shows the well healed amputation area of the big toe. The right panel shows a wooden prosthesis attached to the forefoot. (4)

New developments in the 19th century set the foundation for modern surgery. Painless surgery was first described by Warren and Morton who removed a neck tumour from a patient under ether anaesthesia at the Massachusetts General Hospital in Boston. This method was adopted throughout Europe. Soon afterward, chloroform was introduced as a general anaesthetic, followed by cocaine infiltration as a local anaesthetic several years later. Minor surgery of the extremities became much easier to
perform when epidural cocaine was available for the blocking of nerve trunks (5). Although surgery could now be practised without pain, surgery related mortality was still high due to blood loss and postoperative infection.

In 1848, Semmelweis reported that fever and septicaemia in pregnant women could largely be prevented by hand scrubbing and washing in chlorine water before obstetrical examination. It was only after Lister’s publications in 1867 that it became understood that infections were caused by microbes and that infection was not a normal stage in the wound healing process. Surgical techniques were also markedly improved in these years: intestinal suture techniques were first performed by Dieffenbach in 1836 and further refined with sterilised fine silk thread by Kocher in 1888.

These developments resulted in several new major surgical operations in the late 19th century. Billroth performed his first successful esophagus resection in a dog in 1871, and the first laryngectomy in 1872. He was also the first to attempt reconstruction after partial gastrectomy by gastroduodenostomy (Billroth I; 1881) and by gastroenterostomy distal to the ligament of Treitz (Billroth II; 1885). Sauerbruch became a pioneer in thoracic surgery and performed the first thymectomy in a patient with myasthenia gravis in 1912.

Many surgical procedures were introduced at the end of the 19th century and at the beginning of the 20th century. Operations were performed with increasing success due to a better understanding of anatomy, physiology, increased experience, and the development of anaesthesia. Other supportive techniques were introduced soon afterwards, for example the artificial kidney by Willem Johan Kolff in 1944, the heart-lung machine by Gibbon in 1953, and the treatment of blood loss by blood transfusion. The incidence of postoperative infections decreased due to the implementation of antisepsis measures and the invention of Penicillin by Flemming in 1928 (6).

However, despite the development of surgical skills and improved methods in the treatment of postoperative complications, mortality remained considerable. Many patients died after the progressive failure of one or more vital organs such as the heart, the lungs or the kidneys. Treatment aimed at replacing or restoring the function of individual organs (such as fluid replenishment, mechanical ventilation, haemodialysis
etc.) often failed to stop the fatal course of events. It became clear that more than symptomatic treatment was needed, and that insight into the underlying pathophysiology of the multi-organ failure syndrome would be required for effective intervention. The central mechanism in the pathophysiology of the development and progression of multi-organ failure is called systemic inflammation (7).

**Systemic inflammation**

Theoretically, the inflammatory response which follows surgery facilitates tissue healing. With a small skin wound, platelets initiate the formation of blood clots, thereby stopping bleeding. Activated platelets secrete mediators that attract macrophages and fibroblasts. Chemo-attractants produced by macrophages and fibroblasts attract and activate neutrophils and monocytes. The wound area is cleaned by these neutrophils and an extracellular matrix is formed. After the initial phase of inflammation, the wound is re-epithelialized followed by wound contraction and extracellular matrix reorganization (8). The inflammatory response in patients with small skin wounds is principally a local phenomenon, manifested by redness, swelling, warm skin and mild pain (rubor, tumour, calor, dolor). In patients with extensive trauma, major surgery, severe burns, pancreatitis or sepsis, the inflammatory response generally has important systemic manifestations and has been termed the systemic inflammatory response syndrome (SIRS). SIRS has been defined in a consensus conference, as shown in table 1 (9).

<table>
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<th>SIRS is manifested as 2 or more of the following conditions:</th>
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<td>- Fever (&gt; 38.0 °C) or hypothermia (&lt; 36 °C)</td>
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<tr>
<td>- Tachycardia (&gt; 90 beats/min)</td>
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<tr>
<td>- Tachypnea (&gt;20 breaths/min) or hyperventilation (PaCO₂ &lt; 4.3 kPa)</td>
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<tr>
<td>- Leukocytes &gt; 12 x10⁹/L or &lt; 4 x10⁹/L or &gt; 10% immature neutrophils</td>
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Table 1. Criteria for SIRS
SIRS can progress in an uncontrollable reaction leading to multiple organ failure (heart, lungs, kidneys, liver and bone marrow). The pathogenesis of multiple organ failure is complex and involves several mediators and cytokines. Tumour necrosis factor-alfa (TNF-α) and Interleukin-1 (IL-1) play a key role (10). Activated monocytes and macrophages are able to release massive amounts of these cytokines. This activates the coagulation and complement pathways, and the platelets are triggered by the release of platelet activating factor. The activated endothelium also releases large amounts of pro-inflammatory cytokines, and the expression of adhesion molecules is increased. Polymorphonuclear granulocytes become activated, and through their interaction with the adhesion molecules they transmigrate through the endothelial cells into the targeted tissue. Subsequently granulocytes release large amounts of reactive oxygen radicals and proteases to kill the invading microorganisms. This induces further endothelial damage and activation resulting in an uncontrolled cascade (11). The release of prostaglandins and nitric oxide in combination with the endothelial damage, causes vasodilatation, capillary leakage, generalised oedema, systemic hypotension, and, finally, multiple organ failure (12). In the case of an infection, antibiotics should be administered and (surgical) drainage should be performed whenever possible. Tissue perfusion should be restored by fluid replacement and the administration of vaso-active medication. In case of respiratory failure caused by acute respiratory distress syndrome (ARDS), patients should be mechanically ventilated with high PEEP and low tidal volumes (13). When renal failure occurs, renal replacement therapy by continuous venovenous hemofiltration should be started without delay. Despite aggressive treatment, the mortality rate when multiple organ failure occurs is high (7% for one-organ failure and 48% in three-organ failure) (14). One reason why supportive therapy often fails to save the patient’s life is that supportive therapy alone does not interfere with the systemic inflammation which is driving the whole process. Since this theory was recognised, various efforts have been made to interfere with the immunological processes in an effort to improve outcome.
**Intervention in the inflammatory response**

The inflammatory response which follows major surgery should facilitate tissue healing and eliminate the invading organisms, but this mechanism can overshoot, leading to multiple organ failure and death. Although this hypothesis is plausible, efforts to interrupt the inflammatory cascade such as administering neutralizing antibodies against LPS (lipopolysaccharide), anti-TNF-\(\alpha\), soluble TNF receptors, IL-1 receptor antagonist and high dose steroids have not been successful (15-21). There may be several explanations for these negative results. If the inflammatory response was already activated, it may no longer have been dependent on the initiating stimuli, such as TNF-\(\alpha\) and IL-1. As only single agents were tested in these trials, just one of the many active cascades would be affected, allowing the others to continue fuelling the reaction. A third intriguing explanation is that at the time of the intervention, patients may no longer have been experiencing an overwhelming pro-inflammatory response needing counteraction, but instead were exhibiting a form of immunosuppression that would worsen with anti-inflammatory measures. In this case interventions aimed at restoring immune capacity would have been more appropriate. A compensatory anti-inflammatory reaction in response to the initial pro-inflammatory response has been described to occur and would prevent the excessive tissue destruction resulting from uncontrolled inflammation (22). Theoretically, this anti-inflammatory response could promote a state of immunosuppression which would increase the risk of nosocomial infections. Together with the decreased TNF-\(\alpha\) production by monocytes, these factors make up the compensatory anti-inflammatory response syndrome (CARS) which follows SIRS (22). This model for systemic inflammation combined with the anti-inflammatory response would allow for specific treatments which target the inflammatory cascade. Two different treatment directions are indicated, depending on the response seen in the patient. Patients with an overwhelming systemic inflammatory response should be treated by interfering with the pro-inflammatory cascade, whereas patients exhibiting a dominant anti-inflammatory response should be treated by stimulating the immune response.
Chapter 1

*Monocyte function in systemic inflammation*

The exact mechanism and timing of the anti-inflammatory response was poorly understood until recently. Monocytes play a central role in immunity and inflammation. Hershman et al studied monocyte function in patients after major surgery and sepsis. They discovered that a decreased expression of HLA-DR on peripheral blood monocytes was associated with an increased risk of infectious complications in trauma patients (23). These results were confirmed by other study groups in patients after major surgery and sepsis (24-29). Low HLA-DR expression impairs the immune response, which predisposes the host to generalised infections. Decreased expression of HLA-DR by monocytes is often accompanied by functional abnormalities, such as a low antigen presentation by monocytes to T-cells (30), a critical step in the immune response, and by a diminished capacity to release pro-inflammatory cytokines, such as TNF-α, IL-1, IL-6, IL-8 and interferon-gamma (IFN-γ) (31). This state is known as monocyte deactivation, and constitutes one of the mechanisms underlying CARS. Monitoring monocyte function by measuring HLA-DR expression might be an important parameter to guide intervention in the inflammatory pathway. We hypothesised that a better understanding of the pro- and counter inflammatory response is a prerequisite for successful intervention. This thesis reflects our efforts to better understand these processes.

**LIVER TRANSPLANTATION**

To investigate monocyte function in patients with sepsis we investigated patients who underwent orthotopic liver transplantation (OLT). The first successful OLT was performed by Starzl in 1967 (32). During the following years, the short-term mortality remained high initially because of uncontrolled haemorrhaging during surgery, technical problems, and acute rejection. However, due to major improvements in surgical techniques, anaesthesiology, post-operative care, and the development of potent immunosuppressive drugs, mortality eventually decreased, and liver transplantation became an accepted treatment option for end-stage liver disease.
Unfortunately, the incidence of serious infections remained high and was the principle cause of death during the first months after transplantation (33,34). Although immunosuppressive drug therapy is an important cause of these infections, there are several other factors which contribute as well. For instance: the patient’s poor general condition and nutritional status before surgery; the presence of intravascular, biliary, and urinary catheters; mechanical ventilation; a large surgical wound with drains; and the considerable blood loss that can occur during surgery and afterwards due to poor clotting and low platelet counts. Antimicrobial drugs are important in the treatment of these infections, but may not be sufficient when the patient’s natural protective immune responses fail. In order to enable the immune system to initiate these protective responses, the dosage of immunosuppressive drugs should be strongly reduced. This might result in a dilemma, as insufficient suppression of the immune system predisposes to rejection of the graft. The treatment in these cases depends largely on clinical intuition as there are no presently recognized clinical parameters which indicate immune function. Such parameters would be of great value for calculating dosages of immunosuppressive drugs so that both over-immunosuppression, with its attendant risk of infection, and under-immunosuppression, leading to rejection, may be avoided. To address this issue in our thesis, we note that sepsis was generally preceded by a strong decrease in the expression of HLA-DR on monocytes. Whereas the return of HLA-DR expression to normal values accompanies recovery from sepsis and survival, persistently low values occur in patients who subsequently die from sepsis. As previously discussed, a variety of conditions impact immunoresponsiveness and monocyte function in OLT patients including age, pre-transplant illness, nutritional status, co-existing diseases, and the type and dosage of immunosuppressive drugs. To further investigate the inflammatory response and monocyte function following major surgery, but without many of the confounding factors encountered in OLT patients, we studied a second, much more homogeneous group of patients.
RUPTURED ABDOMINAL AORTIC ANEURYSMS

Patients with ruptured abdominal aortic aneurysms (RAAA) represent a homogeneous patient group (previously healthy, non-immunosuppressed) with a clearly defined catastrophic event, followed immediately by surgery. In RAAA patients, haemorrhagic shock is the primary cause of death both intra-operatively and during the 24 hours following surgery. After this initial post surgical phase, the most frequent cause of death is multiple-organ failure. In fact, more patients die from multiple organ failure than hemorrhagic shock (35,36). Almost all patients who survive surgery develop a systemic inflammatory response syndrome (SIRS), responsible for the development of multi-organ failure (37). The aim of our study was to describe HLA-DR expression on monocytes in RAAA patients after surgery and to establish whether HLA-DR expression correlated with the occurrence of secondary infections and death. In this ‘clean’ model of post-surgical systemic inflammation, we aimed to establish whether RAAA patients die from an overwhelming systemic inflammatory response or from secondary infections with monocyte deactivation and a predominant compensatory anti-inflammatory response. To achieve this, we analysed several clinical and immunological variables.

An abdominal aortic aneurysm is a serious medical condition associated with a high mortality. It is the tenth leading cause of death in men aged 65 to 74 years old, and its incidence is on the rise (38-40). The management of patients with AAA consists of surgical repair with an aortic graft. Endovascular treatment (stenting) was more recently introduced for selected cases. Hospital mortality for the elective repair of AAAs larger than 5.5 cm is reported to be as low as 2% in some centres (41,42). This low mortality is in contrast with the in hospital mortality associated with RAAAs, which ranges from 30% to 70% with surgical repair (43-47).

The advantage of the recently established elective endovascular repair of abdominal aortic aneurysm (EVAR) is the lower incidence of systemic complications (48,49) and a lower hospital mortality (50), compared with open repair. Results for EVAR in symptomatic non-ruptured and ruptured AAAs are promising (51). However,
the survival benefit of avoiding the trauma of surgery might be off-set by the negative influence of the time delay that results from waiting for the required CT-scan. Furthermore, the impact of long-term complications on mortality after EVAR is unknown and EVAR is frequently not feasible in patients with ruptured AAA (51-53).

AIMS AND OUTLINE OF THE PRESENT THESIS

The concept of systemic inflammation is well established in surgical patients. The aim of this study was to describe this response in patients after major surgery with special emphasis on the role of the monocyte. Our studies focused on patients following liver transplantation and patients with a RAAA. These two groups are highly relevant since they show a high incidence of secondary systemic complications, with progression to multiple organ failure and death.

Chapter 2 describes new insights in the pathophysiology of sepsis. The role of monocytes in the inflammatory response is described in detail. Furthermore, possible new interventions based on the patient’s immune status are outlined. Liver transplantation patients are studied in chapter 3. The purpose of this study was to describe the role of HLA-DR expression on monocytes in infectious complications and mortality. Our hypothesis was that a low HLA-DR expression on monocytes is associated with sepsis and mortality. The effect of immunosuppression on monocyte function was also investigated. In postoperative OLT patients this might be especially relevant since patients in whom the anti-inflammatory response predominates might benefit from reduced immunosuppression therapy.

As explained above, patients who have undergone surgical repair of a ruptured aortic aneurysm are also relevant to the study of the systemic inflammatory response.

Systematic data on the organ responses in terms of biochemical parameters of patients after RAAA have only rarely been described. Therefore, we studied the typical biochemical response in RAAA patients in chapter 4. The impact of several parameters on outcome is also described. Although the role of several different cytokines in patients after RAAA has been described in a number of studies, the clinical
significance of biochemical parameters is not always clear. In chapter 5 we describe pro- and anti-inflammatory cytokines and HLA-DR expression on monocytes in RAAA patients. We hypothesised that a low HLA-DR expression was associated with a high mortality in these patients. As multiple organ failure is the most frequent cause of death in our patient population, we evaluated if this was from an ongoing systemic inflammatory response or from immunosuppression and secondary infections as is seen in patients with CARS.

The treatment and outcome of RAAA patients admitted to the University Medical Center Groningen are described in chapter 6. This study will show how the streamlined management of patients directly before surgery, during surgery, and after surgery impacts hospital mortality in patients with symptomatic AAAs.

The Multicentre Aneurysm Screening Study (MASS) has investigated whether screening men aged 65 to 74 for AAA has an effect on AAA related mortality. Screening resulted in a significant survival benefit (54). Screening has also been found to be cost-effective (55). In chapter 7 a letter to the editor in response to the MASS study is presented, stressing that although screening men seems to improve survival, measures to improve survival in RAAA patients by prompt and surgery according to liberal inclusion criteria should not be overlooked.