Diplomarbeit

SPECIFICATION OF IMMUNE MODULATION AFTER CORONARY ARTERY BYPASS GRAFT OPERATION

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ZUSAMMENFASSUNG

Aorto-koronare Bypassoperationen gehören zu den am häufigsten durchgeführten Herzoperationen in Österreich und auf der ganzen Welt. Der Einsatz der Herz-Lungen-Maschine ist begleitet von einer zellulären und einer Kaskaden-Antwort. Dabei ist bekannt, dass die Ausschüttung von Zytokinen in die Richtung einer generalisierten Immunreaktion ähnlich wie bei der Sepsis beeinflusst wird. Es hat den Anschein, dass dieser Effekt eine wichtige Rolle in der Entwicklung von postoperativen infektiösen Komplikationen spielt. Infektionen des Operationsgebiets treten in 2-20% der Patienten nach Herzchirurgie auf und führen zu verlängertem Leiden sowie zu prolongierten Spitalaufenthaltszeiten und höheren Kosten für das Gesundheitssystem.

Die Produktion von IL-10 und die Beteiligung von T_H2 Lymphozyten scheinen wichtige Faktoren in der Entwicklung von Immunsuppression zu sein. Obwohl ST2 eine wichtige Rolle in T_H2 Effektor-Funktionen spielt, wurden die Auswirkungen des Proteins auf die Vorgänge nach Bypassoperationen noch nicht untersucht.

Die hier beschriebene Studie analysiert die Antwort von anti- und proinflammatorischen Zytokinen bei 16 Bypass-Patienten nach Herzoperation. Das Ziel dieser Diplomarbeit ist es, einen Einblick in die Prozesse der Immunmodulation nach Bypassoperationen zu geben und die Rolle von ST2 in diesen Vorgängen näher zu beleuchten.

Die präsentierten Resultate demonstrieren, dass die offene Herzchirurgie eine massive langandauernde Ausschüttung von ST2, einem Protein mit *in vitro* und *in vivo* bekannter immunsuppressiver Eigenschaft, induziert. Diese Daten helfen dabei, die Immunmodulation nach Bypassoperation sowie die assoziierten negativen Konsequenzen besser zu verstehen.

ABSTRACT

Coronary artery bypass graft operation (CABG) is a common surgical procedure in Austria and all over the world. Cardiopulmonary Bypass is known to effect a humoral and cascade response and affect cytokine release leading to a generalized endogenous immune reaction similar to that described in sepsis. This effect may play an important role in development of infectious post-surgical complications. Surgical wound infections occur in 2-20% of patients after heart surgery, leading to increased suffering and costs, and prolonged hospital stay.

The production of IL-10 and the involvement of T_H2-type lymphocytes seem to be important factors in the development of immune suppression. Although ST2 plays an important role in T_H2 effector functions, the protein's part in immune modulation after CABG has not been studied yet.

Analysis of 16 patients undergoing coronary artery bypass graft is described, exploring anti- and pro-inflammatory cytokine responses after heart surgery. The thesis' objective is to obtain insight into immune modulation after CABG operation and to reveal the role of ST2 in this process.

Our results demonstrate that open heart surgery induces a massive long lasting secretion of ST2, a protein that was shown to generate *in vitro* and *in vivo* immune suppression. This data helps to better understand immune modulation seen after CABG surgery and the associated negative consequences.

I. INTRODUCTION

I.I CORONARY ARTERY BYPASS GRAFT OPERATION (CABG)

A coronary artery bypass graft operation is "a surgical procedure in which one or more blocked coronary arteries are bypassed by a blood vessel graft to restore normal blood flow to the heart. These grafts usually come from the patient's own arteries and veins located in the leg, arm, or chest"[1].
In the year 1993 5,514 heart surgeries were performed in Austria, 63.3% of those were coronary artery bypass graft operations. This is equivalent to 446.7 operations per million population.[2]

I.I.I THE OPERATION

I.I.I.I PLANNING AND STRATEGY OF THE OPERATION

Coronary artery surgery requires meticulous planning and careful execution. Planning of the surgery takes into account the patient's general condition, age, symptoms, associated conditions, and diagnostic and angiographic findings. The physical evaluation should include cardiac catheterization, neurologic evaluation, renal function, respiratory status and blood coagulation. If possible, aspirin should be discontinued 1 to 2 weeks prior to the operation because of increased risk of postoperative bleeding.[3-5]

The strategy of the operation is directed toward obtaining complete revascularization by bypassing all severe stenoses in all coronary arteries over 1 mm in diameter. Since the number of conduits to be used conveniently is limited, that means that at least some of the grafts must have sequential anastomoses. The conduits used require adequate preparation, side branches of veins and arteries must be securely controlled and valves and attached fat must be avoided.[3, 4]

Saphenous vein grafts have been the mainstay of coronary artery surgery for a long time but the long-term patency of these conduits is a major concern. The benefit of the left internal mammary artery in terms of survival is well known. Other arterial conduits successfully

utilized are the right internal mammary artery, the gastroepiploic artery, the inferior epigastric artery and the radial artery. The left internal mammary graft with a 90-95% 10-year patency remains the benchmark for long-term patency.[6]

I.I.I.II TECHNIQUE

The heart is approached through a median sternotomy incision (see Figure 1) after general anesthesia and placement of hemodynamic monitoring. At the same time the preparation of the greater saphenous vein is started. Before the pericardium is opened the left internal mammary artery (LIMA) is mobilized. The pericardium is then opened, and stay sutures and purse-string sutures are applied. Cardiopulmonary bypass is established and the aorta is cross-clamped. The heart is covered with cold saline and cold cardioplegia is established. After the heart contraction has stopped and a clear bloodless field is achieved, the planned anastomoses can be made. The currently preferred strategy involves the routine use of the LIMA to the left anterior descendent (LAD) artery and three segments of saphenous vein to the remaining coronary arteries.

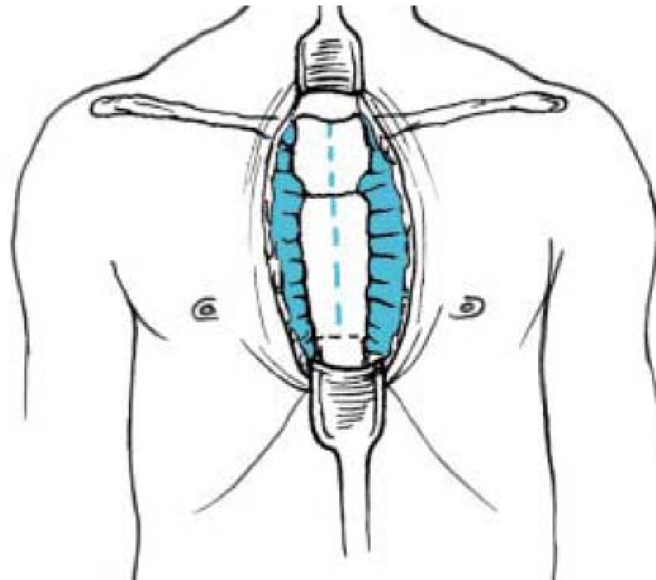


Figure 1: Median sternotomy incision. Modified from [7]

Generally, one vein segment revascularizes the first diagonal artery, one the circumflex system, and one the right coronary system. The venous graft is distended with cold cardioplegic solution and attached to the coronary artery or arteries as well as the aorta. The

internal mammary artery is occluded with a clip, transected proximal of the clip, and brought through a pericardial window to the anastomosis point. After removing any residual air from the ascending aorta the cross-clamp is removed and the controlled aortic reperfusion begins. The mammary artery clip is removed after the hyperkalaemic phase and once the heart beats in a steady rhythm cardiopulmonary bypass is discontinued and decannulation effected. All sutures are inspected to obtain hemostasis and the pericardium is left open to achieve good drainage and prevent cardiac tamponade. Drainage tubes are placed and the sternum is closed with stainless steel wire sutures. The drainage tubes are connected to a container with a negative pressure of 10 to 15 mmHg. Finally the muscles and skin are closed and a dressing is applied to the chest wound.[3, 7]

I.I.I.III EARLY POSTOPERATIVE CARE

Most patients have a smooth and uncomplicated course, but 5 to 10% experience postoperative problems. In the intensive care unit (ICU), a number of parameters are monitored to detect complications at an early stage. Measurements include arterial blood pressure, central venous pressure, urinary output, electrocardiographic tracing, and determination of arterial blood gases. Cardiac function can be assessed with the help of cardiac output and pulmonary capillary wedge pressure. Most patients are extubated either in the operating room or a few hours postoperatively. Criteria for early extubation include good pulmonary gas exchange, adequate ventilatory mechanisms, a clear chest radiograph, absence of dysrhythmias and excess bleeding, and stable neurological and cardiac function. Usually patients can return to the ward on the first postoperative day and discharge from the hospital may occur on the sixth or seventh postoperative day. [3-5]

Pharmacological management should include prevention and treatment of dysrhythmias, because postoperative atrial fibrillation is a major source of morbidity and prolonged hospital stay. In this instance, β-blockers are shown to be beneficial for the reduction of postoperative atrial fibrillation. Aspirin seems to improve vein graft patency significantly throughout the first postoperative year. Administration of statins reduces the progression of atherosclerosis in general and atherosclerotic vein graft disease in particular.[6]

I.I.II EXTRACORPOREAL PERFUSION

I.I.II.I DEFINITION

“Cardiopulmonary bypass is a method of whole-body perfusion in which the pumping action of the heart and the oxygenation of blood by the lungs are replaced by an extracorporeal circuit.”[8]
The patient’s blood, which normally returns to the right atrium, is diverted into a device in which oxygen is supplied to the blood and carbon dioxide is removed. The newly arterialized blood is pumped from the device into the patient’s aorta.[4]

A number of physiologic variables are under direct external control, including total systemic blood flow, systemic arterial and venous pressure, arterial oxygen and carbon dioxide levels, perfusate haematocrit and the temperature of both perfusate and patient.[3]
At low temperatures the basal metabolic activity is reduced and therefore less oxygen is used. Hypothermia is induced to protect the organ system against ischaemic injury while accepting a possible change in the acid-base balance towards an acidotic state.[3]

I.I.II.II TECHNIQUE

To establish cardiopulmonary bypass, cannulae are introduced directly into the ascending thoracic aorta and the right atrial appendage. The aortic cannula is usually chosen of a size that will allow a high flow rate with minimal gradient across the cannula, thus reducing the likelihood of embolic phenomena. Venous blood is returned to the pump by means of a large cannula, selected to be as large as possible to ensure adequate drainage and permit high flow rates as well as low systemic venous pressure during bypass. After connection of the arterial and venous cannulae the cardiac technician opens the line and starts the pump slowly. When no increased resistance or pressure can be found, the venous line is opened and the pump is accelerated. The perfusate is then cooled to a temperature of 28-32°C for brain protection and once the desired temperature is established an aortic cross clamp is applied to allow coronary artery grafting to be performed.[3, 8]

I.I.II.III HEPARIN AND PROTAMINE ADMINISTRATION

About 5 to 10 minutes before cardiopulmonary bypass (CPB) is established the patient is heparinized by the intravenous injection of a dose of 300-400 U/kg body weight of heparin.

Heparin binds to and activates antithrombin III, which is responsible for the anticoagulant activity. The heparin concentration in plasma can be determined with the help of the activated clotting time (ACT), which correlates well with the direct measurement. Protamine sulfate is used to reverse the effect of heparin after terminating CPB and removing all the cannulae. The measurement of ACT and the calculation of a heparin dose-response curve enable the heparin and protamine doses to be individualized for each patient. The ACT should be at least 400s.[4, 8]

I.I.II.IV THE BYPASS CIRCUIT

The main components are an oxygenator and an arterial pump (see Figure 2). The oxygenator is the most important part of the system and probably the most damaging. There are two types of oxygenator, the bubble and the membrane oxygenator. Both have a heat exchanger incorporated in the system to regulate the blood outflow temperature.[4, 8]

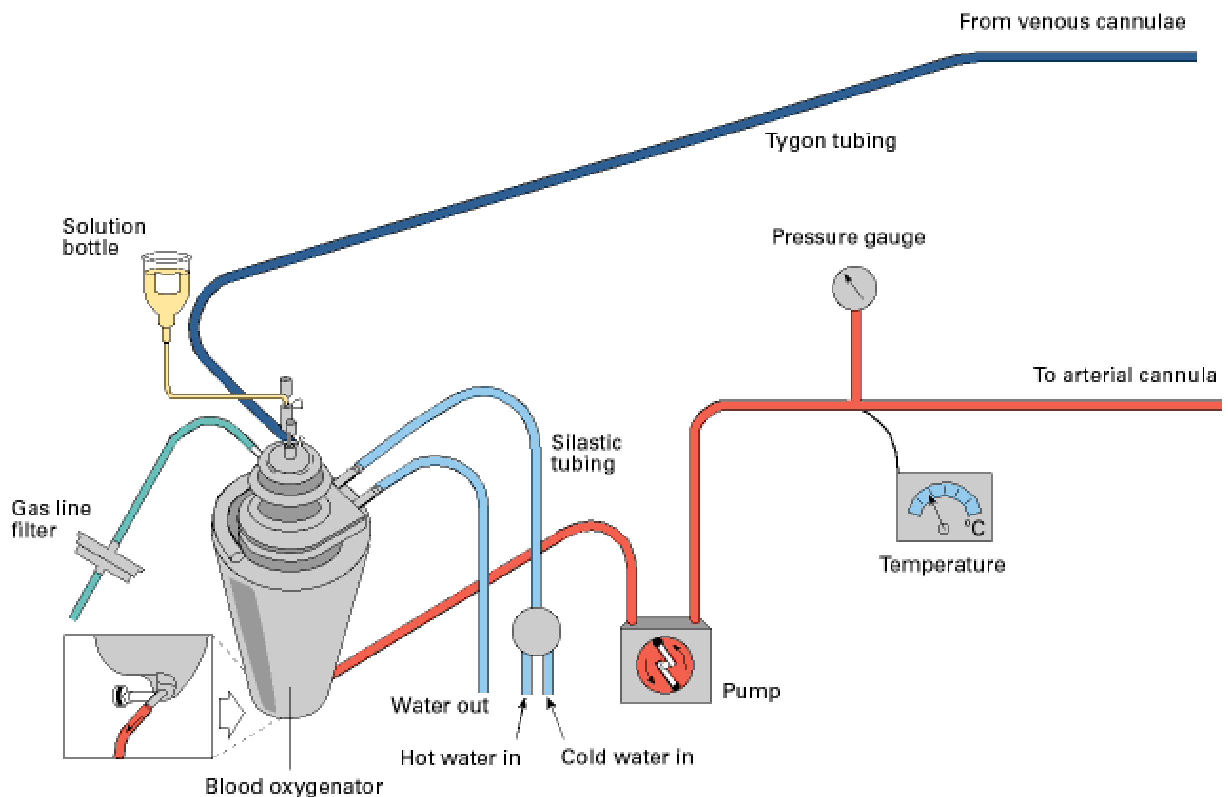


Figure 2: Cardiopulmonary bypass circuit: venous drainage occurs by siphon effect due to the difference in height between the operating table and venous reservoir. [8]

Bubble Oxygenator

The bubble oxygenator works by forcing a rapid jet of oxygen through a volume of blood and thus produces a large blood-gas interface, gas transfer occurs by partial pressure gradients. Smaller bubbles lead to better relative surface area:volume ratio and therefore more efficient oxygenation but are more difficult to remove from the patient's blood. Larger bubbles are more effective for carbon dioxide removal, which is why in practice bubble oxygenators create bubbles in a range of sizes to fulfill both functions. The bubbles are cleared from the system by exposure to surface-action silicone rubber, filtration and settling. While this type of oxygenator is cheap and simple in construction, there are inherent disadvantages: Blood gas control is imprecise, inefficient bubble removal may lead to microembolisation, and the direct blood-gas interface is known to damage blood cells and produce fibrinous microemboli. The bubble oxygenator achieved wide acceptance in the 1960s and the following two decades and is still used today although with declining frequency.[8, 9]

Membrane Oxygenator

A membrane oxygenator separates blood and gas over a membrane permeable to oxygen and carbon dioxide. The membrane itself can consist of hollow fibers or flat sheets, both of which provide a large exchange surface. Arterial oxygen tension is regulated by modulating the oxygen/air mixture and carbon dioxide tension by varying the gas flow or altering its tension in the gas mixture. The advantages are obvious: Fewer microbubbles are able to enter the patient's blood circuit and massive gas embolism is highly unlikely. The propensity for cellular damage is less than that associated with a direct gas-liquid interface and there is greater accuracy in blood gas control. The membrane oxygenator is commonly used worldwide because of its advantages.[8, 9]

Arterial Pump

The arterial pump is used to establish an accurate flow rate and the arterial line pressure must be continuously monitored. A simple mechanical double-arm roller pump with speed control is commonly used. The rollers rotate at a constant flow rate to provide an output similar or exceeding that of the normal cardiac output and act on a tube of silicone rubber less prone to spalliation than the other tubes of the circuit. Non-occlusive rollers are used to avoid damage to red blood cells and haemolysis.[4, 8]

Another possibility is to use a centrifugal head pump. This type of pump seems to be superior

to the roller pump in terms of platelet activation rates, but there was no significant difference in haemolysis rates or neuropsychologic outcome of adult patients.[10-12]

OTHER COMPONENTS OF THE CIRCUIT

The venous cannula is connected to a reservoir, which is positioned below the operating table to provide adequate siphonage from gravity and allows escaping of air returning with the venous blood. A device for ultrafiltration is incorporated into the circuit of the oxygenator to remove excess serum water during the last minutes of CPB. Other components include provision for blood defoaming, monitoring, and safety devices. [4, 8]

Each component adds to the priming volume, that is why the pump-oxygenators are simplified to keep the priming volume low. The diluent, which is used to prime the system, is a balanced electrolyte solution with a near-normal pH and an ionic content resembling that of plasma.[4]

I.I.II.V MYOCARDIAL PROTECTION AND CARDIOPLEGIA

The quiet and bloodless heart through cessation of blood flow is exposed to prolonged episodes of global myocardial ischemia and the anoxic period tolerable does not exceed 30 minutes even with hypothermia. The need for longer periods of cardiac arrest as well as improved myocardial preservation led to the search of alternatives for the widely used intermittent coronary perfusion. Chemical cardioplegia, first used successfully as early as 1955 but then resulting in severe complications, seemed to be the answer. Myocardial oxygen demand is reduced to the point that allows myocardial energy storages to be sufficient to maintain cell structure and gradients of ions, thus myocardial cells remain viable and functional. With the heart electromechanically quiescent the temperature is the primary variable determining energy demand. The cardioplegic solution should be at 4°C and the myocardial temperature should be lowered initially to 12-15°C and maintained at 15-20°C to allow energy production from anaerobic metabolism to suffice.[8]

A potassium dose of 20 to 24 mEq/l rapidly infused promptly depolarizes myocardial cells and produces sustained diastole. Magnesium used at a concentration of 50 mEq/l depresses the inherent rhythmicity of pacemaker cells and the contractility of myocardial cells. An example for a cardioplegic solution is shown in Table 1.[8]

Table 1: St Thomas' cardioplegic solution no. 1 [8]

Substance	Ringer's solution (mmol)	Cardioplegic solution (mmol)	Final concentration (mmol/l)*
Calcium chloride	2.25	0	2.20
Potassium chloride	4.02	15.96	19.59
Sodium chloride	147.13	0	144.25
Magnesium chloride	0	15.99	15.968
Procaine hydrochloride	0	1.00	0.98
Distilled water	1000 ml	20 ml	1020 ml

*Concentration corrected for fluid volume of 1020 ml
Osmolarity = approximately 300 mmol/kg H_2O.

Starting from the 1980s blood-based potassium solutions were studied to further improve myocardial protection and reduce creatine phosphokinase-MB release with mixed results. Cardioplegia with cold oxygenated blood plus an arresting agent is said to intermittently replenish cellular energy levels by aerobic metabolism at the time of reinfusion. In a large high-risk study patients receiving crystalloid cardioplegia were at a significantly increased risk of postoperative myocardial infarction, shock, and development of postoperative conduction defects although this type of cardioplegia is associated with significantly shorter cross-clamp times. In patients with an left ventricular ejection fraction (LVEF) of <36%, blood cardioplegia is associated with more operative stability and a reduction in postoperative morbidity and therefore seems to be superior to crystalloid cardioplegia.[8, 13]

Enhancements of cardioplegic solutions with Krebs' cycle substrates such as glutamate and aspartate have shown to improve ATP preservation. Glucose-insulin-potassium solutions have failed to demonstrate a significant benefit in a large high-risk study group despite good results in smaller non-randomized studies. This solution is commonly used to treat myocardial ischaemia in various medical situations. Supplements of antioxidants such as reduced glutathione seem to be beneficial inactivating free radicals and as scavengers to the intravascular and interstitial compartments. Research with L-arginine as well as inhibitors of leukotrienes synthesis, tumor necrosis factor α (TNF-α), complement factor C5 and neutrophil inflammatory mediators as additives to cardioplegic solutions has been conducted and these substances also seemed to be beneficial and result in increased myocardial protection.[14]

For CABG the infusion of cardioplegia occurs directly through the aortic root after cross-clamping. Reinfusions are often necessary to maintain adequate hypothermia and prolonged

cardiac arrest, usually at intervals of 25 to 30 minutes. After the cross-clamp release there is a high initial rate of spontaneous sinus rhythm, although episodes of ventricular fibrillation are common during the early reperfusion period.[8]

I.I.II.VI GENERAL RESPONSE TO CARDIOPULMONARY BYPASS (CPB)

THE HUMORAL RESPONSE TO CPB

The initial response to cardiopulmonary bypass is a humoral response and is initiated by the contact of plasma with nonendothelialized surfaces of the tubing and the pump-oxygenator. Plastics, glass and metal cause alterations in the structure and function of blood. Specialized plasma proteins are activated and parts of the coagulation cascade respond in spite of the heparinization. Other cascades to respond are the complement, kallikrein and fibrinolytic cascades. The results are increased vascular permeability, smooth muscle contraction, neutrophil aggregation and enzyme release (for an overview of effects see Figure 3).[4, 8]

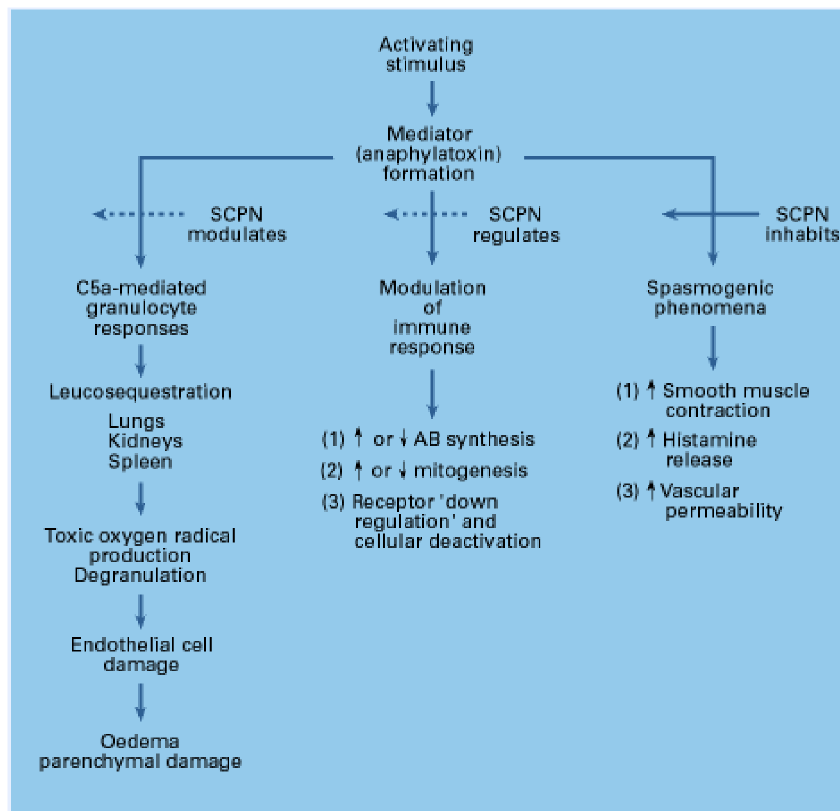


Figure 3: Consequences of complement activation and anaphylatoxin release [6].
[SCPN - Serum carboxypeptidase N; AB – Antibody]

THE CELLULAR RESPONSE TO CPB

The cellular response is mediated by both blood cells and endothelial cells. Neutrophilic granulocytes play a major role in the response to CPB once they are activated by complement or other inflammatory mediators. The neutrophils then migrate toward areas of higher complement concentration and secrete cytotoxic substances, e.g. oxygen free radicals. Platelets are activated within 1 minute of the start of CPB and adhere to the foreign surfaces of the pump-oxygenator and the tubing and start to aggregate. Exposure of the fibrinogen glycoprotein receptors (GPIIb-IIIa complex) and binding of fibrinogen is essential to that process. The reaction of endothelial cells is probably triggered by a combination of shear stress, local ischemia and high local concentrations of substances. Those cells express eicosenoids such as prostaglandins, thromboxanes, leukotrienes and lipoxins.[4]

The stress generated within the bypass circuits causes injury to erythrocytes and the resulting haemolysis can occur immediately as well as over the following 24 hours. Interaction with walls and high shear stress as well as exposure to intermittent positive pressure and subhaemolytic shear rates cause chemical and mechanical changes in the structure of the cell membranes and stimulation of lipid peroxidase systems, thus resulting in the destruction of injured red blood cells.[3]

THE CATECHOLAMINE RESPONSE

The catecholamine response during CPB is characterized by a massive epinephrine release throughout the duration of bypass. Persisting elevation one hour after the operation occurs only in patients with postoperative hypertension. These patients also experience a rise of norepinephrine during CPB.[4]

THE "POSTPERFUSION SYNDROME"

The "postperfusion syndrome" can cause generalized organ dysfunction, consisting of non-cardiogenic haemorrhagic pulmonary oedema, renal impairment, bleeding diathesis, neurological changes, and fever of non-infective origin. This is usually transient and inconsequential but may necessitate treatment, reoperation and respiratory or renal support. Lung biopsies from patients up to 4 hours after bypass show pathological changes and most patients have increased alveolar/capillary oxygen difference and fluid in the tracheobronchial tree. A plausible mechanism for pathological changes in the lung after CPB is that of the

"whole-body inflammatory response" with complement activation as the stimulus of organ dysfunction. Treatment of non-cardiogenic haemorrhagic pulmonary oedema includes infusion of epinephrine and pharmacological doses of steroids, positive end-expiratory pressure is used to improve gas exchange, and aggressive tracheobronchial toilet is necessary for clearance of oedema fluid.[8]

I.I.III COMPLICATIONS OF CORONARY ARTERY BYPASS GRAFT OPERATION

The complexities of a coronary artery bypass graft procedure include the facts that the blood is unaccustomed to traveling through nonendothelially lined channels, to receiving gaseous and particulate emboli, and to experiencing nonphysiologic shear stress. Also the body is unaccustomed to the absence of any appreciable pulmonary blood flow and to the presence of a continuous or only mildly pulsatile aortic pressure. In addition to cardiopulmonary bypass, the patient undergoing cardiac surgery experiences all the stress responses characteristic of major operations and trauma.[4]

Because of improved myocardial protection techniques, especially the introduction of cold potassium cardioplegia, low postoperative cardiac output is now uncommon. Etiologic factors include hypovolaemia, cardiac tamponade, concealed bleeding, dysrhythmias, myocardial insufficiency, and acidosis.[5]

I.I.III.I ATRIAL DYSRHYTHMIAS

Atrial dysrhythmias such as premature atrial contractions, atrial fibrillation, atrial tachycardia and atrial flutter occur in 10 to 30% of patients and are a reflection of postoperative atrial irritability. Treatment includes pharmacologic cardioversion with beta-blockers and anti-arrhythmic agents, and direct-current cardioversion can be effective in some cases. Transient atrial dysrhythmias are usually well tolerated but should be monitored because of occurrence of significantly lower cardiac output or systemic thromboembolism.[5]

I.I.III.II BLEEDING

Bleeding after CABG continues to be a major concern. Prompt and effective treatment reduces morbidity and mortality. Chest tubes placed in the operating room should be closely monitored

and drainage continuously measured. Surgical reexploration is indicated when bleeding is over 250 ml/h, associated with hemodynamic compromise or not responding to medical correction of a coagulation defect. Early reoperation usually stops the source of bleeding, prevents pericardial tamponade and minimizes administration of homologous blood. The incidence of reoperation varies, ranging from 3% to 14% with an average of 6.2%.[15] If a coagulation defect or localized fibrinolysis is suspected, the use of desmopressin, platelet concentrates, and the administration of aprotinin may be indicated. Occasionally the use of fresh frozen plasma or fibrinogen can be effective.[4, 16] Table 2 shows a treatment protocol for bleeding complications.

Table 2: Treatment Protocol for Excessive Mediastinal Bleeding After Cardiopulmonary Bypass in Adults [16]

Problem	Interpretation	Treatment
Bleeding < 50 mL/h		
Stable hemodynamics		No treatment
Abnormal coagulation profile		
Bleeding 50–150 mL/h		
Hypothermia		Correct body temperature
Hypertension		Normalize blood pressure
Diffuse oozing suspected		Trial of PEEP (5–10 cm H_2O), evaluate coagulation status
Coagulopathy		
↑ aPTT, PT, ACT	Heparin excess	Protamine
↑ PT (>1.2 control), ↓ Fibrinogen	Factor deficiency	Fresh frozen plasma
Platelets < 100,000/μL	Thrombocytopenia	Platelet concentrates
Platelets > 100,000/μL, bleeding time > 10 min	Platelet dysfunction	DDAVP, 0.3–0.4 mg/kg
Persistent bleeding 30 min after DDAVP, ↑ FDP, ↑ D-dimers, TEG evidence of fibrinolysis	Fibrinolysis	Tranexamic acid, ϵ-aminocaproic acid, aprotinin (antifibrinolytic dosage)
Massive bleeding		
>250–300 mL/h in first 2 h, >150 mL/h thereafter, arterial bleeding, tamponade (clinical or echographic)		Immediate surgical reexploration

ACT = activated clotting time; aPTT = activated partial thromboplastin time; DDAVP = desmopressin; FDP = fibrin and fibrinogen degradation products; PEEP = positive end-expiratory pressure; PT = prothrombin time; TEG = thromboelastography.

I.I.III.III PERICARDIAL EFFUSION AND CARDIAC TAMPONADE

Pericardial effusion after cardiac surgery is frequent and reaches the maximum dimension in most patients around the tenth postoperative day. Most effusions are asymptomatic and require no particular treatment. Table 3 shows the incidence and size of pericardial effusions in a study population of 780 patients.

Table 3: Incidence and size of pericardial effusions [17]

	Total (%)	CABG (%)	Valve replacement (%)	Other (%)
Number	780	413	324	43
No effusion	282 (36)	104 (25)*	155 (48)	23 (53.5)
Effusion:	498 (64)	309 (75)*	169 (52)	20 (46.5)
Small	341 (68.4)	196 (64)†	128 (76)	17 (85)
Moderate	149 (30)	109 (35)†	38 (22)	2 (10)
Large	8 (1.6)	4 (1)	3 (2)	1 (5)

CABG, coronary artery bypass grafting. *p<0.001 compared with valve replacement group and other group. †p<0.005 compared with valve replacement group and other group.

Cardiac tamponade is a rare complication of CABG operation, developing in only about 1 % of patients with pericardial effusion. Clinical signs and symptoms include progressive weakness and lethargy, progressive dyspnoe on exertion, and orthopnea as well as hepatomegaly, ascites and elevated jugular venous pressure. The initial hemodynamic finding of cardiac tamponade is elevated and equalized central venous and pulmonary wedge pressure, which is usually accompanied with low cardiac output. Sometimes mediastinal widening or blood collection can be seen in the chest radiograph. Once diagnosed, tamponade requires rapid decompression either by percutaneous pericardiocentesis or by subxiphoid pericardiotomy. [4, 5, 17, 18]

I.I.III.IV Infection

Surgical wound infections (SWIs) of the sternal wound and leg occur in 2-20% of patients after CABG and cause increased suffering as well as prolonged hospital stay and increased costs. Postoperative infection in general ranges second among the complications with the highest incremental cost to treat, with an average additional cost of $35,307 (see Table 4).[6, 19, 20]

Table 4: Average cost and length of stay (LOS) of patients with and without complications after CABG [20]

	Average Cost, Mean ± SD $	Incremental Cost of Complication	Average LOS, Mean ± SD Days	Incremental LOS of Complication
Patients without complication	29,477 ± 17,358	–	9.0 ± 5.8	–
Patients with post-op infection	67,115 ± 65,450	+ 35,307	25.2 ± 23.2	+ 15.5
Patients with septicemia	90,843 ± 71,594	+ 59,204	31.0 ± 24.5	+ 21.3

The definition of the Center for Disease Control and Prevention (CDC) of SWIs is commonly used in the US, defining a superficial SWI as one involving skin and subcutaneous tissue and a

deep SWI as one involving muscle and fascia. A superficial sternal wound infection is therefore defined as infection and dehiscence of skin and subcutaneous tissue, but with a stable sternum. A deep sternal infection or mediastinitis (see Figure 4) requires an organism isolated from culture of the mediastinal tissue or fluid or evidence of mediastinitis during surgery or purulent discharge from the mediastinum with one of the following conditions: chest pain, sternal instability, or fever.[19, 21]

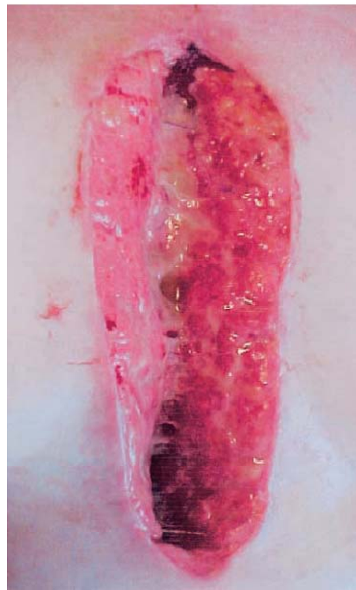


Figure 4: Poststerniotomy mediastinitis[22]

Mediastinitis is reported to occur after 0.5-5% of the CABG procedures and is associated with a mortality of 20% (see Figure 5). General risk factors for deep sternum infections are diabetes and obesity, and reoperation as well as the use of bilateral internal mammary arteries. The resulting devascularization of the sternum appears to contribute to mediastinitis.[5, 6, 23]

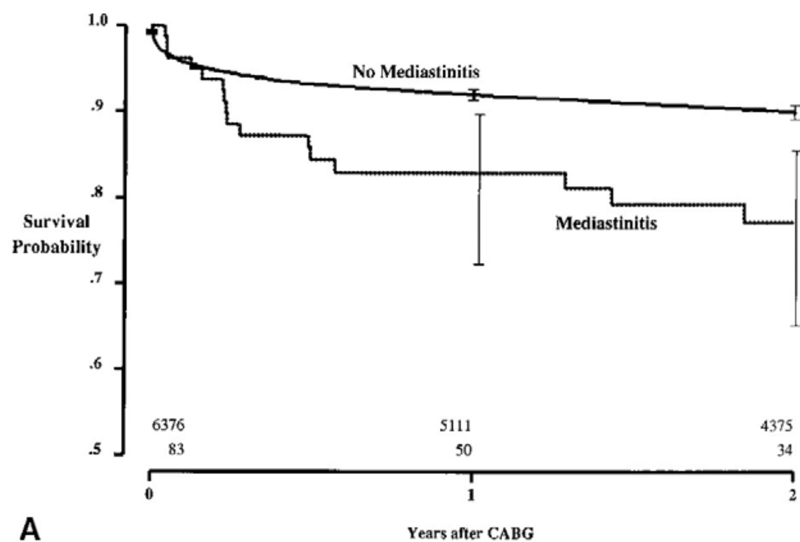


Figure 5: Kaplan-Meier survival plots for patients with and without mediastinitis [23]

Symptoms and signs of sternal wound infection include sternal instability, fever, leukocytosis, and wound drainage. Initial treatment includes broad-spectrum antibiotics and cultures should be obtained. The wound is opened as necessary and thorough debridement should be carried out. This includes removal of all debris, suture material and dead tissue, and in case of sternal instability sternal wires and devitalized parts of the sternum are removed as well. A vacuum-assisted closure (VAC) device has been shown to be useful in treatment of sternal infections (see Figure 6).

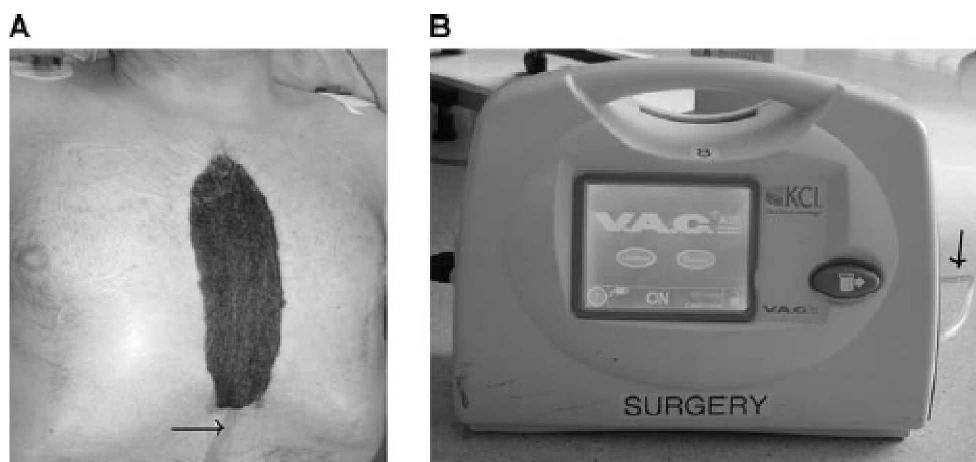


Figure 6: (A) Sternal wound with a polyurethane sponge dressing with tubing (arrow), (B) Vacuum pump with canister (arrow)[21]

The VAC system is placed directly on the sternum or in case of deep involvement into the sternal defect. The system is changed every 48 hours until the wound is well vascularized and covered with granulation tissue, and the bacterial cultures are negative. Wound closure can then be achieved with pectoral or omental flaps. [5, 21, 22, 24]

I.II IMMUNE RESPONSES IN GENERAL AND AFTER CABG

Immunity can be categorized into adaptive and innate immunity. Adaptive immunity is specific and mediated by T- and B-lymphocytes whereas innate immunity is mediated by macrophages and neutrophils. Innate immunity includes all non-specific resistance or immune mechanisms. The response to an initial infection is shown in Figure 7.[25, 26]

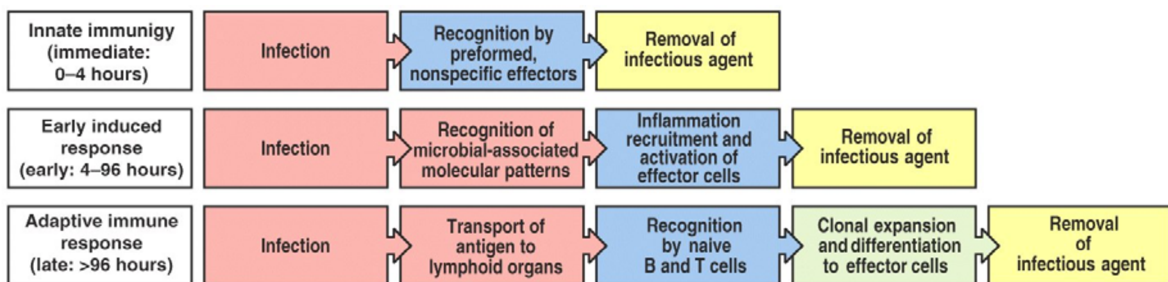


Figure 7: The three phases of initial infection.[27]

I.II.I INNATE IMMUNITY – THE TOLL-LIKE RECEPTOR (TLR) FAMILY

Macrophages, maturing continuously from monocytes and migrating into tissues throughout the body, are usually the first cells to encounter pathogens. The innate immune response starts with the recognition of pathogen-associated molecular patterns (PAMP), components of the pathogens that are not normally found in the host. Antigen-presenting cells (APC), such as macrophages and dendritic cells, initiate a signaling pathway when binding these PAMP, effectively stimulating host defenses through the induction of reactive oxygen and nitrogen intermediates. In addition to that, the activation of APCs starts production of pro-inflammatory cytokines and upregulation of costimulatory molecules, thereby initiating the adaptive immunity, and recruiting natural killer cells and naïve T-cells.[27, 28] An overview of the link between innate and adaptive immunity is given in Figure 8.

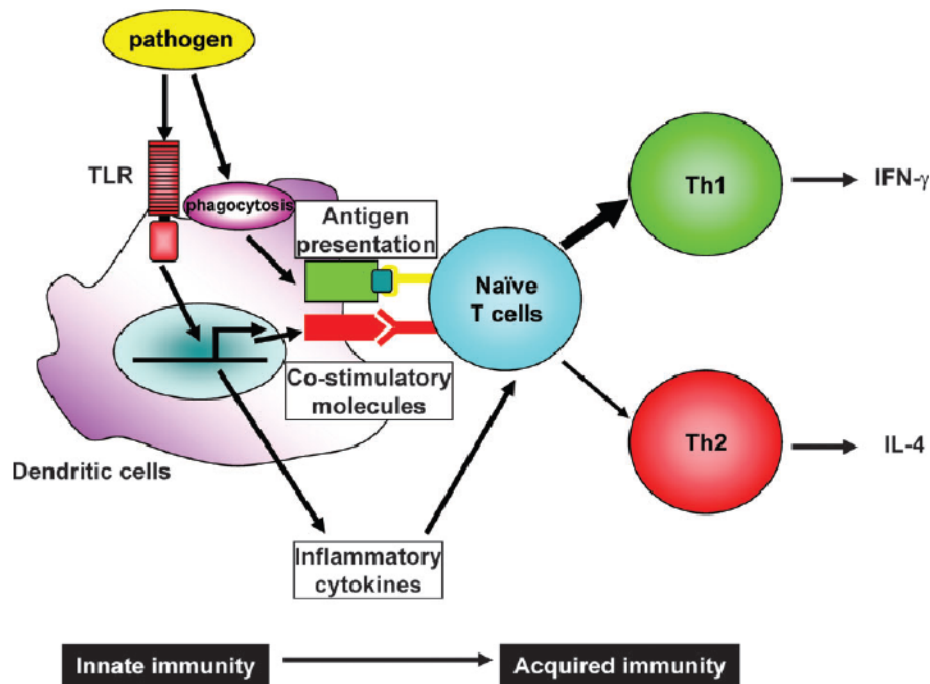


Figure 8: The link between innate and adaptive immunity.[29]

A family of receptors responsible for the recognition of PAMPs, named Toll-like-receptors, can initiate innate immunity via activation of pro-inflammatory proteins which are able to trigger immune response.[29, 30]

I.II.I.I INTRODUCTION TO TLRS

Toll is a *Drosophila* gene essential for ontogenesis and anti-microbial resistance. The product of the Toll gene is a membrane protein consisting of extracellular, transmembrane and cytoplasmatic domains. The cytoplasmatic domain is related to that of the human interleukin-1 receptor (IL-1R) and therefore referred to as the Toll/IL-1R homology (TIR) domain. Toll-like receptors have been identified and cloned in vertebrates and human TLRs are a growing family of molecules involved in innate immunity. Several TLRs have been identified, TLR1 to TLR9 are conserved between the human and mouse, and TLR10 seems to be functional in the human but non-functional in the mouse. Similarly, the human *TLR11* gene has a stop codon, which results in no production, whereas the mouse TLR11 is functional. Generally the TLR family members recognize a variety of microbial components, for a detailed description of the ligands see Figure 9.[29, 31-34]

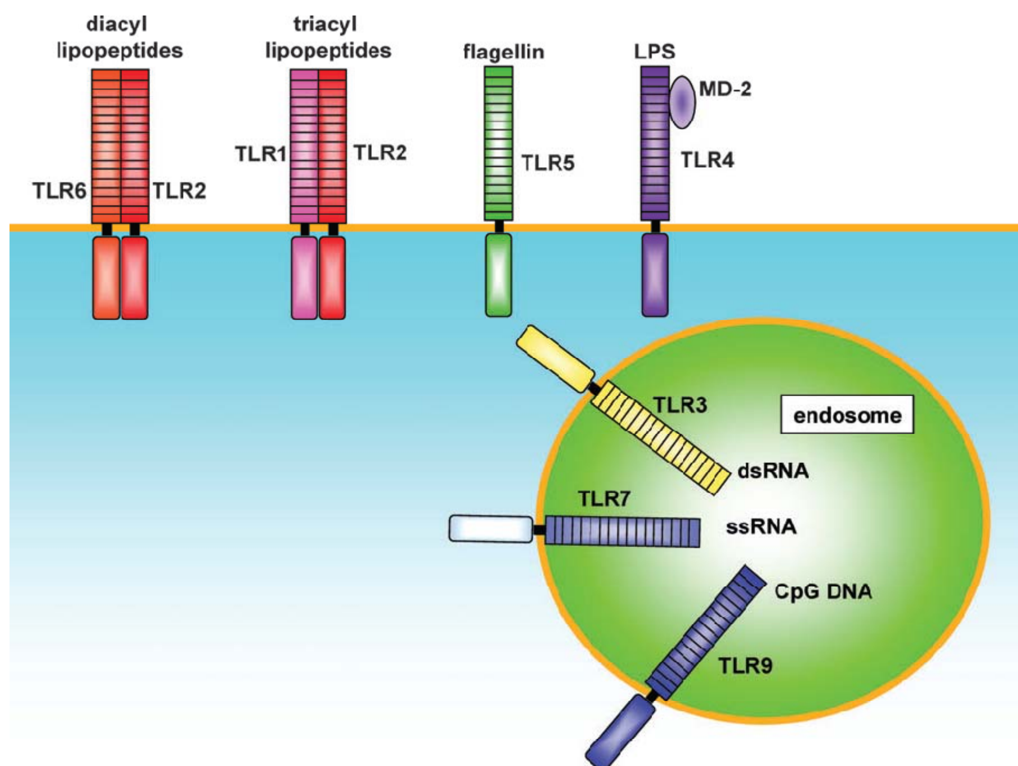


Figure 9: TLRs and their ligands.[29]
TLR2 is essential in the recognition of microbial lipopeptides. TLR1 and TLR6 cooperate with TLR2 to discriminate subtle differences between triacyl and diacyl lipopeptides, respectively. TLR4 is the receptor for LPS. TLR9 is essential in CpG DNA recognition. TLR3 is implicated in the recognition of viral dsRNA, whereas TLR7 and TLR8 are implicated in viral-derived ssRNA recognition. TLR5 recognizes flagellin. Thus, the TLR family members recognize specific patterns of microbial components.
[Abbreviations: CpG DNA – cytosine-phosphatidyl-guanosine deoxyribonucleic acid; dsRNA – double stranded ribonucleic acid; ssRNA – single stranded ribonucleic acid]

TLRs are type-1 orphan receptors with an extracellular domain containing 21 tandemly repeated leucine-rich motifs and a cytoplasmic domain responsible for signal transduction. Small cysteine-rich domains that vary in number and arrangement between different members of the Toll family are included into the ectodomains of Toll proteins. [33]

I.II.I.II SIGNALLING PATHWAYS OF TLRS

Upon ligation of TLRs by their PAMPs, the receptors trigger a signaling cascade, using the same signalling pathways and molecules as the IL-1Rs. The activation of every TLR except TLR3 and TLR4 is dependent on a protein involved in myeloid differentiation called MyD88, hence the signaling cascade is called the MyD88 dependent pathway. Four more adapter proteins have been identified, which are used as triggers of different signaling processes of the TLRs.[30]

MyD88 and interleukin-receptor associated kinase (IRAK) are sequentially recruited and

activate nuclear factor (NF)-κB via TNF receptor-associated factor 6 (TRAF6) and NF-κB-inducing kinase (NIK).[25, 30]

MyD88, a 35 kDa protein, has a modular structure, the carboxy-terminal end binding to the TIR domain of the IL-1R is called the TIR module, and the amino-terminal portion was first recognized in association with apoptotic proteins and is therefore called a "death-domain". The interaction with the IL-1R complex, containing IL-1R, the IL-1R accessory protein and IRAK, is signal dependent and require a bound ligand. IRAK and IRAK-2, two putative serine-threonine kinases, interact with the death domain of MyD88 and in this process get autophosphorylated. Then IRAK disengages from MyD88 and interacts with TRAF6, which is known to immunoprecipitate with NIK. NIK is a member of the mitogen-activated protein kinase kinase kinase (MAPKKK or MKK) family and can activate the IκB kinase (IKK) which is essential for the targeted phosphorylation and degradation of the NF-κB inhibitor IκB. Degradation of IκB releases NF-κB to translocate to the nucleus and induce specific genes.[32, 34]

The MyD88 independent pathway is used by TLR3, which signals through the Toll/IL-1R domain containing adaptor inducing IFN-β (TRIF) protein (also called TRIF dependent pathway). TLR4 uses TRIF and another adapter called TRIF-related adaptor molecule (TRAM). Both signaling cascades induce Interferon (IFN)-α/β through interferon regulatory factor (IRF)-3.[30, 35, 36]

An overview of TLR signaling pathways is given in Figure 10.

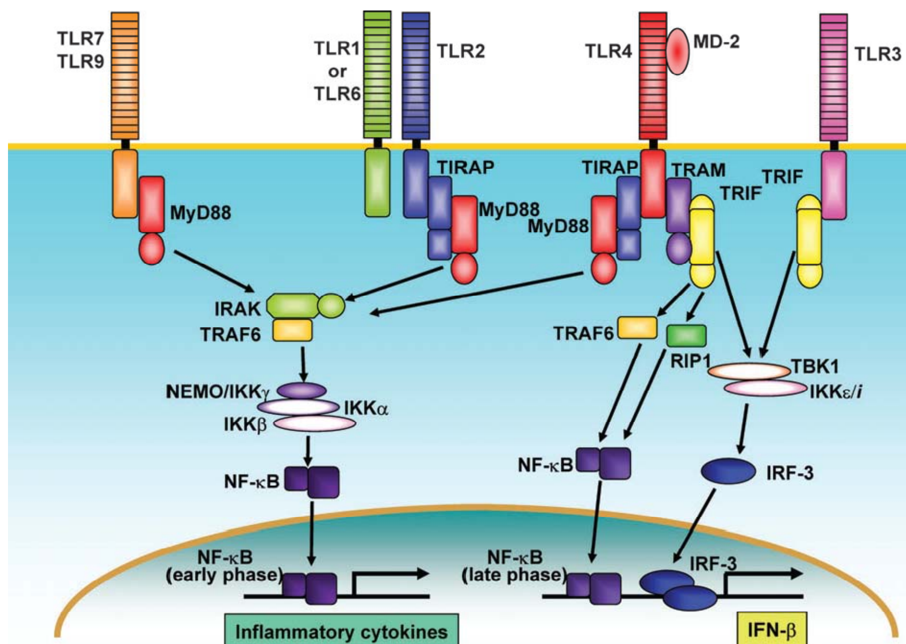


Figure 10: Different signaling pathways of TLR.[29]

I.II.I.III EXPRESSION PATTERN OF TLRS

Immunocompetent cells such as mononuclear phagocytes and polymorphonuclear phagocytes (PMNs), T- and B-lymphocytes, natural killer cells and monocyte-derived dendritic cells (DCs) were analyzed in terms of expression patterns of TLR1 to TLR5. TLR1 seemed to be ubiquitously expressed while TLR2-5 showed a restricted pattern of expression. TLR2, TLR4 and TLR5 are expressed by monocytes, PMNs and DCs. Exposure to lipopolysaccharide (LPS) and proinflammatory cytokines seems to increase TLR4 expression and presence of IL-10 blocks it whereas TLR2 is differentially regulated. TLR3 seemed only present in DCs. As shown in Table 5, these cells express all of the TLRs analyzed, which reflects their unique role in sensing pathogens and causing transition from innate to specific immunity.[32]

Table 5: TLR mRNA expression patterns in human leucocytes (modified from [32])

	T-Lympho-cytes	B-Lympho-cytes	Natural killer cells	PMNs	Mononuclear phagocytes	DCs
TLR1 (ubiquitous)	+	+	+	+	+	+
TLR2. 4 and 5 (restricted)	–	–	–	+	+	+
TLR3 (specific)	–	–	–	–	–	+

I.II.I.IV REGULATION OF TLR SIGNALING

Endotoxin tolerance, the fact that repeated exposure to microbacterial components such as LPS results in reduced responses, prohibits the induction of serious systemic disorders in the host. TLR-mediated signaling is modulated on the adapter level by redistribution, sequestration, denial of access to the TLRs, and degradation of the molecules. The serine/threonine kinase IRAK-M, lacking kinase activity, and the splice variant MyD88s without the intermediate domain, are induced in monocytes and macrophages upon LPS stimulation. Membrane-bound molecules such as single immunoglobulin IL-1 receptor-related (SIGIRR) molecule, also called TIR8, and ST2 seem to be involved in TLR response inhibition.[29, 37, 38]

I.II.II ADAPTIVE IMMUNITY – T-HELPER CELLS AND THEIR SUBGROUPS

The cells responsible for cell-mediated immune response in adaptive immunity are T lymphocytes or T-cells. T-cells involved in cell activation are marked by the expression of CD4 on their cell surface, whereas cytotoxic T-cells typically express CD8.[27]

$CD4^+$ T-cells, also called T-helper (T_H) cells, have various functions in immune protection. They mediate B-cell antigen response, induce macrophages to develop enhanced microbicidal activity, help recruite neutrophils, eosinophils, and basophils, and produce chemokines and cytokines. However, $CD4^+$ T-cells are not a unitary set of cells but can be divided into subgroups of cell populations with different functions.[39] At least four different subsets have been shown to exist, T_H1, T_H2, T_H17, and iTreg cells, the first two will be discussed in detail.[39]

DISCOVERY OF T_H1 AND T_H2 SUBSETS

Mosmann *et al.* first identified two subgroups of murine antigen-specific T_H cells on the basis of cytokine bioactivities, helper function, and biosynthetic labeling patterns. Although responsible for very different biological activities, these cells shared typical helper cell properties.[40] The first subset, the T_H1 cell, produces IL-2, IFN-γ and TNF-β, whereas the second, the T_H2 cell, secretes IL-4, IL-5, IL-6, and IL-10.[40]

In 1991 Del Prete *et al.* succeeded in generating human T cell clones with these characteristics. T cell clones specific for bacterial antigens such as purified protein derivative

of *Mycobacterium tuberculosis* showed a T_H1 secretion pattern and clones specific for helminthic components such as *Toxocara canis* excretory/secretory antigen showed a T_H2 secretion pattern.[41]

An overview of the main properties of T_H1 and T_H2 cells is shown in Table 6.

Table 6: Main Properties of T_H1 and T_H2 Human $CD4^+$ T-Cell Clones (modified from [42])

Properties	T_H1	T_H2
Cytokine secretion		
IFN-γ	+++	−
TNF-β	+++	−
IL-2	+++	+
TNF-α	+++	+
IL-6	+	++
IL-10	+	+++
IL-13	+	+++
IL-4	−	+++
IL-5	−	+++
ST2L expression	+	+++
Regulation by cytokines		
IL-2	up	up
IL-4		up
IFN-γ		down
IL-10	down	down
B-cell help for Ig synthesis		
IgE	−	+++
IgM, IgG, IgA		
At low T:B cell ratios	+++	++
At high T:B cell ratios	−	+++

I.II.II.I FUNCTIONS OF T-HELPER CELLS

The functions of T_H1 and T_H2 cells correlate well with their cytokines. T_H1 cells play a critical role in cell-mediated immunity against intracellular pathogens and delayed type hypersensitivity reactions, with IFN-γ commonly expressed at sites of delayed-type hypersensitivity. T_H1 cells can also provide B-cell help and stimulate production of antibodies of the IgG2a class, but at higher numbers this can switch to suppression.

The T_H2 subset is mainly responsible for mediating phagocyte-independent host defense, e.g. against extracellular parasites including helminths. This response is mediated by IgE and eosinophils with IL-4 as the most important cytokine.[42, 43]

T_H2 cells are commonly found at sites of strong antibody reactions and allergic responses. But

several T_H2 cytokines have anti-inflammatory actions, IL-4 and IL-13 antagonize IFN-γ-induced macrophage activation and IL-10 suppresses macrophage responses. Therefore T_H2 activation can result in the inhibition of acute and chronic inflammation. An important function of these cells seems to be the regulation and limitation of T_H1-mediated immune responses.[44]

When first stimulated by antigen or APC, upon the engagement of the T-cell receptor by the appropriate peptide-MHC complex, the naïve $CD4^+$ T-cell produces IL-2 and subsequently differentiates into phenotypes secreting other cytokines. The phenotype depends on the type of APCs, the nature and amount of antigen, and other micro-environmental factors. IL-2 induces proliferation in both T_H1 and T_H2 cells, but the latter are much more responsive to IL-4.[42, 43] The function and development of T_H1 and T_H2 cells is summarized in Figure 11.

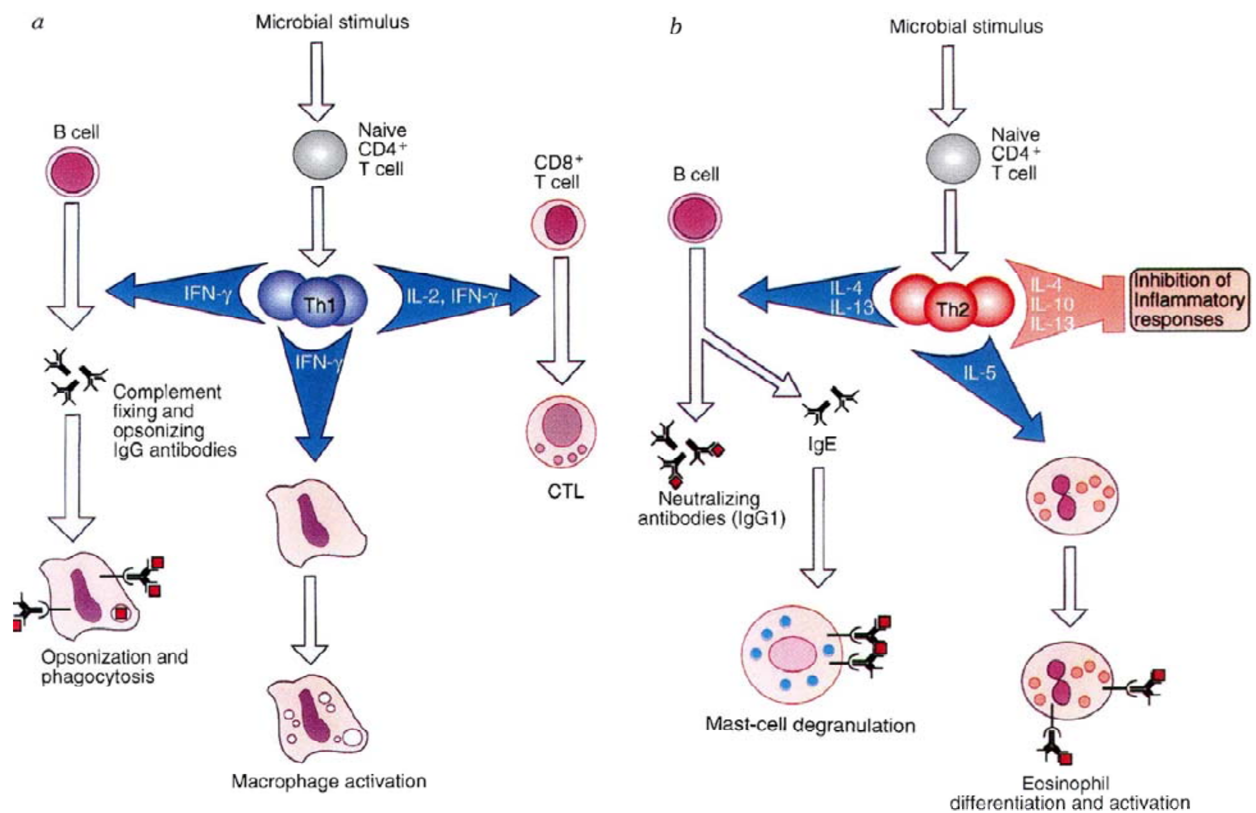


Figure 11: Effector function of T_H1 and T_H2 subsets of $CD4^+$ helper T lymphocytes[44]

a) T_H1 cells induce phagocyte and T-cell-mediated defence reactions against microbes; b) T_H2 cells induce IgE-dependent mast-cell degranulation and eosinophil activation.

The cytokines of T_H1 and T_H2 are capable of acting as inhibitors for the differentiation and effector functions of the reciprocal phenotype, which explains the strong bias toward one subset during many infections.[42, 43] IFN-γ has a selective inhibitory effect on the proliferative response of T_H2 cells.[45] IL-10 inhibits activation of T_H1 cell clones by impairing APC function.[46] An overview of the pathways of inhibition is given in Figure 12.

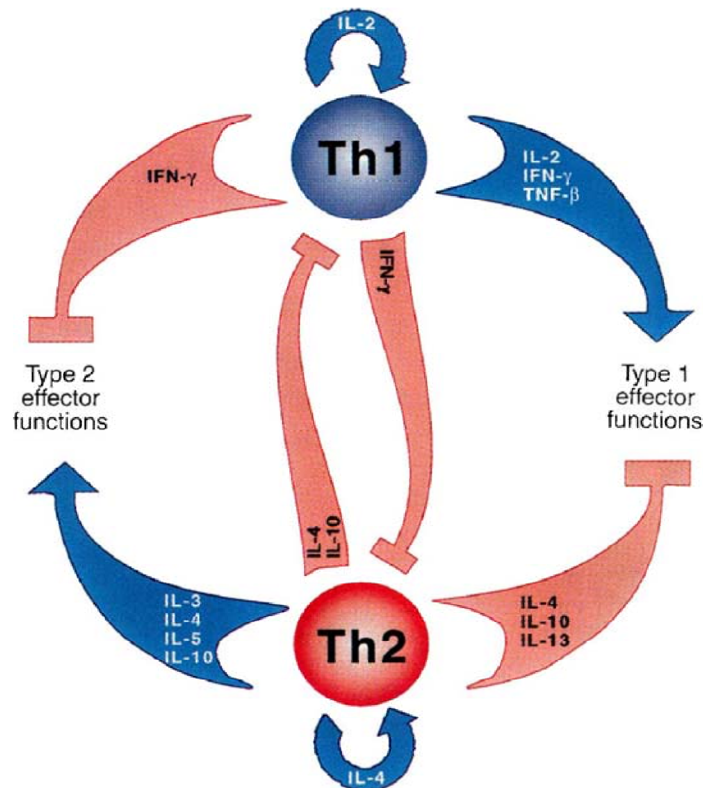


Figure 12: Opposing effector and inhibitory function of T_H1 and T_H2.[44]
TH1 and TH2 pathways are symmetrical, each suppressing the expansion and effector functions of the other subset. IL-2 and IL-4 are shown as autocrine growth factors. Effector functions: blue; inhibitory functions: red.

I.II.II.II Pathophysiological conditions involving T-helper cells

The discovery of polarized forms of T-helper-cells has been critical in the understanding of the pathogenesis of different human diseases. Several pathophysiological conditions have been suspected to be the result of dominant T_H1 or T_H2 responses. Table 7 shows an overview of pathophysiological conditions associated with T_H1 or T_H2 effector responses.

Table 7: Pathophysiological conditions associated with predominant T_H1- or T_H2-type effector responses[42]

T_H cell subset	Condition
T_H1	Autoimmune thyroid diseases Multiple Sclerosis Type 1 diabetes mellitus Crohn’s disease Lyme arthritis Reactive (Yersinia-induced) arthritis Contact (Nickel-induced) dermatitis Acute allograft rejection Rheumatoid arthritis (?) Fetal readsorption
T_H2	Ommen’s syndrome Essential hypereosinophilic syndromes Vernal conjunctivitis Atopic disorders Reduced protection to many infections Successful pregnancy (?) Systemic lupus erythematosus (?) Progression to AIDS in HIV infection (?)

I.II.III ST2 – A MODULATOR OF IMMUNE RESPONSES

In 1989 Tominaga identified a serum-inducible protein with significant similarity to the extracellular portion of the mouse IL-1R that did not have a corresponding transmembrane and cytoplasmatic portion. The protein was called ST2 and found to be expressed in murine fibroblast cells BALB/c-3T3. Nucleotide sequence analysis revealed it as a member of the immunoglobulin superfamily with the unique properties of a lack of transmembrane domain and growth-specific expression.[47]

The ST2 gene, respectively known as T1, DER4, and Fit-1, was identified to be a delayed-early serum response gene in cell growth control.[48-50] It is located on chromosome 2 at q11.2 in close proximity to the loci of other IL-1R family members and its gene products were classified as members of the IL-1R superfamily.[51, 52]

I.II.III.I THE PROTEIN AND ITS VARIANTS

The first variant discovered by the same group in 1993, called ST2L, also has a transmembrane and cytoplasmatic domain and shares 28% amino-acid identity with the IL-1 receptor type 1 as a whole molecule, as can be seen in Figure 13.[52] ST2V, a second variant form of human ST2, expressed by the human leukemic cell line UT-7/GM and various

sublines, lacks a third immunoglobulin-like domain due to alternative splicing and gains a new hydrophobic tail.[53] A third variant, ST2LV, was found to be expressed in the chicken system. This protein shares 318 amino acids with ST2 and ST2L, having lost the transmembrane region of ST2L, which may indicate its function as a soluble secreted protein.[54]

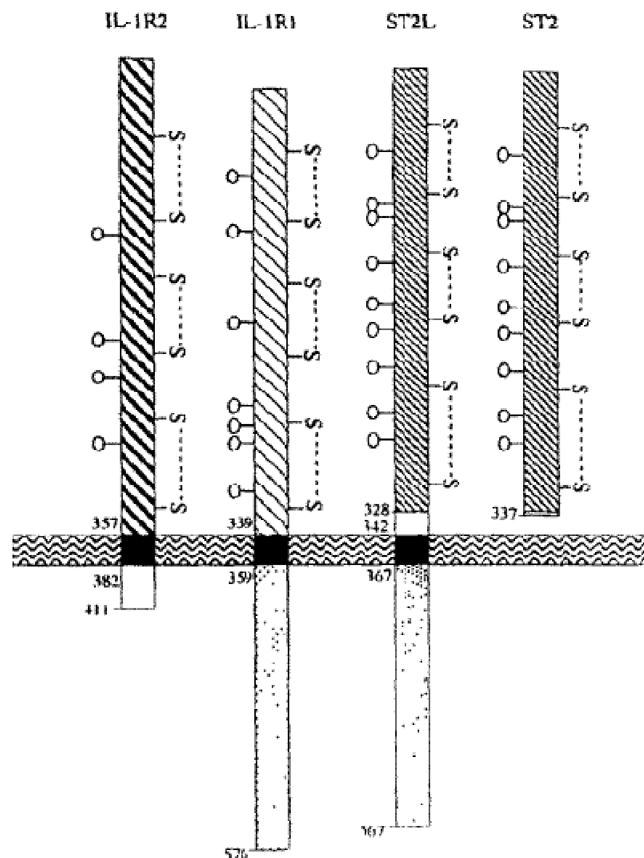


Figure 13: Schematic representation of ST2L-related proteins.[52]
Cross-hatched regions of the proteins indicate the similarity of extracellular domains of IL-1R1, IL-1R2, and ST2L. ST2 is also almost identical with the extracellular domain of ST2L. Striped areas of IL-1R1 (solid line) and ST2L (dotted line) represent a high similarity of cytoplasmatic domains.

I.II.III.II EXPRESSION OF ST2

The membrane bound and the soluble form of the ST2 receptor are expressed in hematopoietic, epithelial, and fibroblast cell lines *in vitro* and the lung and hematopoietic tissues *in vivo*.[48, 50] ST2L is also highly expressed on immature and mature mast cells and their progenitors.[55] The soluble receptor can also be found in embryonic tissues and specific

mammary tumors.[50, 56] The variant form ST2V can be found in stomach, small intestine, and colon.[57]

The importance of ST2 in immune responses became more obvious, when ST2L was found to be a stable cell surface marker expressed strongly on activated T_H2 cells but not T_H1 cells, independent of IL-4. These cells also expressed sST2, which suggests an involvement in the regulation of T_H2 functions.[58-61] The production of type 2 cytokines seemed to precede the expression of ST2L in vitro and it could be upregulated by several APC- or T-cell-derived cytokines. IL-6 had the strongest effect on ST2L expression, an up to 8-fold increase could be seen *in vitro*, whereas IL-1, TNF-α, and IL-5 had smaller effects. Cross-linking of the membrane bound protein resulted in a costimulatory signal for T_H2 cells inducing proliferation and type 2 cytokine production.[62]

A number of models have been used to establish ST2L as a reliable selective marker in various conditions *in vivo*. Treatment of mice with monoclonal anti-ST2L reduced T_H2-mediated lung eosinophilia during respiratory syncytial virus infection, but not TH1-driven pulmonary infiltration.[63] Similar effects could be shown after allergen provocation with ovalbumin.[60, 64, 65] Anti-ST2L antibody increased murine resistance to *Leishmania major* infection, accompanied by enhanced IFN-γ synthesis and diminished IL-4 and IL-5 production.[58] ST2L was highly upregulated in the T_H2-dominated response in murine lungs containing granulomas induced by *Schistosoma mansoni* eggs[66], and eosinophil infiltration, and thus granuloma formation was abrogated in ST2L-deficient mice.[67] The T_H2 shift in HIV patients was shown to be accompanied by a high number of $ST2L^+$ cells.[68] Contrary to these findings, $T1/ST2^{-/-}$ mice showed normal T_H2 responses after infection with *Nippostrongylus brasiliensis* in two independent studies,[69, 70] indicating that the role of ST2L in TH2 differentiation might be auxiliary and involved with advanced commitment to the phenotype.[71]

I.II.III.III THE LIGAND INTERLEUKIN (IL)-33

In the search for a putative ligand of ST2L, many studies have been conducted since its discovery in 1989. Kumar *et al.* and Gayle *et al.* both did not detect binding of IL-1 cytokines IL-1α, IL-1β, and IL-1ra, and identified two proteins as possible ligands, which had no

biological activity.[72, 73]

In 2005, Schmitz *et al.* described IL-33 as a new member of the IL-1 family binding to ST2L, leading to the production of T_H2-associated cytokines and increased serum immunoglobulin levels. IL-1 family members are highly pro-inflammatory and share a common β-trefoil structure. *In vitro*, IL-33 is produced from a 30-kDa propeptide proteolytically cleaved by caspase-1 to generate the mature form, a 18 kDa peptide.[74] Treatment of mice with purified IL-33 leads to blood eosinophilia, splenomegaly, and striking histological changes in the lungs and GI tract as well as elevated serum levels of IgE and IgA. In addition to that, it was shown that IL-33 works as a T_H2 selective chemoattractant both *in vitro* and *in vivo*.[75] IL-33 also seems to have positive effects on the maturation, survival, adhesion, and cytokine production of mast cells independent of IgE [76-78], eosinophil survival and cytokine production [79], and other cells involved in immune responses [80].

At first, the expression pattern of IL-33 seemed to be very restricted. IL-33 mRNA was found only in arterial smooth muscle cells, bronchial epithelial cells, and activated dermal fibroblasts and keratinocytes.[74] Then the cytokine was found to be identical to a chromatin-associated nuclear factor expressed in high endothelial venules (NF-HEF), specialized blood vessels mediating lymphocyte recruitment into lymphoid organs. HEF endothelial cells constituted the first human cell type to express both the mRNA and the protein *in vivo*. Also a homeodomain-like helix-turn-helix motif within the N-terminal part of the molecule was discovered, which is associated with transcriptional repressor properties. *In situ* hybridization showed a major source of IL-33 mRNA to be endothelial cells in chronically inflamed tissues in rheumatoid arthritis and Crohn’s disease. These results suggest a dual function for IL-33 as both a T_H2-inducing cytokine and an intracellular nuclear factor.[81] As a matter of fact, IL-33 seems to be constitutively expressed in the nucleus of human endothelial cells of the vascular tree, multiple tumors, and tissues exposed to the environment, as well as fibroblastic reticular cells of lymphoid tissues. In all these cells the protein only accumulated in the nucleus and no trace was found in the cytoplasma, membrane or extracellular location. Active secretion of IL-33 by dendritic cells or macrophages is unlikely as a major mechanism *in vivo*, since no significant expression of mRNA in hematopoietic cells was detected. These results lead to the possibility that release of IL-33 may be due to infection or trauma of endothelial cells and present an

endogenous alarm system or “alarmin”.[82]

I.II.III.IV IL-33 SIGNAL TRANSDUCTION

IL-33 signal transduction is dependent on expression of ST2L, leading to recruitment of NF-κB and MAP-kinases via IRAK, MyD88 and TRAF6.[74] Signaling of the ST2 receptor seems to resemble typical IL-1 family signaling, the binding to a specific receptor is followed by the recruitment of a coreceptor required to mediate signal transduction.[83] Studies prior to the discovery of IL-33 suggested possible active homodimers based on signaling through antibody-mediated cross-linking. While these signals managed to activate extracellular signal-regulated kinase (ERK), p38, and c-Jun N-terminal kinase (JNK), they could not trigger NF-κB.[62, 84] In the search for a coreceptor the IL-1R accessory protein (IL-1RAcP), a protein necessary for IL-1α and IL-1β-mediated signaling, has been identified *in vitro* and *in vivo* as a member of the signaling complex.[85, 86]

An overview of the binding complexes of ST2 and IL-33 is given in Figure 14.

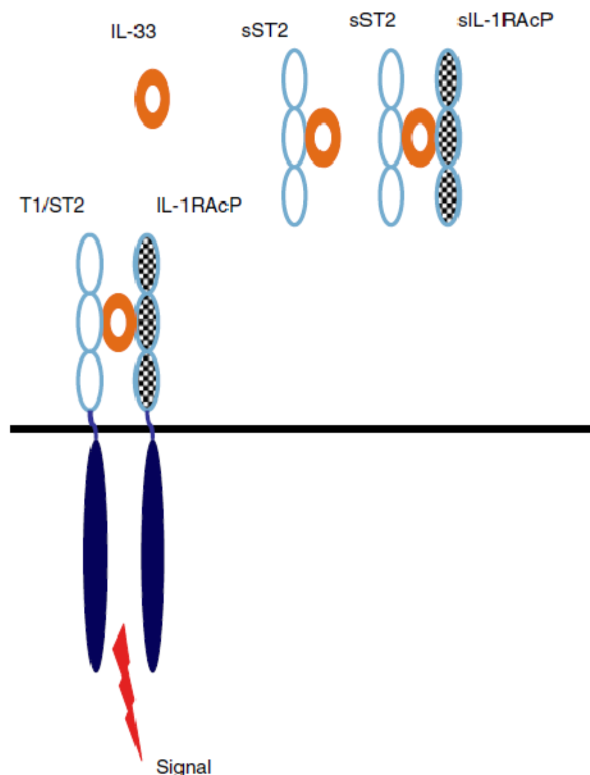


Figure 14: The binding complexes of ST2 and IL-33.[83]
Signaling through the ST2 receptor works starts with the recruitment of the IL-1RAcP as co-receptor. The soluble form sST2 may function as inhibitor of IL-33 with the coreceptor as enhancement.

I.II.III.V SOLUBLE ST2 (SST2) IN HUMAN DISEASE

Whereas ST2L is involved in the positive regulation of T_H2-dependent inflammatory processes, it has been implicated that sST2 attenuates these processes. The role of sST2 as a decoy receptor seems to become clearer with every experiment conducted.

One of the first studies of sST2 in human disease demonstrated significantly elevated sST2 levels in patients with atopic asthma and a correlation with the severity of asthma exacerbation.[87] A murine asthma model also showed increased levels of sST2 after allergen challenge. It is possible that the increased protein production may be required for the suppression of allergic inflammation.[88] Our group showed that significantly increased sST2 levels could be measured in sepsis and trauma patients as compared to abdominal surgery and healthy controls. In addition to that, serum levels of IgG1 and IgG2 were elevated and IL-2 and IFN-γ synthesis decreased in sepsis patients.[89] Soluble ST2 levels were also found to be elevated in the cerebrospinal fluid after subarachnoid hemorrhage, in malignant pleural effusions, and in patients with systemic lupus erythematosus, rheumatoid arthritis, Wegener's granulomatosis, and Behçet disease as well as acute exacerbation of idiopathic pulmonary fibrosis.[90-93] A transient elevation of sST2 protein levels could be measured in the serum of dengue virus infected patients during the late febrile days, a disease with a known shift from a predominant T_H1 to a T_H2 response around the time of defervescence. The upregulation of sST2 could therefore be a mechanism to attenuate that response.[94]

I.II.III.VI THERAPY WITH SST2

An sST2-human IgG1 fusion protein (sST2-Fc) was used to investigate sST2-binding activity of macrophages, which is upregulated by LPS. After binding, the expression of TLR4 and TLR1 was downregulated. Furthermore, sST2 seemed to suppress general inflammatory response induced by LPS both *in vitro* and *in vivo*.[95] A first therapeutic effect of sST2-Fc was reported by Leung *et al.* in the murine model of collagen-induced arthritis. Disease severity was significantly reduced and serum levels of IL-6, IL-12, and TNF-α were downregulated.[96] The same protein was used in a model of warm hepatic as well as intestinal ischemia-reperfusion injury significantly attenuating the damage of both liver and intestine.[97, 98] In a study of 2007, sST2 inhibited the binding of IL-33 to ST2L-positive cells and thus signaling through NF-κB as well as the production of T_H2 cytokines in a murine

model of allergic airway inflammation.[99]

I.II.III.VII sST2 AS A PROGNOSTIC MARKER

The expression of sST2 has also been described in the cardiovascular system, being induced in cardiac myocytes by mechanical strain, IL-1β, and phorbol ester, but not by LPS or TNF-α. Serum levels of sST2 were transiently elevated in humans and mice after myocardial infarction, possibly because of cell injury or increased ventricular stress.[100] Patients who died or developed new congestive heart failure showed significantly higher baseline levels of sST2, suggesting a role in cardiac pathophysiology and the protein's usefulness as a predictory marker.[101, 102] In addition to that, positive correlations between serum sST2 levels and BNP, ProANP and norepinephrine in patients with severe heart failure were reported, identifying the protein as a novel heart failure marker and a sensitive indicator of disease progression.[103, 104] Increased sST2 levels were strongly associated with one-year mortality in patients with acute destabilized heart failure [105, 106] as well as in dyspneic patients with pulmonary disease[107]. Soluble ST2 was not of value in the early identification of acute myocardial infarction in emergency departments.[108]

Il-33 synthesized by cardiac fibroblasts was shown to abrogate angiotensin II- and phenylephrine-induced hypertrophy in cardiac myocytes *in vitro* possibly because of regulation of NF-κB (for an overview of the mechanism see Figure 15). Deletion of the ST2 gene in mice enhanced mechanically induced hypertrophy and fibrosis of the heart while purified recombinant IL-33 improved the pathology in wild type mice probably due to decreased macrophage infiltration or a primary effect on cardiac cells.[109]

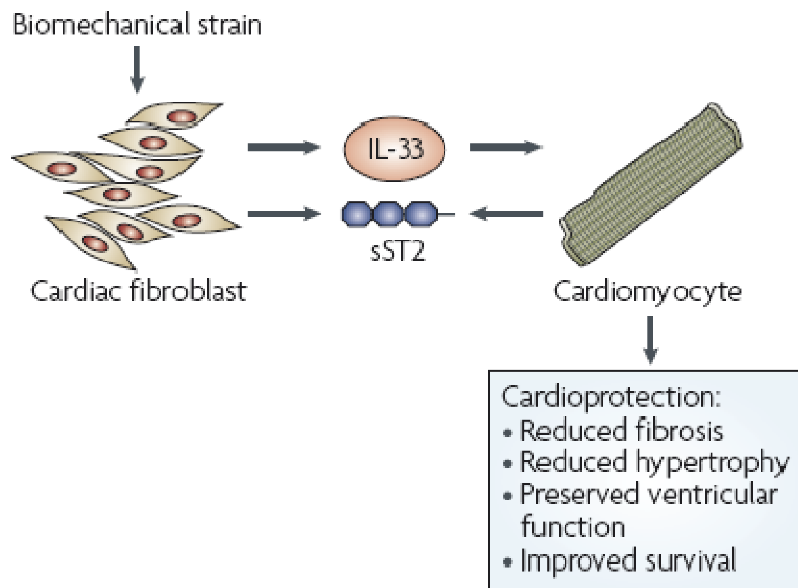


Figure 15: The cardioprotective fibroblast-cardiomyocyte paracrine system of IL-33/ST2.[110]

I.II.III.VIII sST2 IN ATHEROSCLEROSIS

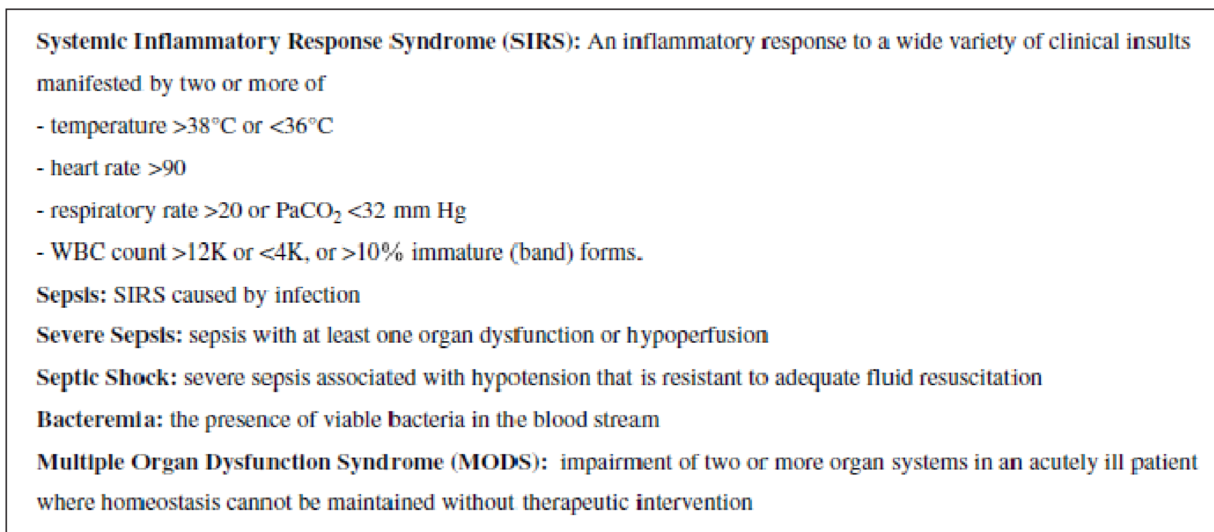
The IL-33/ST2 system also seems to have an effect on atherosclerosis, a chronic inflammation of the arterial wall, which is mediated by T cells and macrophages. Widely expressed throughout vascular cells and tissues, IL-33 leads to substantially smaller atherosclerotic lesions in the murine thoracic aorta and decreased T cell and macrophage infiltration without adversely affecting the assembly of the fibrous cap. Increased levels of IL-4, IL-5, and IL-13 and decreased IFN-γ indicate a T_H1-to-T_H2 switch. Furthermore, the injection of sST2 to neutralize IL-33 activity led to an exacerbation of atherosclerotic lesions and increased IFN-γ production by lymph node cells.[111]

In both heart failure and atherosclerosis, IL-33 signalling seems to be beneficial for disease progression. The idea of sST2 as a decoy receptor to block the IL-33/ST2L pathway is consistent with the findings of the above mentioned studies and the discovery of the protein as a biomarker for increased mortality and worse prognosis.

I.II.IV SYSTEMIC INFLAMMATION AND CABG

I.II.IV.I THE SYSTEMIC INFLAMMATORY RESPONSE SYNDROME (SIRS)

SIRS, for a definition see Figure 16, is clinically characterized by pathological hypotension, fever, disseminated intravascular coagulation, diffuse tissue edema, and injury. In extreme cases organ failure can occur, with pulmonary dysfunction being the most common clinic manifestation. Other organs prone to failure are the myocardium, the kidneys, the gastrointestinal and the central nervous system.[113, 114]



Systemic Inflammatory Response Syndrome (SIRS): An inflammatory response to a wide variety of clinical insults manifested by two or more of

- temperature >38°C or <36°C
- heart rate >90
- respiratory rate >20 or $PaCO_2$ <32 mm Hg
- WBC count >12K or <4K, or >10% immature (band) forms.

Sepsis: SIRS caused by infection

Severe Sepsis: sepsis with at least one organ dysfunction or hypoperfusion

Septic Shock: severe sepsis associated with hypotension that is resistant to adequate fluid resuscitation

Bacteremia: the presence of viable bacteria in the blood stream

Multiple Organ Dysfunction Syndrome (MODS): impairment of two or more organ systems in an acutely ill patient where homeostasis cannot be maintained without therapeutic intervention

Figure 16: Consensus definitions of a spectrum of clinical entities that result in organ failure.[112]

The etiology is characterized by the occurrence of a hyperdynamic circulatory state, marked by an increased cardiac output and reduced systemic vascular resistance, which, if untreated, results in lactic acidosis and increased risk of multiorgan failure and postoperative infections. The treatment includes vasoconstrictive agents and fluids.[115]

SIRS seems to occur predominantly in adult patients after complex cardiac interventions, but also to a lesser amount in routine procedures such as CABG.[115]

I.II.IV.II MEDIATOR RESPONSE TO CPB

The inflammatory response after cardiac surgery has been a subject of extended research. A wide spectrum of agents is released, either acting as triggers, such as complement, or mediators, like cytokines and adhesion molecules, or effectors, such as proteolytic enzymes, oxygen free radicals, and arachidonic acid metabolites.[116] The group of mediators will be

discussed in detail. During the development of SIRS the immune response consists of different cytokines, released at different times and for different periods.[117]

Pro-inflammatory responses to CPB

The activation of inflammatory cascades due to mediator release was deemed to be responsible for deleterious effect of CPB and therefore has been of interest in the past years. One of the first studies to investigate cytokine responses after CPB reported a peak of IL-6, a cytokine mediating the acute-phase response, 4 hours after the operation and continuing elevation until 48 hours postoperatively while there was no significant IL-1β response.[118] Another study reported IL-8, a chemoattractant for neutrophils, to be increased 10 minutes after CPB and a steady decrease over the next 67 hours, no significant change in TNF-α was found.[115] In general, TNF-α seems to be released in a biphasic pattern in response to CPB, with a typical first peak toward the end of CPB and a second one 18 hours after CPB.[116, 119] Heparin-coated circuits and the administration of aprotinin were discovered to subdue production of the cytokine.[113, 120]

Inflammatory mediators seem to be influenced by CPB in particular, as was suggested by many studies comparing conventional cardiac surgery with off-pump CABG. Especially IL-8, IL-10 and TNF-α levels appeared to be higher in the first patient collective. IL-6 and C-reactive protein (CRP) did not show significant differences.[114]

Anti-inflammatory responses to CPB

Anti-inflammatory cytokines also respond to the stimulus of the CPB. IL-10 is significantly affected by CPB, a peak is described one hour after declamping as well as on postoperative day 1.[119, 121-124] IL-1 receptor antagonist (IL-1ra) peaked 2 hours after the operation and the increase of TNF soluble receptors 1 and 2 (TNFsr-1 and -2), the endogenous antagonists of TNF-α, was maintained throughout 24 hours.[124]

In vitro studies showed a significantly reduced IFN-γ and IL-2 response of peripheral blood mononuclear cells (PBMCs) obtained during and after CPB, resulting in a decreased proliferation of these cells.[125] These results also indicate the release of immunosuppressive factors during cardiac surgery.

The nonspecific inflammatory response after CPB seems to be counteracted by an anti-inflammatory response, starting with IL-10.[119, 124] This product of TH2 cells then activates other anti-inflammatory cytokines, such as TNFsr-1 and -2 and IL-1ra. Interestingly enough, this reaction does not occur in an experiment with isolated CPB circuits.[124] An impairment of T_H1 cell function seems to occur, since IL-2 and IFN-γ production are decreased, and T_H2 cell function is upregulated. The immunodepression observed after CPB may occur because of the cytokine shift to T_H2, and the T_H1 depression gains clinical significance whenever a reaction of this type is needed, like infection caused by bacteria or viruses.[123, 126, 127]

I.II.IV.III HEAT SHOCK PROTEINS (HSP) IN CARDIAC SURGERY

Heat shock proteins, also termed stress proteins, belong to a group of highly conserved molecules ranging from 8 to 110 kDa which are constitutively expressed in all species and comprise 5 to 10% of the total protein count during normal growth. The induction of these proteins, which then accounts for up to 15% of cell protein, can occur after a range of cellular insults. They also fulfill a variety of functions, including serving as cellular chaperones in folding other proteins, cytoprotection, and participation in protein synthesis and transport of antigens.[128-130]

HSPs are not obligate intracellular molecules, they can be secreted from a variety of cell types in addition to being released into the extracellular compartments after severe cell damage. The innate immune system seems to be highly reactive to these “danger signals” and HSPs are commonly perceived as inflammatory mediators.[130]

Exogenous HSP70 can act as a cytokine stimulating a pro-inflammatory signal cascade in monocytes, which results in an upregulation of IL-1β, IL-6, and TNF-α.[131] This signal transduction is mediated via the MyD88/NF-κB pathway and can utilize both the TLR2 and TLR4 receptor in a CD14-dependent way.[132, 133] Similar to HSP70, exogenous HSP60 is also able to activate innate immune cells through TLR2 and TLR4, triggering the production of IL-6, IL-12, and TNF-α.[134]

Compounds that can induce HSP response are often inhibitors of NF-κB either directly by

stabilizing I-κBα or indirectly by inducing I-κBα gene expression, therefore acting as anti-inflammatory regulators.[135] HSP60 and HSP70 in particular, seem to be able to trigger immunoregulatory pathways resulting in suppression of responses that occur in human inflammatory diseases. Clinical trials with HSPs as immunoregulatory peptides in patients with type I diabetes and rheumatoid arthritis have been promising. Data on human diseases are still incomplete but HSPs seem to downregulate inflammation in these models.[136] This regulation is mediated by T cells and associated with an increase of IL-10.[137-139] An overview of the reaction of the immune system to HSPs is given in Table 8.

Table 8: Reaction of the immune system to “self” stress proteins in contrast to exogenous stress proteins[130]

Stress protein	Endogenous	Exogenous
HSP60	Anti-inflammatory	Pro-inflammatory
HSP70	Anti-inflammatory	Pro-inflammatory
Qualitative response	T_H2, IL-4, IL-10	T_H1, IFN-γ

In a study of our group comparing several heat shock proteins and 20S proteasome in sera of patients undergoing on-pump versus off-pump CABG, another interesting insight into immune response after cardiac surgery was obtained.[140] Results are depicted in Figure 17.

Serum levels HSP27, which experienced a twofold increase 60 minutes after CABG in the on-pump group, lead to the assumption that apoptosis and alterations in cell cytoskeleton are taking place in on-pump patients. Concordant with previous results [141], HSP70 serum levels are elevated in the on-pump group, which may indicate increased cellular stress and a possible protective effect of the protein in the immune reaction after CABG operation. HSP90α may also have immunomodulatory effects due to its association with receptor-mediated endocytosis and antigen presentation [142]. The described twofold increase of 20S proteasome in the on-pump group was assumed to be due to a release into the vascular bed after hemolysis of red blood cells and degeneration of epithelial and endothelial cells. The increase was significantly correlated with the secretion of HSP but not with the described increase of IL-6.[140] Another marker of endothelial and epithelial apoptosis, caspase-cleaved cytokeratin 18 (ccCK 18), is also released after on-pump CABG.[143] In general, the release of the above described proteins seems to occur because of stressful stimuli associated with the on-pump CABG procedure, resulting in an activation of the innate immune system.

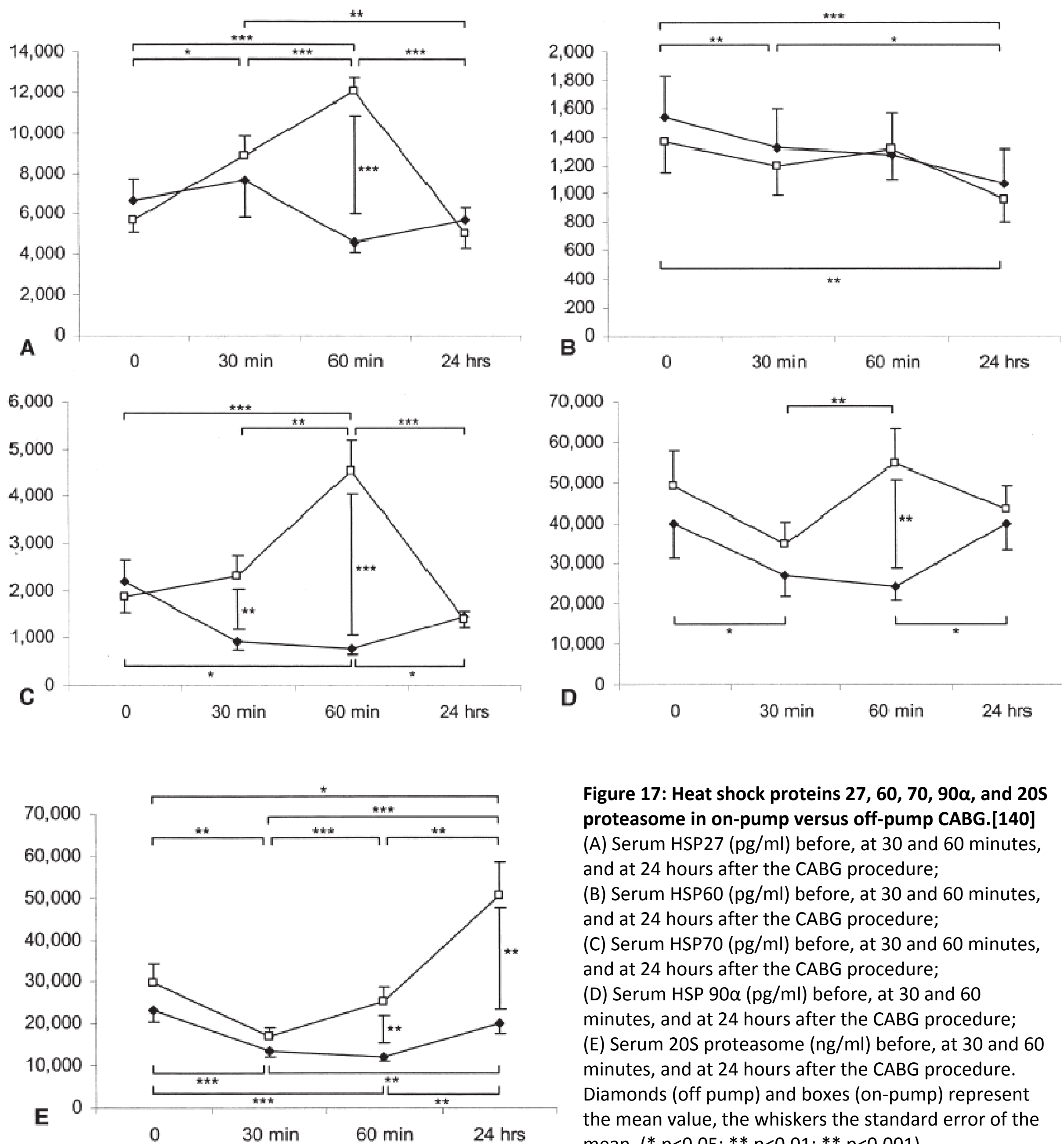


Figure 17: Heat shock proteins 27, 60, 70, 90α, and 20S proteasome in on-pump versus off-pump CABG.[140]
(A) Serum HSP27 (pg/ml) before, at 30 and 60 minutes, and at 24 hours after the CABG procedure;
(B) Serum HSP60 (pg/ml) before, at 30 and 60 minutes, and at 24 hours after the CABG procedure;
(C) Serum HSP70 (pg/ml) before, at 30 and 60 minutes, and at 24 hours after the CABG procedure;
(D) Serum HSP 90α (pg/ml) before, at 30 and 60 minutes, and at 24 hours after the CABG procedure;
(E) Serum 20S proteasome (ng/ml) before, at 30 and 60 minutes, and at 24 hours after the CABG procedure.
Diamonds (off pump) and boxes (on-pump) represent the mean value, the whiskers the standard error of the mean. (* $p<0.05$; ** $p<0.01$; ** $p<0.001$)

II. MATERIAL AND METHODS

II.I STUDY POPULATION

The study protocol was approved by the "Ethics commission of the Medical University of Vienna and the General Hospital of Vienna" (EC 356/2006). All study subjects or their legal designees signed a written informed consent.

Sixteen consecutive patients with multivessel coronary artery disease undergoing CABG surgery with extracorporeal circulation were included in Table 9. All patients received the following anaesthetic regimen: For premedication patients received morphine (0.1 mg/kg), midazolam (0.05-0.1 mg/kg) and atropin (0.005 mg/kg), for induction of anaesthesia midazolam (0.1-0.15 mg/kg), fentanyl (0.005 mg/kg), pipecuronium (0.08 mg/kg) or atracurium (0.5 mg/kg) were administered. For maintenance of anaesthesia patients received a continuous IV propofol infusion (0.07-0.14 mg/kg/min) and isoflurane (0.1-1.5 vol%) as well as repetitive administration of fentanyl boli (0.0025 mg/kg). In order to obtain constant muscle relaxation atracurium (0.5 mg/kg/h) was administered. Patients were heparinized with 3 mg/kg heparin (= 300 IU/kg) to achieve an activated clotting time (ACT) of ≥ 400 s. Mild hypothermia (32-34°C) was instituted, the composition of the priming solution was 1200-1750 ml crystalloid solution + 1000 IU heparin + 100 ml mannitol (20%) + 150 ml Na-bicarbonate solution (4.2%). The heparin effect was neutralized with protamine after going off bypass.

Table 9: Patient demographics (n=16)

Parameter	Mean±SEM
Age (years)	58.8±2.8
Sex (%male)	81.3
BMI	27.8±2.1
NYHA (class)	3.1±0.1
EF (%)	45.6±2.9
Euroscore	5.1±0.7
Average number of grafts	3.8±0.2
Aortic clamping time (min)	79.3±7.6
ECC	107.9±11.1
Transfused Units	1.4±0.5
Creatine kinase-MB after 24 hours (%)	8.1±0.7

II.II EXCLUSION CRITERIA

Infection, re-do operation or emergency operation, malignancies, verified immunological disorders, acute myocardial infarction less than 2 weeks ago and medication with immune-modulating agents such as steroids or non-steroidal anti-inflammatory drugs (NSAIDs) were causes for exclusion from the study.

II.III BLOOD SAMPLES

Blood samples were drawn at the beginning of surgery, 60 minutes thereafter and once on each postoperative day during the following eight days. Serum samples were centrifugated, aliquoted and kept frozen until the specific tests were performed.

II.IV ENZYME-LINKED IMMUNOABSORBENT ASSAY (ELISA)

Cytokines and proteins can be assayed by immunological recognition, and immunoassays are reproducible and specific. These tests use a combination of polyclonal and monoclonal antibodies to detect target proteins.[144]

II.IV.I QUANTIFICATION OF SERUM SOLUBLE ST2 LEVELS

A commercial ELISA kit was used to determine levels of soluble ST2 (R&D Systems, Minneapolis, MN, USA). Standards were prepared and the appropriate volume of sample or standard was added to a 96-well microtiter plate precoated with anti-human ST2 antibody. All samples were run in duplicate. Each well was then aspirated and the plates were washed with washing solution. Peroxidase-conjugated anti-human ST2 antibody was added to the microwells and incubated. Substrate and stop solution were added to each well, and the optical density was measured at 450 nm. The amount of protein in each sample was calculated according to a standard curve of optical density values for known levels of ST2. The sensitivity of the ELISA kit is 25 pg/ml.

II.IV.II QUANTIFICATION OF SERUM IL-4 LEVELS

A commercial ELISA kit was used to measure the serum levels of IL-4 (Bender Med Systems, Vienna, Austria). Standards were prepared and the appropriate volume of sample or standard was added to a 96-well microtiter plate, precoated with the monoclonal antibody for the appropriate marker. All samples were run in duplicate. Each well was then aspirated and the plates were washed with the specific washing solution provided with the kit. An enzyme-linked polyclonal antibody against the marker was added. Substrate and stop solution were added to each well, and the optical density was read at the appropriate wavelength for each assay. The amount of protein in each sample was calculated according to a standard curve of optical density values for known levels of protein.

II.IV.III QUANTIFICATION OF SERUM IL-10 LEVELS

A commercial ELISA kit was used to measure the serum levels of IL-10 (Bender Med Systems, Vienna, Austria). Standards were prepared and the appropriate volume of sample or standard was added to a 96-well microtiter plate, precoated with the monoclonal antibody for the appropriate marker. All samples were run in duplicate. Each well was then aspirated and the plates were washed with the specific washing solution provided with the kit. An enzyme-linked polyclonal antibody against the marker was added. Substrate and stop solution were added to each well, and the optical density was read at the appropriate wavelength for each assay. The amount of protein in each sample was calculated according to a standard curve of optical density values for known levels of protein.

II.IV.IV QUANTIFICATION OF SERUM IL-6 LEVELS

A commercial ELISA kit was used to measure the serum levels of IL-6 (Bender Med Systems, Vienna, Austria). Standards were prepared and the appropriate volume of sample or standard was added to a 96-well microtiter plate, precoated with the monoclonal antibody for the appropriate marker. All samples were run in duplicate. Each well was then aspirated and the plates were washed with the specific washing solution provided with the kit. An enzyme-

linked polyclonal antibody against the marker was added. Substrate and stop solution were added to each well, and the optical density was read at the appropriate wavelength for each assay. The amount of protein in each sample was calculated according to a standard curve of optical density values for known levels of protein.

II.IV.V QUANTIFICATION OF SERUM IL-8 LEVELS

A commercial ELISA kit was used to measure the serum levels of IL-8 (Bender Med Systems, Vienna, Austria). Standards were prepared and the appropriate volume of sample or standard was added to a 96-well microtiter plate, precoated with the monoclonal antibody for the appropriate marker. All samples were run in duplicate. Each well was then aspirated and the plates were washed with the specific washing solution provided with the kit. An enzyme-linked polyclonal antibody against the marker was added. Substrate and stop solution were added to each well, and the optical density was read at the appropriate wavelength for each assay. The amount of protein in each sample was calculated according to a standard curve of optical density values for known levels of protein.

II.IV.VI QUANTIFICATION OF SERUM IFN-GAMMA LEVELS

A commercial ELISA kit was used to measure the serum levels of IFN-γ (Bender Med Systems, Vienna, Austria). Standards were prepared and the appropriate volume of sample or standard was added to a 96-well microtiter plate, precoated with the monoclonal antibody for the appropriate marker. All samples were run in duplicate. Each well was then aspirated and the plates were washed with the specific washing solution provided with the kit. An enzyme-linked polyclonal antibody against the marker was added. Substrate and stop solution were added to each well, and the optical density was read at the appropriate wavelength for each assay. The amount of protein in each sample was calculated according to a standard curve of optical density values for known levels of protein.

II.IV.VII QUANTIFICATION OF IMMUNOGLOBULIN LEVELS

Commercial ELISA kits were used to measure the serum levels of IgM, IgG and IgE (Bethyl Laboratories, Montgomery, AL, USA). Standards were prepared and the appropriate volume of sample or standard was added to a 96-well microtiter plate, precoated with anti-human IgM, IgG or IgE antibody. All samples were run in duplicate. Each well was then aspirated and the plates were washed with washing solution according to the ELISA kit. Goat anti-human HRP conjugate was added to the microwells and incubated. Substrate and stop solution were added to each well, and the optical density was measured at 450 nm. The amount of protein in each sample was calculated according to a standard curve of optical density values for known levels of immunoglobulines.

II.V STATISTICAL ANALYSIS

Statistical analysis was performed for a descriptive study with no main a priori hypothesis using SPSS software (SPSS Inc., Chicago, IL, USA). Results are presented as mean ± standard error of mean (SEM) if not otherwise stated. Statistical analysis was conducted using one-way ANOVA (analysis of variance) to calculate significance. Post-hoc group comparisons were corrected for multiple testing with the use of Dunnet and Tukey-HSD. A p value of 0.05 was deemed to be significant.

III. Results

III.I Serum sST2 increases significantly at 24 hours

Figure 18 demonstrates that a significant rise of sST2 occurs at 24 hours after CABG. Serum levels of soluble ST2 (pg/ml) started to increase after 60 minutes (1,481.95±890.23) and peaked 24 hours after surgery (13,356±2,838.84, $p<0.001$) relative to preoperative amounts (38.32±13.49). Then, serum levels decreased gradually, reaching a postoperative nadir of 105±48.21 on the eighth day (day 2: 3,846.25±1,203.63; day 3: 1,340.58±422.49; day 4: 868.07±401.43; day 5: 668.07±251.27; day 6: 398.12±200.50; day 7: 186.06±48.21).

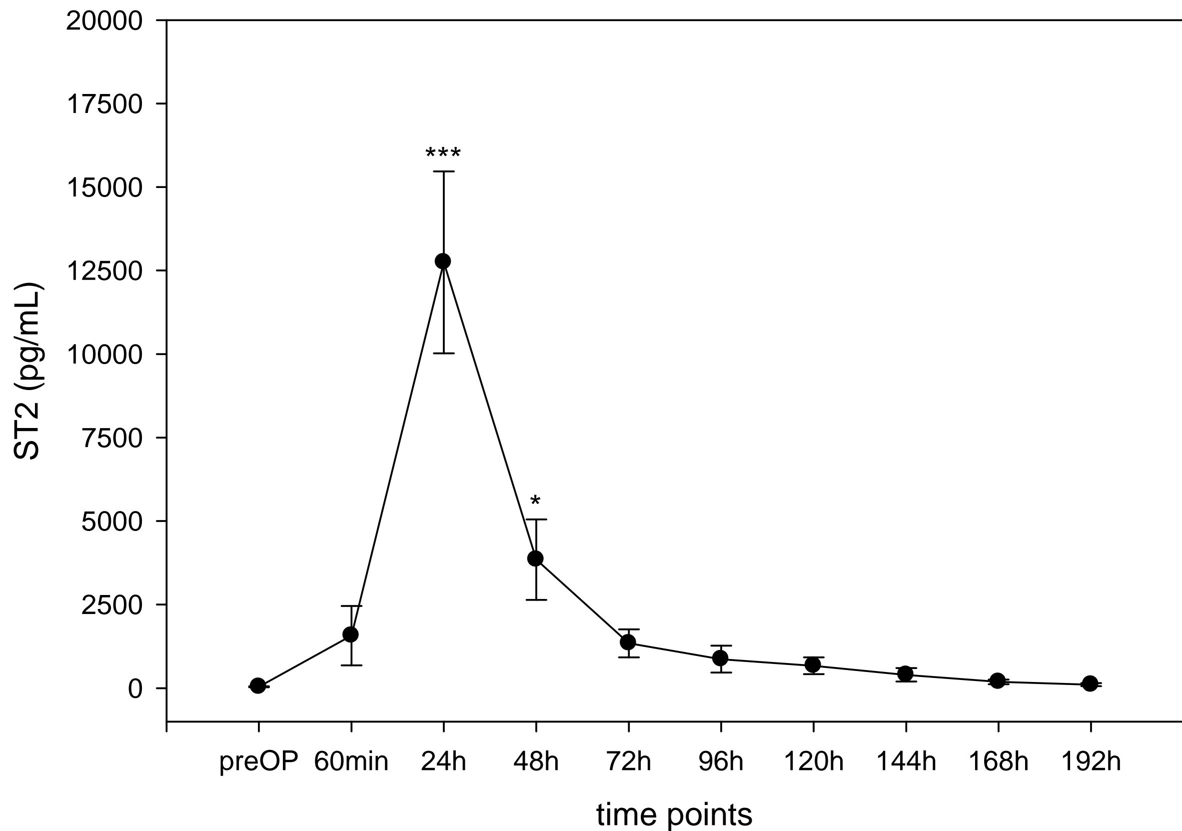


Figure 18: Serum soluble ST2 (pg/ml) before the coronary artery bypass graft operation, at 60 minutes, 24 hours, 48, 72, 96, 120, 144, 168, and 192 hours. Dots represent the mean value; the whiskers, the standard error of the mean (*p < 0.05; **p < 0.01; ***p < 0.001).

III.II SERUM IL-10 INCREASES SIGNIFICANTLY AT 60 MINUTES

Figure 19 shows serum levels of IL-10 (pg/ml). We evidenced a significant increase of IL-10 ($p<0.001$) from preoperative values of 2.57±0.98 to a maximum of 42.68±9.84 60 minutes after surgery. Serum levels then decreased to nearly baseline levels 24 hours after the operation (6.31±1.74). Further levels were: day 2: 5.00±1.07; day 3: 4.35±1.38; day 4: 3.79±1.40; day 5: 3.53±0.96; day 6: 2.41±0.84; day 7: 1.97±0.55; day 8: 2.75±0.83.

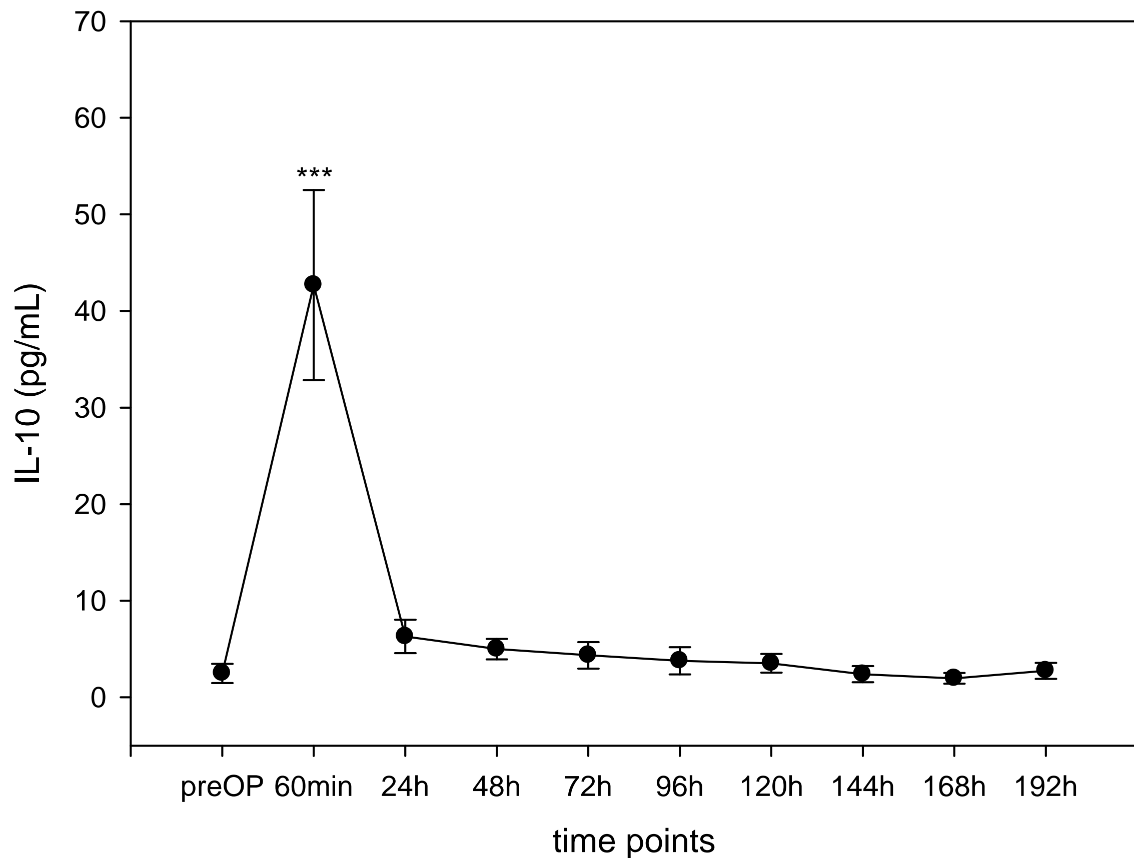


Figure 19: Serum IL-10 (pg/ml) before the coronary artery bypass graft operation, at 60 minutes, 24 hours, 48, 72, 96, 120, 144, 168, and 192 hours. Dots represent the mean value; the whiskers, the standard error of the mean (*p < 0.001).**

III.III SERUM IL-4 EVIDENCES NO SIGNIFICANT ALTERATION AT ANY TIME POINT

Serum levels of IL-4 (pg/ml) are depicted in Figure 20. IL-4 did not change significantly within the study period. Values were: preOP: 4.71±2.10; 60min: 2.23±1.43; d1: 2.33±0.98; d2: 2.90±1.17; d3: 1.83±0.75; d4: 1.54±0.71; d5: 1.25±0.46; d6: 1.77±0.93; d7: 1.39±0.68; d8: 0.62±0.43.

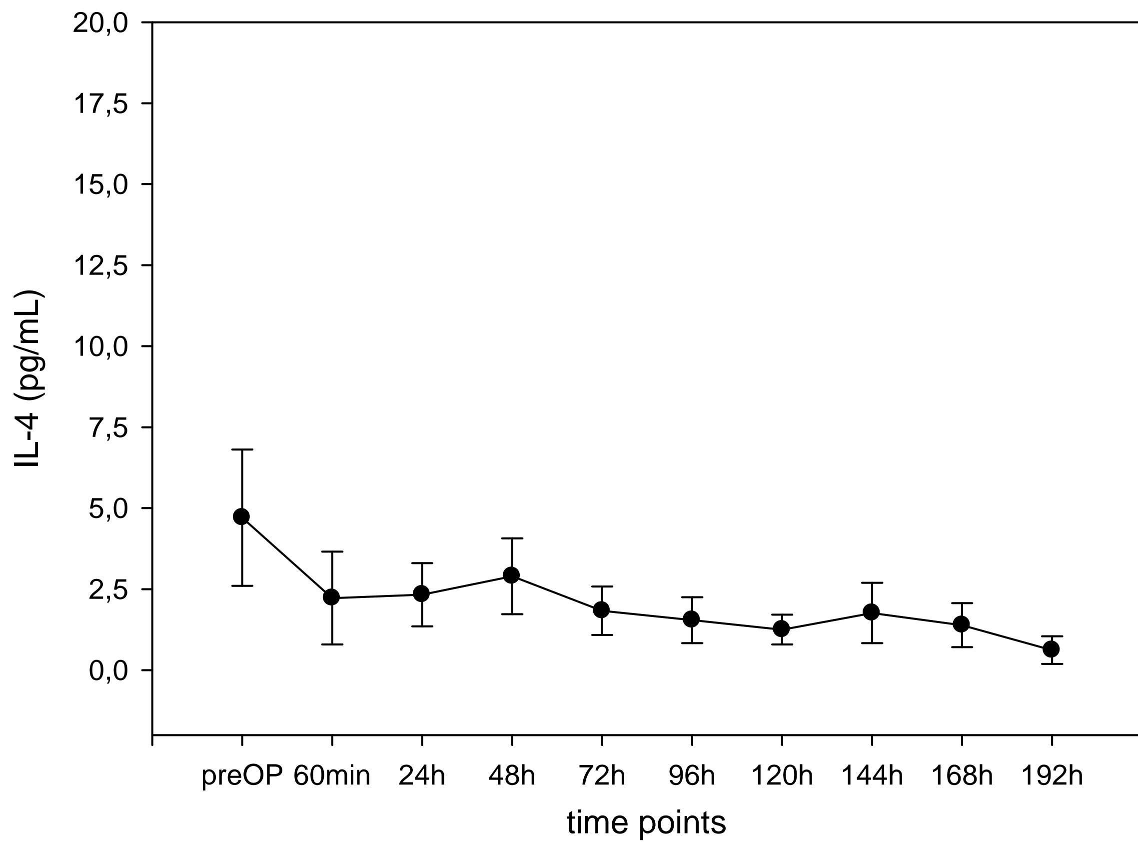


Figure 20: Serum IL-4 (pg/ml) before the coronary artery bypass graft operation, at 60 minutes, 24 hours, 48, 72, 96, 120, 144, 168, and 192 hours. Dots represent the mean value; the whiskers, the standard error of the mean.

III.IV SERUM IL-6 INCREASES SIGNIFICANTLY AT 60 MINUTES

Figure 21 shows serum levels of IL-6 (pg/ml), evidencing a significant increase 60 minutes after surgery (256.99±49.44; $p<0.001$) in comparison to preoperative values (6.87±2.13). IL-6 decreased to normal levels on day 4 (day 1: 76.51±9.67, $p<0.05$, day 2: 42.92±4.73, day 3: 21.11±2.98, day 4: 12.14±2.79). Further values were: day 5: 13.32±2.43; day 6: 11.22±2.29; day 7: 12.54±2.69; day 8: 22.82±4.17.

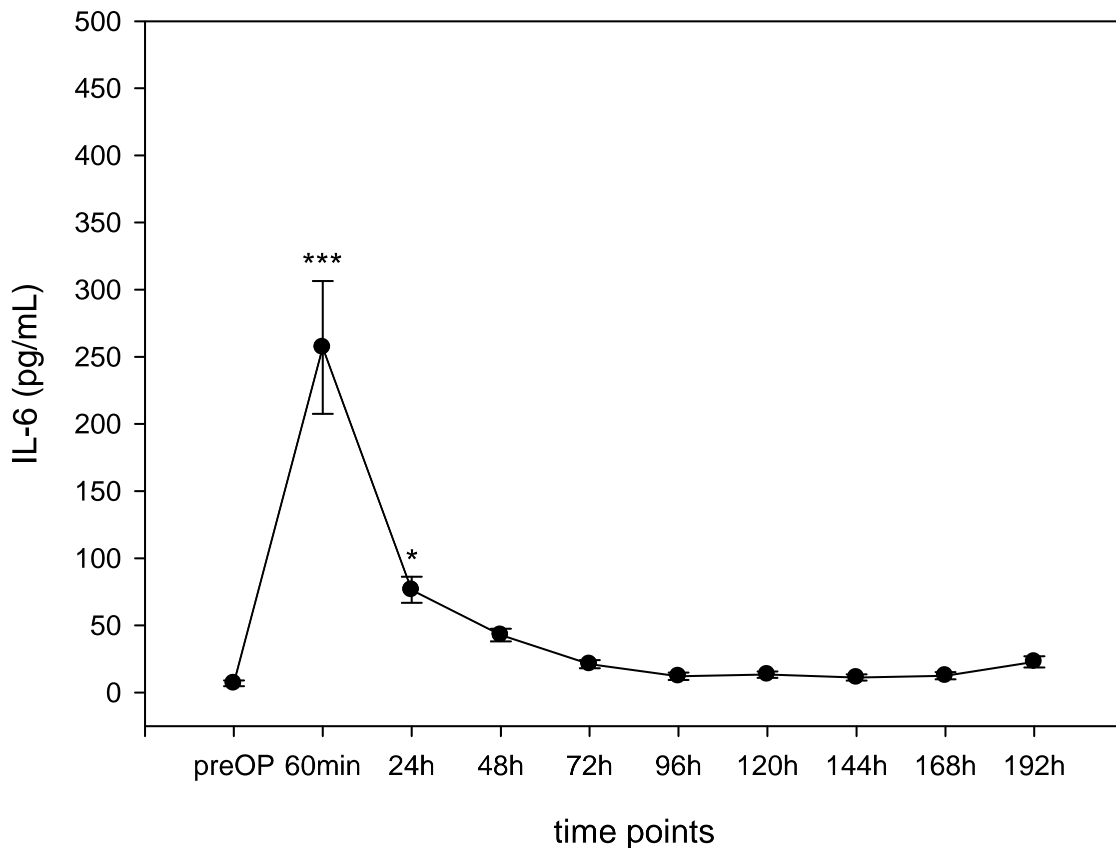


Figure 21: Serum IL-6 (pg/ml) before the coronary artery bypass graft operation, at 60 minutes, 24 hours, 48, 72, 96, 120, 144, 168, and 192 hours. Dots represent the mean value; the whiskers, the standard error of the mean (*p < 0.05; ***p < 0.001).

III.V SERUM IL-8 INCREASES SIGNIFICANTLY AT 60 MINUTES

As depicted in Figure 22, IL-8 (pg/ml) levels also peaked 60 minutes after surgery (108.66±17.48; p<0.001) in comparison to preoperative values (36.46±4.25). IL-8 leveled to initial values 24 hours after the operation (52.16±5.22). Further values were: day 2: 52.67±5.40; day 3: 44.03±4.62; day 4: 40.36±4.52; day 5: 47.43±5.71; day 6: 48.18±5.95; day 7: 50.14±6.24; day 8: 47.23±7.20.

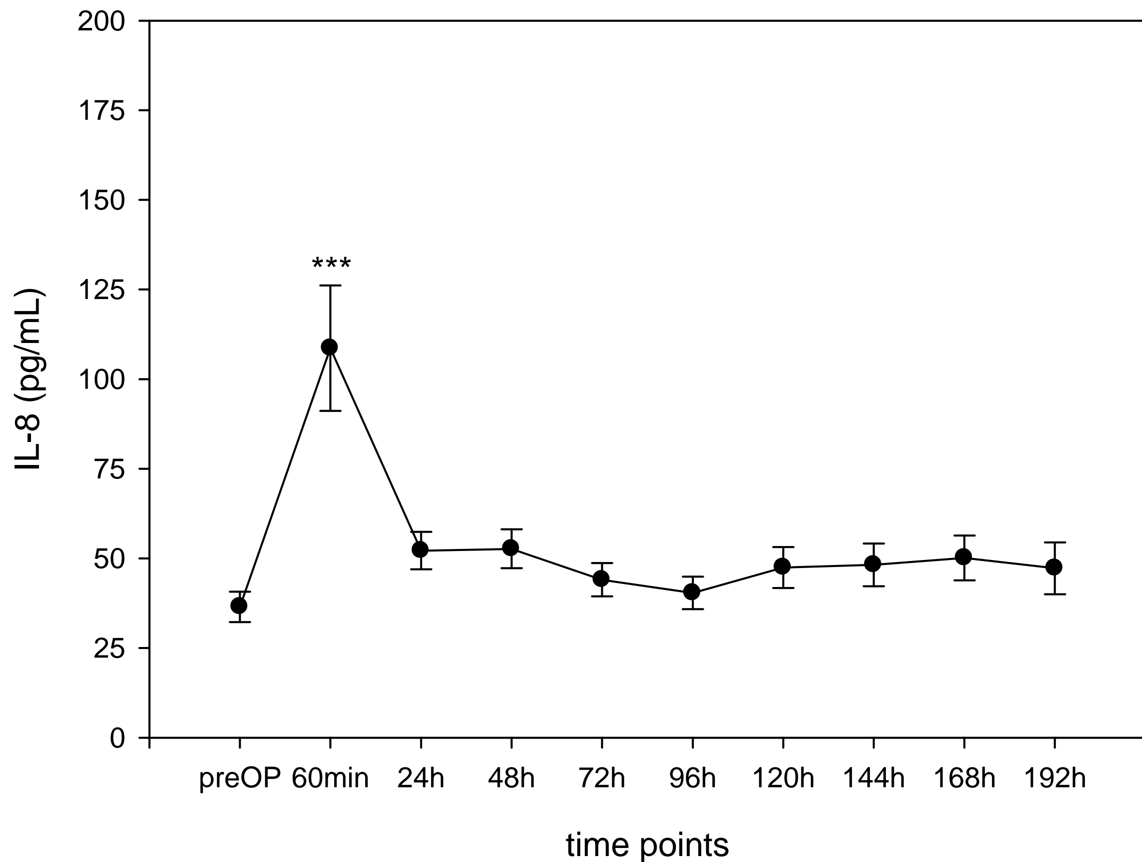


Figure 22: Serum soluble IL-8 (pg/ml) before the coronary artery bypass graft operation, at 60 minutes, 24 hours, 48, 72, 96, 120, 144, 168, and 192 hours. Dots represent the mean value; the whiskers, the standard error of the mean (*p < 0.001).**

III.VI Serum IFN-gamma evidences no significant alteration at any time point

Figure 23 shows serum levels of IFN-γ. The mean levels (pg/ml) did not change significantly during the study period. Values were: preOP: 13.62±5.09; 60min: 15.59±3.98; d1: 20.54±6.49; d2: 20.07±6.07; d3: 16.55±6.56; d4: 16.49±3.31; d5: 27.37±8.15; d6: 21.25±8.62; d7: 30.79±10.01; d8: 27.69±6.41.

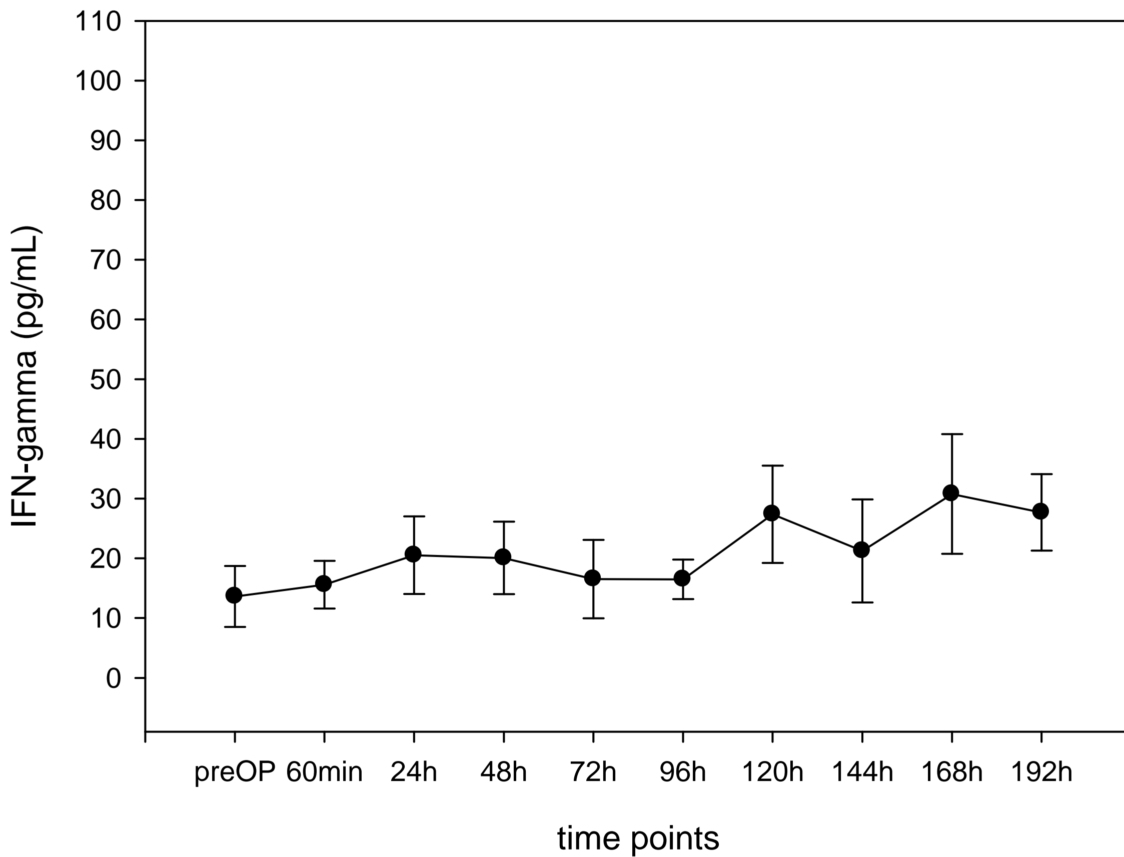


Figure 23: Serum soluble IFN-gamma (pg/ml) before the coronary artery bypass graft operation, at 60 minutes, 24 hours, 48, 72, 96, 120, 144, 168, and 192 hours. Dots represent the mean value; the whiskers, the standard error of the mean.

III.VII IMMUNOGLOBULIN SUBTYPE ANALYSIS

III.VII.I IGM CONTENT FIRST DECREASES SIGNIFICANTLY ON DAY THREE AND THEN INCREASES UNTIL DAY EIGHT

As demonstrated in Figure 24, levels of immunoglobulin subtype IgM (g/L) decreased from preoperative values of 9.5±0.88 to 7.55±0.71 (p=0.605) three days after surgery. Then values started to climb significantly reaching 14.94±1.13 (p<0.05) on day 5 and peaking on day 8 (17.31±1.38; p<0.001).

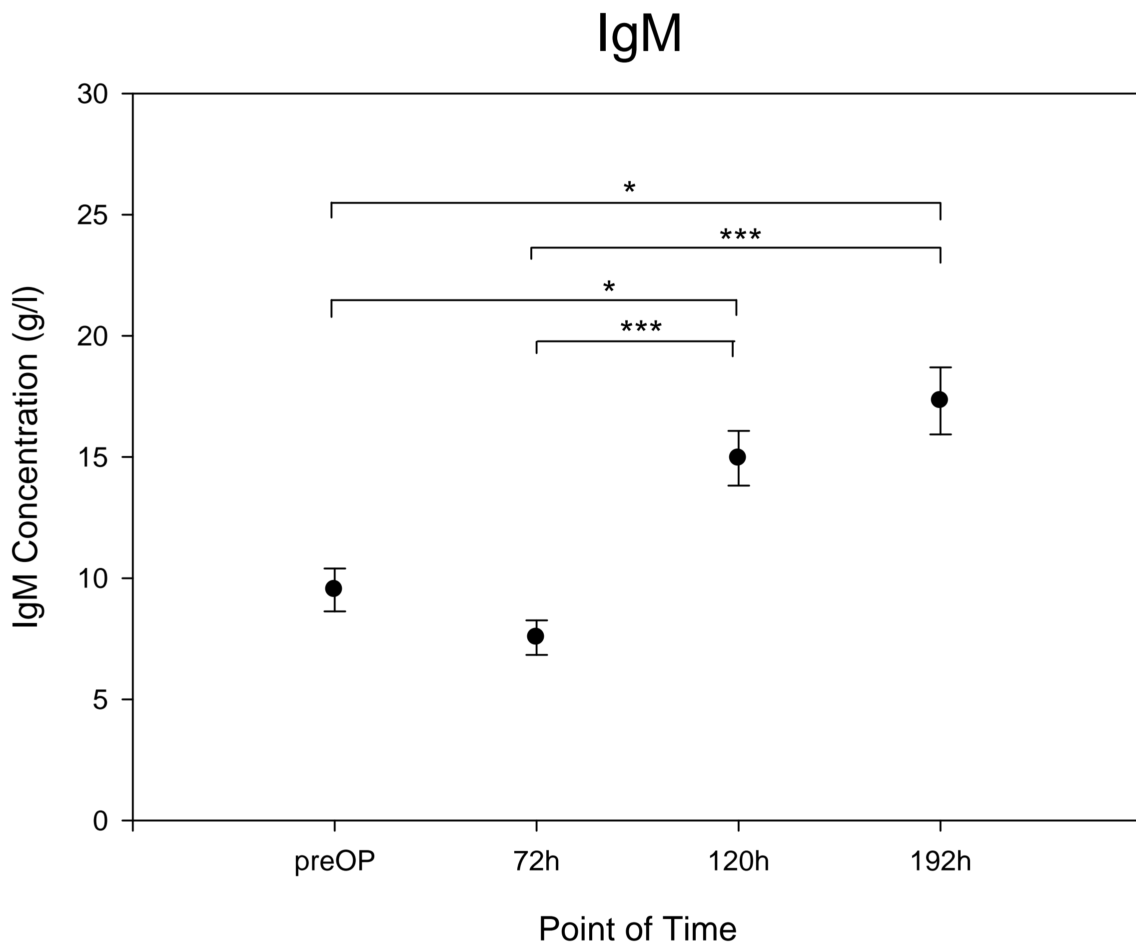


Figure 24: Serum IgM (g/l) before the coronary artery bypass graft operation, at 72 hours, 120, and 192 hours. Dots represent the mean value; the whiskers, the standard error of the mean (*p < 0.05; ***p < 0.001).

III.VII.II IgE and IgG content evidences no significant alteration at any time point

The amount of serum immunoglobulin subtypes IgE and IgG are shown in Table 10. Immunoglobulin levels before as well as three, five and eight days after surgery were analyzed. No significant changes occurred.

Table 10: Serum IgE and IgG (g/l) before the coronary artery bypass graft operation, at 72 hours, 120, and 192 hours.

	pre-OP	Day 3	Day 5	Day 8
IgE	390.79 ± 67.12	367.18 ± 67.55	412.77 ± 68.64	419.81 ± 79.65
IgG	2.10 ± 0.09	2.17 ± 0.09	2.26 ± 0.15	2.24 ± 0.25

IV. DISCUSSION

The development of immune modulation is commonly seen in patients after CPB, conventional CABG seems to result in a general immunosuppression. This effect may play an important role in development of infectious postsurgical complications, especially surgical wound infections of the sternal wound and leg. These complications cause increased suffering as well as prolonged hospital stay and increased costs.

The inhibition of T_H1 responses by T_H2 cells and the production of IL-10 seem to be important factors in the development of immunosuppression.[123] Although ST2 was revealed to play an important role in T_H2 effector functions, the part of the protein in immune response after CABG remained unclear. The aim of the study was to obtain insight into immune modulation after CABG operation and to reveal the role of ST2 in this process.

We could show for the first time that patients undergoing conventional CABG exhibit a mean 350 fold increase of sST2 within 24 hours after the operation. The serum level decreases gradually until the eighth day when patients were discharged. Concordant with prior investigations [115, 118] elevated levels of pro-inflammatory cytokines such as IL-6 and IL-8 were found. However, these increases, although directly related to the CPB [114], were only detectable 60 minutes after surgery and therefore seem to be of minor clinical importance.
The observed elevation of IL-10 has also been described before, along with an increase of IL-1ra, and TNFsr-1 and -2 [119, 121, 124], indicating a counteracting anti-inflammatory response to the inflammatory stimulus of the CPB. The involvement of ST2 in this process and the significant increase of IgM within 192 hours after operation corroborate the T_H2 bias that occurs after CABG.

Our results raise three important questions:

- How does the massive secretion of sST2 occur?
- What influence does sST2 have on the immune response after CABG?
- Could the increase have a negative effect on the innate and adaptive immune system leading to increased risk of infection?

IV.I HOW DOES THE MASSIVE SECRETION OF SST2 OCCUR?

The secretion of sST2 may be explained by two different mechanisms. Firstly, the recently discovered cardioprotective fibroblast-cardiomyocyte paracrine system may be involved. Due to mechanical strain and cell necrosis as a direct effect of the operation IL-33 is released into the blood stream. Soluble ST2 in its function as a decoy receptor may act as a negative regulator to the proinflammatory stimulus of IL-33 and the protein may also be involved in ventricular matrix remodeling.[110] This theory is consistent with the fact that sST2 represents a biomarker for poor prognosis in patients with cardiovascular disease [103], whereas IL-33 appears to be beneficial in both heart failure and atherosclerosis.[110]

The second possible mechanism for sST2 expression may occur because of the application of CPB. We speculate that the contact of the innate immune system to the extracorporeal circuits mimics exposure to microbial products such as peptidoglycan, bacterial lipoproteins, lipoteichoic acid, mycobacterial lipoarabinomannan, and yeast cell wall components, which signal through pathogen-associated molecular pattern receptors like TLRs. The pro-inflammatory stimulus due to the use of cardiopulmonary bypass is well documented, resulting in an increase of T_H1 cytokines. This first response is followed by a counteracting T_H2 answer, starting with IL-10 and may be subsequently followed by an IL-33 production. Figure 25 shows the involvement of sST2 and IL-33 in the immune response. The release of sST2 after CABG may be due to the regulation of T_H2 response as a decoy, binding free IL-33, possibly with the help of IL-1RAcP.

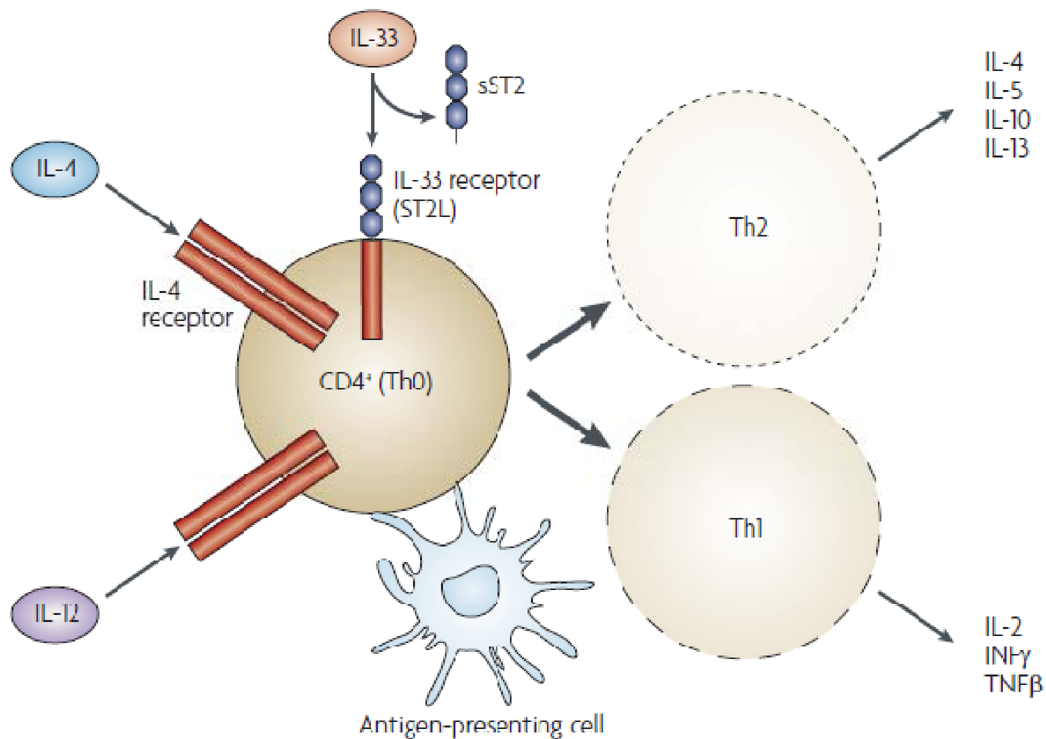


Figure 25: IL-33 and sST2 in the T_H2 immune response.[110]

IV.II WHAT INFLUENCE DOES SST2 HAVE ON THE IMMUNE RESPONSE AFTER CABG?

It has been demonstrated recently that cardiac myocytes and fibroblasts produce mature IL-33 in response to biomechanical strain, which in turn inhibits ventricular hypertrophy and fibrosis. This effect can be reversed by sST2, suggesting its possible role as a decoy receptor.[110] Concordant with these findings, it has been shown that sST2 can bind directly to THP-1 cells, a human monocytic leukemia line, leading to the inhibition of IκB degradation and the downregulation of IL-6 after LPS stimulation. NF-κB is unable to translocate to the nucleus and bind to the IL-6 receptor.[145] In a number of *in vivo* models, sST2 was able to inhibit the proinflammatory response, e.g. in a murine arthritis or asthma model.[96, 99] More examples of the immunomodulatory function of sST2 can be found in chapter I.II.III.VI.

It is commonly accepted that pre-exposure with LPS reduces the sensitivity to a second challenge with LPS resulting in a diminished production of certain cytokines.[146] Soluble ST2 expression can be induced by IL-1a, IL-1b, and TNF-α in macrophages after LPS challenge. The sST2-Ig fusion protein is able to bind directly to bone marrow-derived macrophages resulting in the downregulation of TLR4 and TLR1. Mortality after LPS challenge was significantly reduced as well as serum levels of IL-6, IL-12 and TNF-α, while the blocking of endogenous sST2 resulted in exacerbation of the toxic effects of LPS. Therefore, sST2 seems to have anti-inflammatory effects directly acting on macrophages, although the mechanism is currently not well understood.[95] Figure 26 shows the assumptive mechanism of macrophage regulation by sST2.

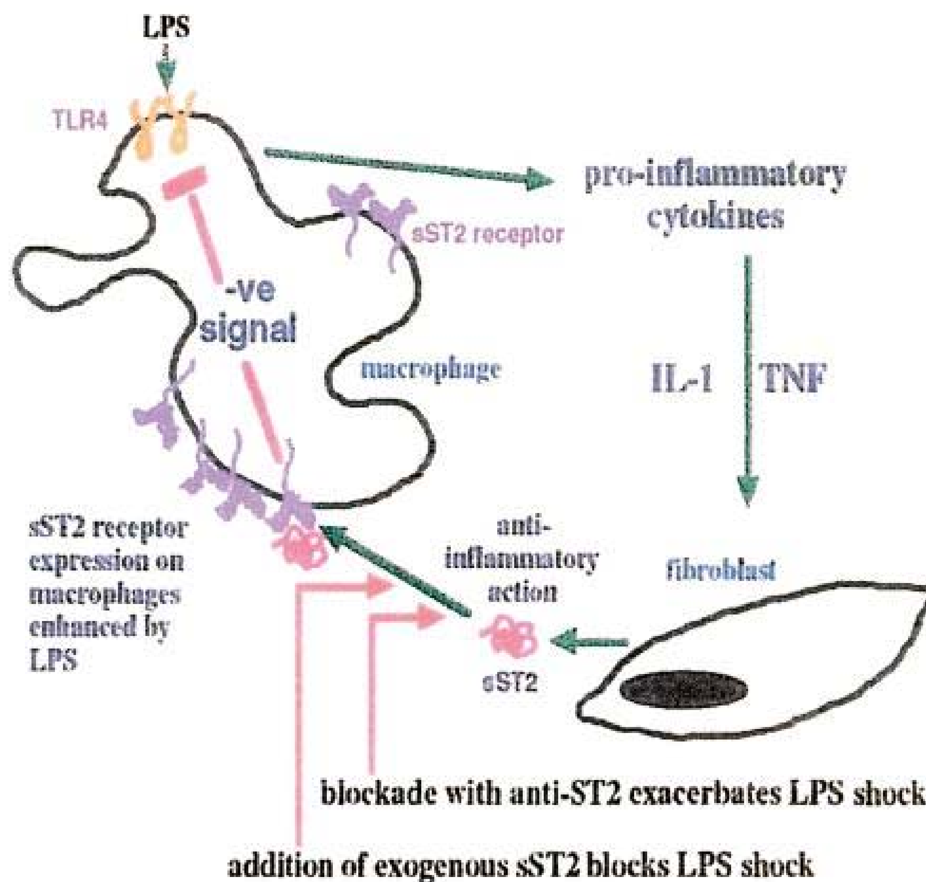


Figure 26: The functional role of sST2 in the regulation of inflammatory response by LPS-induced macrophages.[95]

IV.III COULD THE INCREASE HAVE A NEGATIVE EFFECT ON THE INNATE AND ADAPTIVE IMMUNE SYSTEM LEADING TO INCREASED RISK OF INFECTION?

Based on the obtained data we conclude that coronary artery bypass graft operation induces a massive secretion of soluble ST2, a protein associated with *in vitro* and *in vivo* immune suppression. This observation contributes to the fact that patients after CABG operation are susceptible to local and systemic infection. Significantly elevated levels of sST2 were measured to persist for 120 hours after the operation assuming a long lasting immune deviation. Concomitantly, the IgM content evidenced a significant rise within the hospital stay.

According to the data from our group and that of others, a long lasting systemic immune suppression might be induced in patients undergoing cardiac surgery that may have negative consequences on the short and long term outcome of patients as well as subsequently higher costs for the health care system. The evidence of immune suppression after CABG should result in the mandatory administration of antibiotics after surgery in order to reduce incidence of infectious complications. These observations might serve as an additional model for the development of immune modulation seen in patients after CBP.

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VI. ABBREVIATIONS

ACT	activated clotting time
APC	antigen-presenting cell
ATP	adenosine triphosphate
BMI	body-mass index
BNP	brain natriuretic peptide
CABG	coronary artery bypass graft
ccCK 18	caspase-cleaved Cytokeratin 18
CD	cluster of differentiation
CDC	Center for Disease Control and Prevention
CPB	cardiopulmonary bypass
CK-MB	creatine phosphokinase - muscle brain
CRP	C-reactive protein
DC	dendritic cell
ECC	extracorporeal circulation
EF	ejection fraction
ELISA	enzyme-linked immunoabsorbent assay
ERK	extracellular signal-regulated kinase
GI	gastrointestinal
GPIIb-IIIa	glycoprotein IIb-IIIa
HEF	high endothelial venules
HSP	heat shock protein
ICU	intensive care unit
IU	international unit
Ig	immunoglobulin
IFN-α/β	Interferon-α/β
IL	Interleukin
IL-1R	interleukin-1 receptor
IL-1ra	IL-1 receptor antagonist
IL-1RAcP	IL-1R accessory protein
IκB	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor

IKK	IκB kinase
IRAK	interleukin-receptor associated kinase
IRAK-2	interleukin-receptor associated kinase 2
IRF-3	interferon regulatory factor-3
iTreg cells	induced regulatory T cells
JNK	c-Jun N-terminal kinase
kDa	kilo Dalton
LAD	left anterior descendent
LIMA	left internal mammary artery
LOS	length of stay
LPS	lipopolysaccharide
LVEF	left ventricular ejection fraction
MAPKKK	mitogen-activated protein kinase kinase kinase
mEq/l	milliequivalent per liter
MHC	major histocompatibility complex
MKK	mitogen-activated protein kinase kinase kinase
mRNA	messenger ribonucleic acid
MyD88	myeloid differentiation factor 88
NF-HEF	nuclear factor expressed in high endothelial venules
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
NIK	NF-κB-inducing kinase
NSAID	non-steroidal anti-inflammatory drug
NYHA	New York Heart Association
PAMP	pathogen-accociated molecular patterns
PBMC	peripheral blood mononuclear cell
pH	pondus Hydrogenii
PMN	polymorphonuclear phagocytes
proANP	pro-atrial natriuretic peptide
SEM	standard error of mean
SIGIRR	single immunoglobulin IL-1 receptor-related
SIRS	systemic inflammatory response syndrome
sST2	soluble ST2

sST2-Fc	sST2 IgG1 fusion protein
SWI	Surgical wound infection
T_H	T-helper
TIR	Toll/IL-1R homology
TLR	Toll-like receptor
TNF-α/β	tumor necrosis factor α/β
TNFsr	TNF soluble receptor
TRAF 6	TNF receptor-associated factor 6
TRAM	TRIF-related adaptor molecule
TRIF	Toll/IL-1R domain containing adaptor inducing IFN-β
SD	standard deviation
SIRS	systemic inflammatory response syndrome
SWI	surgical wound infection
U/kg	Unit per kilogram
VAC	vacuum-assisted closure

VII. APPENDIX

Teile der vorliegenden Arbeit wurden im Journal *Thoracic and Cardiovascular Surgeon* publiziert. Die vollständige Publikation ist im Anschluss angefügt.

Secretion of Soluble ST2 – Possible Explanation for Systemic Immunosuppression after Heart Surgery

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Key words
○ immunology
○ CABG
○ inflammation

Abstract

Background: Cardiopulmonary bypass is known to affect cytokine release leading to a generalized endogenous immune reaction similar to that described in sepsis, without having been explored in great detail. Therefore we evaluated the anti- and pro-inflammatory cytokine responses after heart surgery.
Methods: 16 patients who underwent coronary artery bypass graft (CABG) surgery with extracorporeal circulation were included. ST2, IL-4 and IL-10 served as markers for TH2 cytokine response; IL-6, IL-8 and IFN-gamma as TH1 markers. Furthermore, total immunoglobulin subtype analysis (IgM, IgG, IgE) was performed.
Results: Serum levels of soluble ST2 started to climb at 60 minutes (from 38 ± 14 preoperatively to 1480 ± 890 pg/ml) and peaked 24 hours after surgery (13 360 ± 2 840 pg/ml, $p < 0.001$). IL-10 reached a maximum at 60 minutes and returned to baseline levels 24 hours later. IL-6 and IL-8 levels peaked 60 minutes after surgery. IL-4 and IFN-gamma did not change. Only IgM showed a significant peak on day eight ($p < 0.001$).
Conclusion: Our results demonstrate that CABG surgery induces a massive long-lasting secretion of ST2, a protein related to immune suppression.

Introduction

Cardiopulmonary bypass (CPB) utilized in on-pump coronary artery bypass graft (CABG) procedures induces a systemic immune response [1]. The release of a variety of mediators has been implicated in the pathogenesis of immunologic dysfunction after CABG surgery [2]. Previous reports have shown a release of interleukin (IL)-6 and IL-8 during and in the first 24 hours after CABG surgery, thus many clinicians consequently employ the term "systemic inflammatory response syndrome" (SIRS) [3–6]. Recently, reports have appeared that demonstrated a direct effect of CBP on the secretory capacity of T cells to produce immune activating cytokine IL-2 and interferon (IFN)-γ after CABG surgery. These studies suggested that the observed alterations may be the result of the extracorporeal circulation (ECC). Attenuation of the secretory capacity of pro-inflammatory cytokines after CABG surgery was linked to ECC. However, it was deemed unlikely that the production of an anti-inflammatory TH2 cytokine IL-10 is causative for this observation [7–8]. Since IFN-γ and IL-2 are produced by TH1-type lymphocytes it can be concluded that ECC induces impairment of TH1 cell function, implying a generalized mitigated immune response. IL-10, which is produced by TH2-type lymphocytes and macrophages, adds to this effect by inhibiting TH1-type cytokine release. According to the literature, severe systemic infections after CABG operation occur in 17–22% of cases within 30 days after surgery [9]. From the existing evidence it seems conclusive that the concept of inflammation after heart surgery is not supported by the clinical picture. Since a TH2 cytokine bias in patients undergoing CABG surgery was reported, we referred to publications studying nosological entities, such as asthma and autoimmune diseases, which are reported to have a TH2 bias. These diseases have been associated with a novel distinct TH2 specific product, named T1/ST2 [10]. T1/ST2, an IL-1 receptor related molecule, which has been shown to distinguish a subset of CD4+ T-helper cells of the TH2 subtype, are characterized by their elevated expression of the cytokines IL-4, IL-5, and IL-13 [11]. The gene expressing the

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T1/ST2 receptor was originally described as a serum-induced gene in fibroblasts. The biological function of the T1/ST2 receptor remains unclear, but its homology with the Toll receptor and other IL-1 family members suggests that it may play a central role in adaptive immune responses.

Previously published data from our group showed that patients undergoing on- and off-pump CABG surgery exhibit increased serum levels of soluble endothelial/epithelial apoptosis-specific products (caspase-cleaved cytokeratin 18) within 24 hours, and furthermore a dilution of soluble VCAM and ICAM due to hypooncotic hemodilution and a rapid secretion of soluble ST2 [4, 6] within 24 hours after heart surgery. In contrast, the secretion of heat shock proteins (HSP) was solely increased after on-pump CABG surgery [5]. Although the immediate immune response has been studied extensively, data on the further time course of the triggered immune reaction in CABG patients who are operated with CPB are not available. Therefore, as no clear picture is presented in the literature pertaining to a sequential TH1/TH2 cytokine milieu after CABG, we sought to investigate soluble proteins that represent either inflammatory (TH1) or anti-inflammatory cytokines (TH2) in a sequential fashion until day eight (discharge of patients). Moreover, we wanted to explore the relationship between secretion of ST2, IL-10 and immunoglobulin isotype production in a single cohort.

Materials and Methods

The study protocol was approved by the "Ethics Commission of the Medical University of Vienna and the General Hospital of Vienna" (EC 356/2006). All study subjects or their legal representatives signed a written informed consent.

Sixteen consecutive patients with multivessel coronary artery disease undergoing CABG surgery with extracorporeal circulation at the Department of Cardiothoracic Surgery in Debrecen were included in this newly designed clinical investigation (Table 1). All patients received the following anesthetic regimen: for pre-medication, patients received morphine (0.1 mg/kg), midazolam (0.05–0.1 mg/kg) and atropin (0.005 mg/kg); for the induction of anesthesia, midazolam (0.1–0.15 mg/kg), fentanyl (0.005 mg/kg), pipecuronium (0.08 mg/kg) or atracurium (0.5 mg/kg) were administered. For maintenance of anesthesia patients received a continuous IV propofol infusion (0.07–0.14 mg/kg/min) and isoflurane (0.1–1.5 vol%) as well as repetitive administration of fentanyl boli (0.0025 mg/kg). In order to obtain constant muscle relaxation, atracurium (0.5 mg/kg/h) was administered. Patients were heparinized with 3 mg/kg heparin (~300 IE/kg) to achieve an activated clotting time (ACT) of ≥ 400 s. Mild hypothermia (32–34 °C) was instituted; the composition of the priming solution was 1200–1750 ml crystalloid solution + 1000 IE heparin + 100 mannitol (20%) + 150 ml Na-bicarbonate solution (4.2%). The heparin effect was neutralized with protamine after weaning off bypass.

Exclusion criteria

Infection, redo or emergency operation, malignancies, verified immunological disorders, acute myocardial infarction less than 2 weeks ago and medication with immune-modulating agents such as steroids or non-steroidal anti-inflammatory drugs (NSAIDs) were causes for exclusion from the study.

Table 1 Characteristics of study patients.

	Patients (n = 16) mean ± SEM (median)
Age (years)	58.8 ± 2.8 (59)
Sex (% male)	81.3
BMI (kg/m²)	27.8 ± 2.1 (28.5)
NYHA class	3.1 ± 0.1 (3)
EF (%)	45.6 ± 2.9 (49)
EuroScore	5.1 ± 0.7 (4)
Average number of grafts (n)	3.8 ± 0.2 (4)
Aortic clamping time (min)	79.3 ± 7.6 (78)
ECC time (min)	107.9 ± 11.1 (104)
Transfused units of blood (n)	1.4 ± 0.5 (1)
Creatine kinase-MB % after 24 hours (%)	8.1 ± 0.7 (8)

Values are mean ± SEM (standard error of the mean) or percent. BMI = body mass index; NYHA = New York Heart Association; EF = ejection fraction; ECC = extracorporeal circulation

Blood samples

Blood samples were drawn at the beginning of surgery, 60 minutes thereafter and once on each postoperative day during the following eight days. Serum samples were centrifuged, aliquoted and kept frozen until the specific tests were performed.

Quantification of serum soluble ST2 levels

A commercial ELISA kit was used to determine levels of soluble ST2 (R&D Systems, Minneapolis, MN, USA). Standards were prepared and the appropriate volume of sample or standard was added to a 96-well microtiter plate precoated with antihuman ST2 antibody. All samples were run in duplicate. Each well was then aspirated and the plates were washed with washing solution. Peroxidase-conjugated antihuman ST2 antibody was added to the microwells and incubated. Substrate and stop solution were added to each well, and the optical density was determined at 450 nm. The amount of protein in each sample was calculated according to a standard curve of optical density values for known levels of ST2. The sensitivity of the ELISA kit is 25 pg/ml.

Quantification of serum cytokine levels

Commercial ELISA kits were used to measure the serum levels of IL-4, IL-10, IL-6, IL-8 and IFN-γ (Bender Med Systems, Vienna, Austria). Standards were prepared and the appropriate volume of sample or standard was added to a 96-well microtiter plate, precoated with the monoclonal antibody for the appropriate marker. All samples were run in duplicate. Each well was then aspirated and the plates were washed with the specific washing solution provided with the kit. An enzyme-linked polyclonal antibody against the marker was added. Substrate and stop solution were added to each well, and the optical density was read at the appropriate wavelength for each assay. The amount of protein in each sample was calculated according to a standard curve of optical density values for known levels of protein.

Quantification of immunoglobulin levels

Commercial ELISA kits were used to determine serum levels of IgM, IgG and IgE (Bethyl Laboratories, Montgomery, AL, USA). Standards were prepared and the appropriate volume of sample or standard was added to a 96-well microtiter plate, precoated with antihuman IgM, IgG or IgE antibody. All samples were run in duplicate. Each well was then aspirated and the plates were

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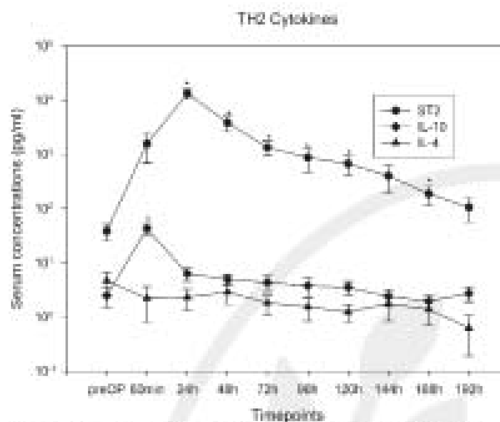

Fig. 1 Serum concentration of TH2 cytokine levels (ST2, IL-4 and IL-10) before, 60 minutes and every 24 hours up to 192 hours after conventional CABG with ECC. Data are expressed as mean ± SEM.

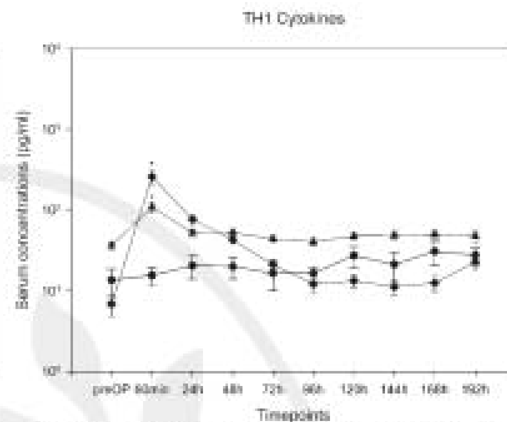

Fig. 2 Serum concentration of TH1 cytokine levels (IFN-gamma, IL-6 and IL-8) before, 60 minutes and every 24 hours up to 192 hours after conventional CABG with ECC. Data are expressed as mean ± SEM.

washed with washing solution according to the ELISA kit. Goat antihuman HRP conjugate was added to the microwells and incubated. Substrate and stop solution were added to each well, and the optical density was determined at 450 nm. The amount of protein in each sample was calculated according to a standard curve of optical density values for known levels of immunoglobulins.

Statistical analysis

Statistical analysis was performed for a descriptive study with no main *a priori* hypothesis using SPSS software (SPSS Inc., Chicago, IL, USA). Results are presented as mean ± standard error of mean (SEM), unless otherwise stated. Two-sided Student's *t*-tests for paired and unpaired comparisons were used to calculate significance. A *p* value of 0.05 was deemed to be significant.

Results

TH2 cytokine response

Serum levels of soluble ST2 (pg/ml) started to increase 60 minutes after bypass (1482 ± 890, $p = 0.107$) and peaked 24 hours after surgery (13360 ± 2840, $p < 0.001$) relative to preoperative amounts (38 ± 14). ST2 levels decreased gradually, reaching a postoperative nadir of 105 ± 48 on the eighth day (day 2: 3850 ± 1200; day 3: 1341 ± 422; day 4: 868 ± 401; day 5: 668 ± 251; day 6: 398 ± 201; day 7: 186 ± 48; day 8: 105 ± 48).

Levels of IL-10 (pg/ml) rose from 2.57 ± 0.98 preoperatively to a maximum of 42.68 ± 9.84 ($p < 0.001$) 60 minutes after surgery before decreasing again to nearly baseline levels 24 hours after the operation (6.31 ± 1.74). The following serum concentrations were: day 2: 5.00 ± 1.07; day 3: 4.35 ± 1.38; day 4: 3.79 ± 1.40; day 5: 3.53 ± 0.96; day 6: 2.41 ± 0.84; day 7: 1.97 ± 0.55; day 8: 2.75 ± 0.83.

IL-4 (pg/ml) did not change significantly within the study period. Values were: preOP: 4.71 ± 2.10; 60 min: 2.23 ± 1.43; day 1: 2.33 ± 0.98; day 2: 2.90 ± 1.17; day 3: 1.83 ± 0.75; day 4: 1.54 ± 0.71; day 5: 1.25 ± 0.46; day 6: 1.77 ± 0.93; day 7: 1.39 ± 0.68; day 8: 0.62 ± 0.43.

Fig. 1 depicts the time course of the TH2 cytokine response.

TH1 cytokine response

IL-6 (pg/ml) levels peaked 60 minutes after surgery (256.99 ± 49.44; $p < 0.001$) compared to preoperative values (6.87 ± 2.13). IL-6 decreased to normal levels on day 4 (day 1: 76.51 ± 9.67; day 2: 42.92 ± 4.73; day 3: 21.11 ± 2.98; day 4: 12.14 ± 2.79). Further values were: day 5: 13.32 ± 2.43; day 6: 11.22 ± 2.29; day 7: 12.54 ± 2.69; day 8: 22.82 ± 4.17.

IL-8 (pg/ml) levels also peaked 60 minutes after surgery (108.66 ± 17.48; $p < 0.001$) in comparison to preoperative values (36.46 ± 4.25). IL-8 levelled to initial values 24 hours after the operation (52.16 ± 5.22). Further values were: day 2: 52.67 ± 5.40; day 3: 44.03 ± 4.62; day 4: 40.36 ± 4.52; day 5: 47.43 ± 5.71; day 6: 48.18 ± 5.95; day 7: 50.14 ± 6.24; day 8: 47.23 ± 7.20.

IFN-gamma (pg/ml) did not change significantly during the study period. Values were: preOP: 13.62 ± 5.09; 60 min: 15.59 ± 3.98; day 1: 20.54 ± 6.49; day 2: 20.07 ± 6.07; day 3: 16.55 ± 6.56; day 4: 16.49 ± 3.31; day 5: 27.37 ± 8.15; day 6: 21.25 ± 8.62; day 7: 30.79 ± 10.01; day 8: 27.69 ± 6.41. Fig. 2 demonstrates the TH1 cytokine response during the study period.

Immunoglobulin subtype analysis

We analyzed immunoglobulin levels before as well as three, five and eight days after surgery. Levels of immunoglobulin subtype IgM (g/L) decreased from preoperative values of 9.50 ± 0.88 to 7.55 ± 0.71 three days after surgery. Then values started to climb significantly, reaching 14.94 ± 1.13 on the fifth day and peaking on day eight (17.31 ± 1.38; $p < 0.001$). These results are shown in Fig. 3. The amount of serum immunoglobulin subtypes IgE and IgG are given in Table 2. No significant changes occurred.

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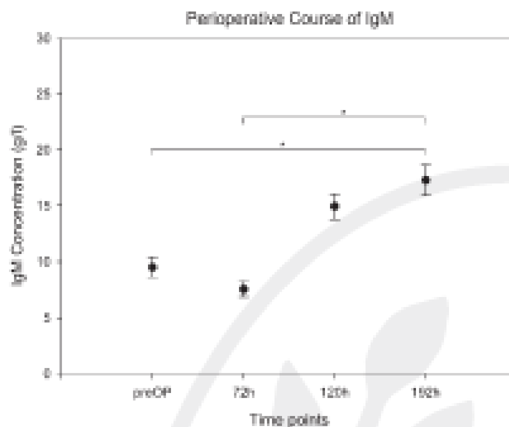

Fig. 3 Serum concentration of IgM before, 72, 120 and 192 hours after conventional CABG with ECC. Data are expressed as mean ± SEM. * = $p<0.001$.

Table 2 Serum concentration of immunoglobulin subtypes IgE and IgG in patients before and on day 3, 5 and 8 after conventional coronary artery bypass grafting with cardiopulmonary bypass (CPB). Data are expressed as mean ± SEM.

IgE (ng/ml)	preOP	390.8 ± 67.1
	72 h	367.1 ± 67.5
	120 h	412.8 ± 68.6
	192 h	419.8 ± 79.6
IgG (g/l)	preOP	2.1 ± 0.1
	72 h	2.2 ± 0.1
	120 h	2.3 ± 0.1
	192 h	2.2 ± 0.2

Discussion

Our study showed for the first time that patients undergoing conventional CABG surgery exhibit a mean 350-fold increase of soluble ST2 within 24 hours with a gradual decrease until the eighth day when patients were discharged. Since our investigation has revealed only a marginal increase of pro-inflammatory cytokines, we argue that this observation is of minor clinical importance. In contrast, IgM subtype analysis showed a significant increase within 192 hours after operation. This finding corroborates a clear TH2 bias after CABG surgery. The observed increase of soluble ST2 raises the following question: Could the findings have an impact on the adaptive immune system after open heart transplantation and utilization of ECC?

Recent work has shown that Toll-like receptors (TLRs) function as mammalian pattern-recognition receptors signalling the presence of microbial components to the innate immune cells [12]. At least 10 members of the mammalian TLR family have been reported, with each TLR showing a distinct specificity for the molecular pattern of microbes. We speculate that contact of the innate immune system to ECC mimics exposure to microbial products such as peptidoglycan, bacterial lipoproteins, lipoteichoic acid, mycobacterial lipoarabinomannan and yeast cell wall components. It is commonly accepted that pre-exposure to lipopolysaccaride (LPS) reduces the sensitivity to a second challenge with LPS resulting in a diminished production of numerous cytokines in both rodents and humans [13]. The molecular mechanisms of TLR-induced tolerance may involve the downregulation of the TLR4-MD2 complex [14] which may lead to diminished TLR signalling ST2, negatively regulated TLR4 and IL-1R signalling via sequestration of myeloid differentiation factor 88 (MyD88) [15]. In the clinical context, this may mean that primary TLR activation of the innate immune systems induces a state of tolerance towards secondary infections in patients undergoing CABG operation; hence surgery creates a "state of anergy" or an "immunocompromised state" due to secretion of soluble ST2.

In a murine asthma model, the administration of recombinant soluble ST2 fusion proteins has antagonistic effects on IL-33 signalling in allergic airway inflammation, suppressing IL4 and IL-5 production in the process [17]. Furthermore, in murine LPS-induced shock models, the soluble ST2 fusion protein has direct anti-inflammatory properties acting directly on macrophages. Treatment enhances the survival rate after LPS challenge and suppresses IL-6, IL-12 and TNF-α production by acting directly on macrophages via the ST2-TLR4 route [18]. In a murine collagen-induced arthritis model, the administration of sST2-Fc fusion protein downregulated levels of IL-6, IL-12 and TNF-α and effectively suppressed symptoms even after evident onset of disease [19]. ST2 expression was shown to be induced by IL-1b, IL-1a, and TNF-α in fibroblasts, macrophages, muscle and spleen cells following an LPS challenge that is comparable to the sepsis syndrome [18]. Thus, macrophages respond to LPS by producing TH2-biased cytokines that in turn can induce expression of soluble ST2. The mechanism by which soluble ST2 protein suppresses the production of pro-inflammatory cytokines is presently not well understood. Nuclear factor (NF)-κB is an important transcription factor required for the expression of inflammatory cytokines. Takezako et al. showed that soluble ST2 inhibits the binding of the NFκB complex to the IL-6 promoter as well as IκB degradation in THP-1 cells [19]. We deduce from this data that secretion of soluble ST2 most probably induces immunosuppressive activity.

The study protocol we employed has limitations. Due to the time-points we chose for our measurements we most likely missed the peak in the secretion of the various parameters we determined, which is known to occur within the first 24 hours after surgery [4]. Nevertheless, the peak in ST2 secretion has already been characterized by our group in a previous investigation. Furthermore, we were interested in elucidating the further time-course of these markers of immunologic response, as this has not been described before. Unfortunately, as only low-risk patients were included in our study we cannot comment on the alterations seen in high-risk patients whose odds ratio for postoperative complications is much greater. Additionally, our data do not allow a differentiation to be made between the effect of ECC and that of the surgical procedure itself on the course of the triggered immune response. As far as the immediate ST2 increase is concerned we did not find a difference between patients operated with CPB and off-pump patients as described previously [4]. Studies are currently being designed in our department to answer this clinical question.

Based on our presented data we conclude that open heart surgery utilizing CPB induces a massive secretion of ST2, a protein

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that has been shown to generate *in vitro* and *in vivo* immune suppression. We are convinced that this observation provides an additional explanation why patients after CABG operation are susceptible to local and systemic infection after heart operation. In our understanding this is partially due to the massive release of the described protein. Moreover, it is interesting to see that the massive increase of ST2 lasted for 120 hours after surgery and could be responsible for a long-lasting immunodepression. Concomitantly, the IgM content showed a significant rise during the hospital stay. This is immunologically important as immune globulins are known to have immune suppressive activity *in vitro* [20].

Although the observations presented in this manuscript are purely descriptive in patients who underwent uneventful on-pump CABG operation, they still show an increase in biological substances rarely seen in humans. According to our data, and those of others, a long-lasting systemic immune suppression might be induced that may have negative consequences for short- and long-term outcomes in patients undergoing cardiac surgery. We are deeply convinced that only further scientific efforts to understand immune suppression after CABG and its adequate treatment (*e.g.*, modification of surgical procedure, antibiotics) will lead to an attenuation of infection-related morbidity and mortality in cardiac surgery.

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VIII. CURRICULUM VITAE

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PERSONAL BACKGROUND

Nationality: Austrian
Family Status: Married
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Date of Birth: March 5th, 1981

EDUCATION

2006/06 – Present	Student Research Fellow at the Department of Cardio-Thoracic Surgery, General Hospital Vienna, Medical University of Vienna, Austria
2003/10 – Present	**Medical Student at the Medical University of Vienna, Austria**
1999/09 – 2003/06	Student of Business Consultancy at the University of Applied Sciences Wiener Neustadt, Austria, graduation with the degree Mag. (FH)
1999/06	Matura (High School Graduation) with Distinction
1991 - 1999	Don-Bosco-Gymnasium (High School), Unterwaltersdorf, Austria
1987 - 1991	Lower School

CLINICAL TRAINING

2009/02	Clinical Clerkship at the Department of Pediatric Surgery, Charité Hospital, Berlin, Germany (3 weeks)
2009/01	Clinical Clerkship at the Department of Pediatrics, Hospital St. Anna, Vienna, Austria (2 weeks)
2008/11	Clinical Clerkship at the Department of Psychiatry, General Hospital Vienna, Medical University of Vienna, Austria (2 weeks)
2008/10	Clinical Clerkship at the Department of Neurology, Hospital Goettlicher Heiland, Vienna, Austria (2 weeks)
2008/06	Clinical Clerkship at the Department of Orthopedic Surgery, Orthopedic Hospital Speising, Vienna, Austria (3 weeks)
2008/05	Clinical Clerkship at the Department of Surgery, Hospital Barmherzige Schwestern, Vienna, Austria (3 weeks)
2008/01	Clinical Clerkship at the Department of Trauma Surgery, General Hospital Vienna, Medical University of Vienna, Austria (3 weeks)
2007/11	Clinical Clerkship at the Department of Internal Medicine, Hospital Barmherzige Schwestern, Vienna, Austria (5 weeks)
2007/09	Clinical Clerkship at the Department of Cardiac Surgery, Charité Hospital, Berlin, Germany (4 weeks)
2007/07	Clinical Clerkship at the Department of Clinical Pathology, General Hospital Vienna, Medical University of Vienna, Austria (2 weeks)
2007/02	Clinical Clerkship at the Department of Gynaecology, Hospital of Mödling, Austria (2 weeks)
2006/08	Clinical Clerkship at the Department of Trauma Surgery, Hospital Meidling, Vienna, Austria (4 weeks)
2006/07	Clinical Clerkship at the Department of Internal Medicine, Hospital of Mödling, Austria (4 weeks)
2005/07	Clinical Clerkship at the Department of Dermatology, General Hospital Vienna, Medical University of Vienna, Austria (4 weeks)

CONTINUING EDUCATION

2007/05 — Methodenseminar „Statistik“ – Methods Seminar „Statistics“, a.o. Univ. Prof. Dr. Martin Posch, Vienna, Austria

2007/03 — Methodenseminar „Medizinische Informatik“ – Methods Seminar „Medical Information Technology“, a.o. Univ. Prof. Dr. Ernst Schuster, Vienna, Austria

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CONGRESSES AND MEETINGS

2008/05 — 49. Österreichischer Chirurgenkongress – 49th Annual Meeting of the Austrian Society of Surgery, Innsbruck, Austria

2007/10 — Austrotransplant – 21st Annual Meeting of the Austrian Society of Transplantation, Transfusion and Genetics, St. Wolfgang, Austria

2006/10 — Austrotransplant – 20th Annual Meeting of the Austrian Society of Transplantation, Transfusion and Genetics, Hof bei Salzburg, Austria

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RESEARCH ACTIVITY AND PUBLICATIONS

Articles:

Szerafin T, Hoetzenecker K, Hacker S, Horvath A, Pollreisz A, Arpád P, Mangold A, **Wliszczak T**, Dworschak M, Seitelberger R, Wolner E, Ankersmit HJ.
Heat shock proteins 27, 60, 70, 90alpha, and 20S proteasome in on-pump versus off-pump coronary artery bypass graft patients.
Ann Thorac Surg. 2008 Jan;85(1):80-7.

Hoetzenecker K, Hacker S, Hoetzenecker W, Sadeghi K, Sachet M, Pollreisz A, Mangold A, **Wliszczak T**, Bielek E, Muehlbacher F, Klepetko W, Ankersmit HJ.
Cytomegalovirus hyperimmunoglobulin: mechanisms in allo-immune response in vitro.
Eur J Clin Invest. 2007 Dec;37(12):978-86.

Manuscripts accepted:

Szerafin T[x], **Niederpold T**[x], Mangold A[x], Hoetzenecker K, Hacker S, Roth G, Lichtenauer M, Dworschak M, Wolner E, Ankersmit HJ.
Secretion of Soluble ST2 – Possible Explanation for Systemic Immunosuppression after Heart Surgery.
[x] Szerafin, Niederpold and Mangold share the first authorship

Published Abstracts:

Niederpold T, Hoetzenecker K, Hacker S, Mangold A, Pollreisz A, Lichtenauer M, Szerafin T, Krenn C, Ankersmit HJ.
Th1 and Th2 cytokine response in coronary artery bypass graft (CABG) patients.
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Mangold A, Hoetzenecker K, Hacker S, Pollreisz A, **Wliszczak T**, Lichtenauer M, Wolner E, Klepetko W, Gollackner B, Szerafin T, Auer J, Ankersmit HJ.
Alpha-Gal Specific Humoral Immune Response after Implantation of Bioprostheses in Cardiac Surgery.
6th EAACI-GA2LEN Davos Meeting, Pichl, Austria. 2008/02.
published in Abstractbook.

Hacker S, Soleiman A, Hoetzenecker K, Lukschal A, Pollreisz A, Mangold A, **Wliszczak T**, Lichtenauer M, Horvat R, Muehlbacher F, Wolner E, Klepetko W, Ankersmit HJ.
Degenerative Cardiac Pigment Lipofuscin Contains Cytokeratin-18 and Caspase-cleaved Cytokeratin-18.
Annual Meeting of the Austrian Society of Allergology and Immunology, Alpbach, Austria. 2007/12. published in Abstractbook.

Hoetzenecker K, Hacker S, Hoetzenecker W, Sadeghi K, Sachet M, Pollreisz A, Mangold A, **Wliszczak T**, Bielek E, Muehlbacher F, Wolner E, Klepetko W,

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CMV Hyperimmunoglobulin Influence NK cell Viability and Function in vitro.
Annual Meeting of the Austrian Society of Allergology and Immunology, Alpbach, Austria. 2007/12. published in Abstractbook.

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Pollreisz A, Hacker S, Hoetzenecker K, **Wliszczak T**, Volf I, Ankersmit HJ.
CMVIg and IVIg induce CD32-mediated platelet aggregation in vitro: implication of therapy induced thrombocytopenia and thrombosis in vivo.
21st Annual Meeting of the Austrian Society of Transplantation, Transfusion and Genetics, St. Wolfgang, Austria. 2007/10. European Surgery 2007;39:Suppl 218:28.

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48th Annual Meeting of the Austrian Society of Surgery, Graz, Austria. 2007/06. European Surgery 2007;39:Suppl 215:16.

Poster Presentations:

Mangold A, Hoetzenecker K, Hacker S, Pollreisz A, **Wliszczak T**, Lichtenauer M, Wolner E, Klepetko W, Gollackner B, Szerafin T, Auer J, Ankersmit HJ.
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Alpha-Gal Specific Humoral Immune Response after Implantation of Bioprostheses in Cardiac Surgery.
21st Annual Meeting of the Austrian Society of Transplantation, Transfusion and Genetics, St. Wolfgang, Austria. 2007/10.

Oral Presentations:

Th1 and Th2 cytokine response in coronary artery bypass graft (CABG) patients.
(oral presentation)
49th Annual Meeting of the Austrian Society of Surgery, Innsbruck, Austria. 2008/05

Specification of immune modulation after Coronary Artery Bypass Graft operation.
(Project Presentation)
Medical University of Vienna, Vienna, Austria. 2007/05.

Diploma Thesis: **Wliszczak T.** Support of Business Cooperations in Terms of Supply Chain Management by integrated Enterprise Portals

Project Thesis: **Wliszczak T.** Implementation of selected Elements of the SCOR-Reference Process PLAN in SAP Advanced Planner & Optimizer (APO) using a Case Study of Collaboration

High School Graduation Thesis: **Wliszczak T.** Italian Design of the 20th Century.

AWARDS AND GRANTS

2008/12 Leistungsstipendium – Scholarship for Academic Achievement, Medical University of Vienna
2006/12 Leistungsstipendium – Scholarship for Academic Achievement, Medical University of Vienna
2006/12 Förderungsstipendium – Student Research Scholarship, Medical University of Vienna -„Specification of immune modulation after CABG operation"
1999/10 Special Award for Outstanding Academic Achievement, Austrian Minister of Education
1999/06 Matura (High School Graduation) with Distinction

MEMBERSHIPS

2005/10 Austrian Society of Transplantation, Transfusion and Genetics

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OTHER OCCUPATIONS

2000/05 – Present Certified Judge for Athletics Championships
1999/01 – Present Technical Assistant at a Psychotherapist's Office
2004/09 – 2005/04 Barista at a Coffee Shop
2001/08 – 2002/08 Project Assistant at an International Consulting Company

2000/05 – 2000/08	Accountant at an Athletics Club
2000/04 – 2000/05	Part-time worker at a Wholesaler

LANGUAGE SKILLS

Native German Speaker
Proficient in English (BEC Higher Certificate)
Good Knowledge of French
Basic Knowledge of Spanish

REFERENCES

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