Summary

Blood contact with unphysiological surfaces initiates activation of plasma proteins and cells involved in the host defense mechanism, which leads to an inflammatory reaction. This reaction is mostly localized to counteract penetrating microorganisms or to protect for bleeding and to stimulate tissue repair in case of tissue damage. This inflammatory reaction can become generalized during sepsis or extensive exposure of blood to biomaterials and then might affect the host.

Patients undergoing cardiopulmonary bypass (CPB) suffer from the "whole body inflammatory reaction" induced by the blood-material contact. The main factors involved in this reaction and the subsequent impairment of the host defense mechanism are investigated in this thesis.

In the literature review *(Chapter 1)* we indicate that the complement system and the population of polymorphonuclear leucocytes are the major factors of the natural host defense mechanism. Therefore changes of complement components, the appearence of complement split products and changes in leucocyte function and number are important parameters in the subsequent studies. During CPB the host defense mechanism, including the plasmatic systems (complement, clotting, fibrinolytic, kinin) and cells (white cells, platelets, erythrocytes, endothelium) becomes massively activated. Therefore a wide range of assays is needed to explore the complex interactions of these systems.

In a study on patients undergoing CPB we determined activation of the complement system and of blood cells while the extracorporeal circuit was composed with a bubble or a membrane oxygenator *(Chapter 2)*. Both circuits activated similarly the *alternative* pathway of complement, shown by high C3a plasma concentrations. Bubble oxygenators also activated the *classical* pathway, shown by decreased native complement C2. Bubble oxygenators also significantly decreased opsonizing capacity and serum bactericidal activity, both important plasmatic host defense mechanisms against infection. The decreased the host defense mechanisms can be explained in part by the consumption of complement factors.

The continuous activation of the alternative pathway of complement observed in this study indicates complement activation by sources additional to the biomaterial, since complement activation on biomaterial will cease by saturation of binding sites. We hypothetized that during CPB complement could also be activated by the interaction with enzymes which are released during inflammation. Because inflammation can potentially be inhibited by corticosteroids one group of patients was treated with corticosteroids before CPB started *(Chapter 3)*. During recirculation of heart and lungs, just after release of the aortic crossclamp, a sudden rise of C3a and fibrin(ogen) degradation products, and a decrease of leukocyte and platelet numbers was observed in the untreated but not in the corticosteroid treated group. This was explained by activation of the fibrinolytic and complement system in the heart and lung vasculature in the untreated group during ischemia. This biological source of complement activation could indeed be inhibited by corticosteroids. However, C3a generation induced by the materials before crossclamp release was not inhibited by steroids. In this period the use of biocompatible materials is needed to prevent complement activation.

Since the increased fibrinolytic activity in CPB patients plays a role in the whole body inflammatory reaction, a plasmin inhibitor, aprotinin, was given in high doses during CPB *(Chapter 4)*. In aprotinin treated patients the fibrinolytic system was effectively inhibited, demonstrated by the absence of fibrin(ogen) degradation products during CPB. A remarkable observation was that thromboxane was not released by platelets from aprotinin treated patients, whereas thromboxane was released to tenfold baseline concentrations in untreated patients. This effect of aprotinin on platelets correlated with reduced capillary bleeding during CPB and with a significant reduction of bloodloss and blood requirements postoperatively.

To clarify the protective effect of aprotinin on platelets the effect on the platelet von Willebrand receptor (GP Ib) was studied *(Chapter 5)*. The number of GP Ib receptors decreased by 40% already in the first five minutes of CPB in untreated patients, but remained unaffected in aprotinin treated patients. Since fibrinolytic activity in plasma could only be measured after 30 minutes of CPB, a local effect of aprotinin on the platelet membrane is the most likely explanation for the protective effect.

In standardized animal experiments sham CPB was performed to investigate host defense mechanisms related to contamination during CPB and to the occurrence of infection after CPB.

Contamination of the extracorporeal circuit by exogenous sources was evaluated with typable bacteria sprayed into the air of the operating room *(Chapter 6)*. In this study it was demonstrated that airborne bacteria contaminate the circuit by sedimentation into the open thorax cavity and by transport into the circulation by the aspiration of shed blood from the thorax cavity. Postoperative infection with the airborne bacteria develops in these contaminated animals even when peroperative swab and blood samples were negative due to low concentrations of airborne bacteria. An enhanced susceptibility for infection due to affected plasmatic and cellular factors of host defense may explain the increased risk of infection after CPB *(Chapter 7)*. It was demonstrated by means of opsonizing capacity and serum bactericidal activity as well as by means of chemiluminescence and bacterial killing performed by phagocytes that CPB conducted with a membrane oxygenator did not affect the host defense mechanism. When cardiotomy suction with aspiration of air was included in this system the host defense mechanism was affected, and when a bubble oxygenator instead of a membrane oxygenator in combination with cardiotomy suction was used the host defense mechanism was severely impaired. These findings demonstrate a relationship between the amount of blood-air contact during CPB and the impairment of the host defense mechanism against infection. Because most cardiac centers still use bubble oxygenators and perform blood suction with aspiration of air, a substantial decrease of host defense will be acquired, as indeed

was documented in our preceeding clinical study *(Chapter 2)*. This decreased host defense against infection necessitates the use of prophylactic treatment with antibiotics. The effect of two regimens often used for antibiotic prophylaxis were tested in our dog experiments with use of bubble oxygenator perfusion and cardiotomy suction *(Chapter 8)*. A broad spectrum cephalosporin regimen (cefuroxime) was not effective to eliminate gram-positive or gram-negative bacteria instantaneously. In contrast a regimen consisting of benzylpenicillin, gentamicin, and flucoxacillin markedly improved serum bactericidal activity and thus eliminated contaminating bacteria in these dogs with an impaired host defense mechanism. Despite the different way of action of the two regimens, both prevented postoperative infection.

In conclusion. The inflammatory reaction induced by CPB involves mainly the complement and fibrinolytic system, the white cells and platelets. A part of the inflammatory reaction can be inhibited by prophylactic treatment with corticosteroids and aprotinin. This results in reduced postoperative complications such as bleeding tendency.

The activation of the alternative pathway of complement by the extracorporeal circuit cannot yet be abolished by pharmaceutic intervention nor by the presently used biomaterials. Activation of the classical pathway of complement can significantly be reduced by membrane oxygenation instead of bubble oxygenation. When bubble oxygenators and traumatic cardiotomy suction are used the cellular and humoral host defense will be severely impaired. Since patients are exposed to contamination with airborne bacteria during CPB, the risk of postoperative infection appears to be high. Antibiotics only eliminate bacteria if the proper antibiotic regimen is used. To decrease the risk of infection with bacteria that are not sensitive for the applied regimen, it is recommended to maintain an optimal host defense by membrane oxygenation and cardiotomy suction without air aspiration.