On the induction of inflammatory reactions by Streptococcus pyogenes

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Abstract

Streptococcus pyogenes is an important human pathogen that causes significant morbidity and mortality worldwide. In order to successfully infect the human host, S. pyogenes is equipped with tools that modulate the human immune defence. The present thesis explores how different streptococcal proteins interfere with innate immunity, adaptive immunity, and blood coagulation.

M protein is a surface-bound streptococcal virulence factor with antiphagocytic properties. M proteins can be released from the bacterial surface, and in the present thesis we report that soluble M1 protein is a potent activator of monocytes and T cells. M1 protein-induced monocyte activation involves Toll-like receptor (TLR) 2, and results in secretion of the pro-inflammatory cytokines IL-6, IL-1β and TNFα. In addition, monocytes treated with M1 protein up-regulate Tissue Factor (TF) expression on the cell surface, leading to activation of the extrinsic pathway of coagulation. T cell induction upon M1 protein stimulation results in a potent Th1 type response. Activation does not require normal antigen processing, it is MHC class II dependent, and Vβ restricted with preferential expansion of Vβ2 and 4 bearing T cells, suggesting that M1 protein functions as a superantigen.

Thrombin activatable fibrinolysis inhibitor (TAFI) is a human procarboxypeptidase circulating in plasma. When activated, TAFI inhibits fibrinolysis and modulates the immune system by cleaving inflammatory mediators such as C5a, fibrinopeptides and bradykinin. Here we find that TAFI binds to Streptococcal collagen-like surface protein A and B on S. pyogenes. These bacteria can also recruit the two TAFI activators thrombin and plasmin to the bacterial surface, leading to the activation of surface-bound TAFI.

Taken together, the findings may help explain how S. pyogenes modulates the immune response in order to successfully infect its human host.

Key words: Streptococcus pyogenes, M protein, Streptococcal collagen-like surface protein, monocyte, T cell, coagulation, TAFI

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Cover image: *Streptococcus pyogenes* bacteria growing in chains, visualized by scanning electron microscopy (Picture provided by courtesy of Dr. Matthias Mörgelin).

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Contents

List of papers

This thesis is based on the following papers, referred to in the text by their roman numerals (I-IV).

I. **Lisa I. Påhlman, Matthias Mörgelin, Jana Eckert, Linda Johansson, Wayne Russell, Kristian Riesbeck, Oliver Soehnlein, Lennart Lindbom, Anna Norrby-Teglund, Ralf R. Schumann, Lars Björck, and Heiko Herwald**. Streptococcal M protein – a multipotent and powerful inducer of inflammation. *Journal of Immunology* (2006) 177:1221-1228

II. **Lisa I. Påhlman, Erik Malmström, Matthias Mörgelin, and Heiko Herwald.** M protein from *Streptococcus pyogenes* induces tissue factor expression and pro-coagulant activity in human monocytes. *Under consideration*

III. **Lisa I. Påhlman, Jessica Darenberg, Anders I. Olin, Malak Kotb, Heiko Herwald, and Anna Norrby-Teglund.** Soluble M1 protein of *Streptococcus pyogenes* triggers potent T cell activation. *Manuscript*

IV. **Lisa I. Påhlman, Pauline F. Marx, Matthias Mörgelin, Slawomir Lukomski, Joost C. M. Meijers, and Heiko Herwald.** Thrombin Activatable Fibrinolysis Inhibitor binds to *Streptococcus pyogenes* by interacting with collagen-like surface proteins A and B. *Under consideration*

Abbreviations

BLS	bare lymphocyte syndrome
HBP	heparin binding protein
HLA	human leukocyte antigen
IFNγ	interferon γ
Ig	immunoglobulin
IL	interleukin
IVIG	polyspecific immunoglobulins
LPS	lipopolysaccharide
LTA	lipoteichoic acid
Mga	multiple gene regulator of group A streptococcus
MHC	major histocompatibility complex
NALP	NACHT-, LRR- and pyrin domain-containing protein
NFκB	nuclear factor kappa B
NOD	nucleotide-binding oligodimerization domain
PBMC	peripheral blood mononuclear cell
PIP2	phosphatidylinositol 4,5-bisphosphate
RIG-I	retinoic acid inducible gene I
Scl	streptococcal collagen-like surface protein
Spe	streptococcal pyrogenic exotoxin
STSS	streptococcal toxic shock syndrome
TAFI	thrombin activatable fibrinolysis inhibitor
TCR	T cell receptor
TF	tissue factor
Th	T helper
TLR	Toll-like receptor
TNF	tissue necrosis factor

Introduction

The human body is constantly exposed to various microorganisms, and without our immune system, we would rapidly succumb to severe infections. In response to invading microorganisms, the immune system triggers an inflammatory reaction, serving to destroy or wall off the infectious agent. As these processes often induce tissue damage, inflammation also has an important function in triggering tissue repair. Although the immune system is fundamentally protective, it can cause significant harm if the balance between tissue damage and repair is altered. For example, inappropriate inflammatory reactions underlie hypersensibility reactions to food or insect bites, and autoimmune disorders such as rheumatoid arthritis. Moreover, severe bacterial infections, such as sepsis and septic shock, are believed to be caused by an over-activation of the human immune system.

The present thesis explores how surface proteins of the important human pathogen *Streptococcus pyogenes* interact with the three cornerstones of the human immune system, namely innate immunity, adaptive immunity, and blood coagulation.

1 Innate immunity

The innate immune system constitutes our first line of defense against invading pathogens. It is mobilized within minutes to hours upon infection, and serves to attack and eliminate the microbe. In addition, it has an important role in activating the slower but more specific adaptive immune system. Innate immunity is phylogenetically conserved and present in almost all multicellular organisms [1]. In humans, it consists of proteins such as complement and antimicrobial peptides, as well as immune cells, which are described in more detail below.

1.1 Polymorphonuclear Neutrophils

Polymorphonuclear Neutrophils are the first immune cells to arrive at the site of infection. They normally circulate in the blood stream, but are guided to the infectious site by endothelial cells that up-regulate adhesins, such as selectins and integrins, in response to infection. As a result, neutrophils adhere to the vascular wall, cross the endothelium via so-called diapedesis, and start to migrate towards the infectious site. Here they attack microorganisms by for example phagocytosis, followed by killing via oxygen radicals or microbicidal substances [2]. Neutrophils are rich in cytosolic granules, containing for example inflammatory mediators, antimicrobial peptides, matrix degrading enzymes, components involved in oxidative burst, and membrane-bound receptors mediating adhesion. Based on the granular content and the tendency to undergo exocytosis, at least four different subsets of granula have been characterized; azurophilic (primary) granules, specific (secondary) granules, gelatinase (tertiary) granules, and secretory vesicles. (reviewed in [3]). In order to mount an effective antimicrobial response while minimizing damage of host tissues, granula are mobilized in a regulated fashion. In the early phase of infection, secretory vesicles containing adhesion receptors are exocytosed. Specific and gelatinase granules are released next, secreting matrix-degrading proteases that facilitate tissue migration. At a later stage, azurophilic granules rich in microbicidal compounds are mobilized to the cell exterior or to the phagolysosomal compartment [4]. Of the many proteins released from neutrophilic granula, heparin binding protein (described below) is of special interest for this thesis.

1.1.1 Heparin binding protein

Heparin binding protein (HBP), also known as azurodicin and cationic antimicrobial protein of molecular mass 37 kDa (CAP37), is stored in both azurophilic granules and secretory vesicles [5]. HBP belongs to the serine proteinase family and shares sequence homology with elastase, proteinase 3 and cathepsin G from human neutrophils [6]. However, the protein lacks enzymatic activity due to the exchange of two out of three essential catalytic amino acids [6, 7]. Many of the HBP effects specifically target monocytes. For example, the protein functions as a strong chemoattractant to monocytes both *in vitro* [8] and *in vivo* [9], it selectively induces adhesion of monocytes to the endothelium [10], it enhances monocyte survival [11], and it potentiates cytokine production in monocytes treated with lipopolysaccharide (LPS) [12, 13]. Moreover, HBP is antimicrobial against a wide spectrum of Gram-negative bacteria, whereas Gram-positive bacteria, including *S. pyogenes*, are resistant [14, 15]. The protein also binds the lipid A portion of LPS with high affinity [16], and functions a potent inducer of vascular leakage [17].

1.2 Monocytes and macrophages

Monocytes are mononuclear phagocytes circulating in the blood stream. From the blood, monocytes migrate into tissues, where they mature into different kinds of macrophages. Both monocytes and macrophages constitute heterogeneous cell populations with an impressing ability to vary surface markers and functional patterns according to the microenvironment. Monocytes can broadly be divided into two subpopulations, in the following referred to as inflammatory and resident monocytes (table 1). Resident monocytes are recruited to tissues in the absence of inflammation.

Table 1. Differences between inflammatory and resident monocytes in surface markers, adhesion molecules, and chemokines involved in monocyte trafficking. (BRAK, breast and kidney-expressed chemokine; CD62L, L-selectin; C(X3)CR, chemokine receptor; ICAM, intercellular adhesion molecule; MCP, monocyte chemotactic protein; MIG, monokine induced by IFNγ; MIP, macrophage inflammatory protein; VCAM, vascular cell adhesion molecule)

	Inflammatory monocytes	*Resident monocytes*
Surface markers	$CX3CR1^{low}$, $CCR2^{+}$, $CD62L^{+}$	$CX3CR1^{high}$
Adhesion	β2-integrin, ICAM1, ICAM2, VCAM1, VLA4, CD62L	β2-integrin
Chemokines	MCP-1, MIG	MIP1α, BRAK, fractalkine

They adhere to non-inflamed endothelium via β2-integrins, and migrate into the tissues where they differentiate into tissue-specific macrophages such as peritoneal macrophages, alveolar macrophages, Kupffer cells in the liver, brain microglial cells and pericytes. Under normal conditions, these cells maintain homeostasis of tissues by clearing senescent and apoptotic cells [18]. Resident monocytes can also give rise to specialized cells such as dendritic cells and osteoclasts [19]. In contrast, inflammatory monocytes are specifically recruited to sites of infection, governed by adhesins up-regulated by inflamed endothelium and guided by inflammatory chemokines [18](table 1). At the infectious site, monocytes mature into macrophages and become the main effectors of innate immunity. Firstly, they function as efficient phagocytes that engulf and kill microbes. Secondly, they secrete inflammatory mediators such as interleukins 1 and 6 (IL-1 and IL-6), and tissue necrosis factor α (TNFα) that induce a local inflammatory reaction. Macrophages can also up-regulate tissue factor (TF) on their cell surfaces, which then initiates blood coagulation and amplifies the inflammatory response (see chapter 3.1.1). Thirdly, they activate the adaptive immune system by presenting antigen peptides on major histocompatibility complex (MHC) class II to T cells [18]. In addition, macrophages are responsible for polarizing the adaptive immune system towards a cell- or an antibody-mediated response due to their ability to secrete different co-stimulatory signals in response to different stimuli (see chapter 2.1).

1.3 Pattern recognition receptors

Cells of innate immunity express so-called pattern recognition receptors (PRRs) in order to distinguish between self and non-self. Pattern recognition receptors share some common features: (i) they recognize so-called pathogen-associated molecular patterns (PAMPs) rather than specific epitopes, (ii) they are constitutively expressed by the host, (iii) they are non-clonal, i.e. identical receptors are expressed by all cells of a given type, and (iv) they are independent of immunologic memory [20]. In the following section, some important pattern recognition receptors are described.

1.3.1 Toll-like receptors

The *toll* gene was initially described to govern the embryonic development of the *Drosophila* fruit fly [21], but it later became apparent that the corresponding

protein also protected the fly from fungal infections [22]. Soon after, Toll-like receptors (TLRs) were found in humans [23], where they are primarily expressed by immune cells, and in particular by phagocytes [24]. TLRs belong to the family of type 1 transmembrane receptors with an extracellular domain containing leucine-rich repeats regions, and a cytoplasmic domain (Toll/IL-1 receptor or TIR domain) that shares homology with the IL-1 receptor. When activated, signaling occurs via a MyD88 dependent or independent pathway that eventually leads to the activation of nuclear factor kappa B (NFκB). NFκB controls the expression of genes involved in innate immunity, such as inflammatory cytokines (reviewed in [25]). 11 different TLRs have been described so far in humans, although human TLR11 seems to be non-functional. Most of the known TLR ligands are of bacterial or viral origin, such as LPS, peptidoglycan, microbial DNA and dsRNA, but host-derived substances such as heat shock proteins, fibrinogen and mRNA have also been reported to activate the receptors (table 2). TLRs can be divided into two groups based on their sub-cellular localization, also reflecting their ligand specificities. As shown in figure 1, TLR1, 2, 4, 5, and 6 are expressed at the cell surface. In contrast, TLR3, 7, 8, and 9, which

Table 2. Ligands of human TLRs.

TLR	*Microbial ligands*	*Endogenous ligands*
1	Triacyl lipopeptides (in conjunction with TLR2) [26]	
2	Peptidoglycan [27] LTA [27] Lipoproteins from Gram-pos. and Gram-neg. bacteria and *Mycoplasma* [28, 29] Zymosan [30] *Yersinia* V-antigen [31] Streptococcal M protein (paper I, this thesis)	
3	dsRNA [32]	mRNA [33]
4	LPS [34, 35]	Heat shock protein 60 [36] Heat shock protein 70 [37] Fibrinogen [38] Extra domain A of fibronectin [39]
5	Flagellin [40]	
6	Diacyl lipopeptides (in conjunction with TLR2) [41]	
7	ssRNA [42]	
8	ssRNA [43]	
9	CpG DNA [44]	IgG/chromatin complexes [45]
10	Unknown	
11	Not applicable	

sense microbial nucleic acids and therefore require digestion of the pathogen, are found in endosomal compartments [25]. Many of the cell surface-expressed TLRs require co-receptors for efficient ligand recognition. For example, CD14 and MD-2 are involved in LPS-recognition via TLR4 [46], and dectin-1 has been identified as an important binding partner of TLR2 in the response to β-glucans [47]. In addition, TLR2 forms heterodimers with either TLR1 or 6, allowing discrimination between di- and triacyl fatty acids [48]. The diversity of co-receptors may explain the broad ligand specificity of TLRs. It may also be a mechanism by which immune cells discriminate between pathogens and commensals, as the downstream TLR signaling depends on the co-receptor involved [49].

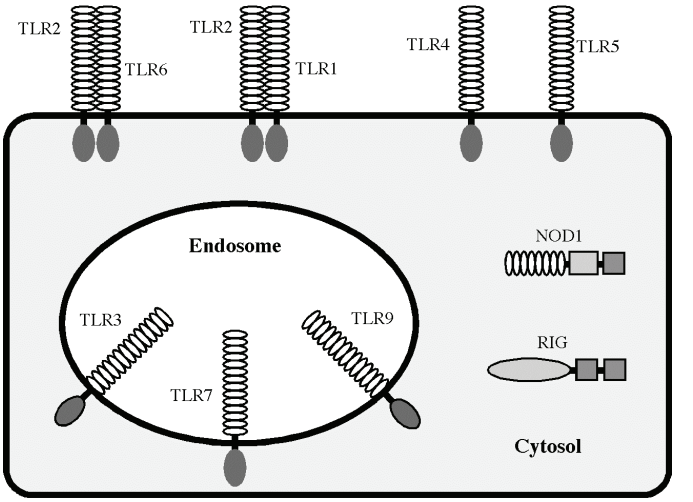

Figure 1. Sub-cellular location of pattern recognition receptors. TLRs are expressed at the cell surface or in the endosomal compartment, whereas NOD-like receptors and RIG-I-like receptors are found in the cell cytosol.

1.3.2 NOD-like receptors

NOD-like receptors are intra-cellular receptors (Fig. 1) that recognize non-self structures in the cell cytosol. The proteins contain carboxyterminal series of leucin-rich repeats, which are believed to be involved in ligand recognition [50]. The NOD-like receptor family can be divided into two sub-classes, namely the nucleotide-binding oligodimerization domains (NODs) and the NACHT-, LRR- and pyrin domain-containing proteins (NALPs). The most well-characterized members are

NOD1 and NOD2, which recognize peptides derived from peptidoglycans. While NOD1 responds to γ-D-glutamyl-*meso*-diaminopimelic acid present in peptidoglycan from Gram-negative bacteria [51], NOD2 senses muramyl dipeptide present in peptidoglycan from both Gram-positive and Gram-negative bacteria [52]. How these ligands enter the cytosol is still unknown, but it has been suggested that they are generated during bacterial digestion in the phagolysosome, or taken up by a specific transporter [53]. NOD proteins are mainly expressed by antigen presenting cells and epithelial cells, and similar to TLRs, their activation leads to an inflammatory response via NFκB activation [53]. In contrast, NALPs are activated by pathogen-associated molecular patterns including flagellin and bacterial RNA, but they also respond to so-called danger-associated molecular patterns such as low concentrations of intracellular K^+ and mechanical rupture [54, 55]. NALP signaling leads to the activation of caspase-1, which converts pro-forms of IL-1β and IL-18 into biologically active proteins [55].

1.3.3 RIG-I-like receptors

Retinoic acid inducible gene I (RIG-I)-like receptors are cytosolic proteins (Fig. 1) that are activated by viral dsRNA [56]. Their signaling is similar to that of TLR3, TLR7, TLR8, and TLR9, all of which are activated by viral products [55]. However, while TLRs are mainly expressed by dendritic cells, RIG-I-like receptors are widely distributed among human cell types. It has therefore been suggested that TLRs are viral sensors of dendritic cells, whereas RIG-I-like receptors react to viral infections in other cells [57].

2 Adaptive immunity

While innate immunity depends on pattern recognition, the adaptive immune system recognizes specific antigen epitopes. B and T cells, which are the main effectors of adaptive immunity, express individual receptors with unique antigen-specificities. As a consequence, only a small fraction of the total lymphocyte population responds to a given antigen. Activated cells start to proliferate, and the clonal expansion of antigen-specific cells leads to a highly adapted and specialized immune response. A second function of the adaptive immune system is the generation of immunologic memory, which enables rapid and efficient immune activation upon re-infection with the same pathogen. A general overview of B and T cells is given below.

2.1 T lymphocytes

In order to induce T cell activation, antigens have to be processed and presented to the T cell as peptide fragments by MHC class I or class II molecules. In humans, these are represented by several human leukocyte antigen (HLA) alleles. MHC class I is expressed by all nucleated cells and presents peptides derived from the cell cytosol. In contrast, MHC class II is mainly expressed by antigen presenting cells and displays peptides from the endosomal compartment [58]. T cell activation is initiated when the T cell receptor (TCR) binds to the antigen-MHC complex. In addition, co-stimulatory signals from the antigen presenting cell, such as the binding of CD80 or CD86 to CD28 on the T cell, are required for activation to take place [59].

The TCR is expressed as a dimer consisting of an α and a β chain ($\alpha\beta$T cells) or a γ and δ chain ($\gamma\delta$T cells). Each chain is encoded by large pools of gene segments, distributed on different chromosomes. During the course of T cell maturation, the gene segments are randomly combined, giving rise to an unique TCR on each T cell [60]. Only a small fraction of the developing lymphocytes are allowed to mature, as cells expressing TCRs that lack affinity for self-MHC class II, or that react to host-derived peptides on MCH molecules, are eliminated [61]. As a consequence of the enormous diversity of TCRs, only a minor subset of the T cell population is activated in response to a single antigen. Activated cells start to proliferate, generating large

clones of antigen-specific T cells with different effector functions depending on the T cell type [62]. The majority of T cells express TCRs consisting of α and β chains. These cells are subgrouped into $CD4^+$ and $CD8^+$ T cells based on their surface markers. $CD4^+$ cells, also called T helper (Th) cells, recognize peptides presented by MHC class II. Upon activation, the $CD4^+$ cell mounts either a Th1 or Th2 response. The Th1 response is important for combating bacterial infections. It is characterized by the secretion of IL-2 and IFNγ, leading to enhanced phagocytosis, oxidative burst and intracellular killing of microbes. In contrast, Th2 cells release cytokines such as IL-4, IL-5, IL-10 and IL-13, promoting B cell activation and antibody production [63]. Skewing towards the Th1 or Th2 phenotype is largely dependent on the antigen presenting cell and how this cell was activated. For example, Toll-like receptor activation of dendritic cells generally leads to a Th1 response, whereas parasites such as helminths trigger dendritic cells to induce Th2 cells [64].

$CD8^+$ cells, also referred to as cytotoxic T lymphocytes, recognize peptides displayed on MHC class I. Tumor cells and host cells infected with viruses or intracellular bacteria will present foreign peptides to the cytotoxic T cell, which will then induce apoptosis of the transformed cell [65].

Other subpopulations of T cells are for instance regulatory T cells and γδT cells. Regulatory T cells downregulate the immune response, which may be an important mechanism to prevent autoimmunity. γδT cells constitute only 1-5% of the total T cell population and are mostly found in epithelial tissues such as the skin or the gut. Their activation is not necessarily dependent on normal antigen processing, which places them on the border between innate and adaptive immunity [59].

2.2 B lymphocytes

The most important function of B cells is to secrete antigen-specific immunoglobulins (Ig). These Y-shaped molecules consist of two heavy and two light chains, and are divided into five different classes (IgM, IgD, IgG, IgA, and IgE) based on the constant region of the heavy chain [58]. Antibodies bind their corresponding antigen epitopes on the invading microbe, thereby greatly enhancing the clearance of pathogens through Fc-mediated phagocytosis. In addition, antibodies coating a bacterium activate the classical pathway of complement, leading to direct destruction

of the microbe via formation of the membrane attack complex, as well as to facilitated phagocytosis via complement opsonization [66]. B cells recognize antigens via B cell receptors, consisting of surface-bound antibodies. B cell activation is initiated upon binding of an antigen to the B cell receptor. The antigen is taken up, processed, and presented on MHC class II to T helper cells, which then provides a secondary co-stimulatory signal. Activation leads to clonal expansion of the antigen-specific B cell, which also matures into a plasma cell that produces large amounts of soluble antibodies [58].

Similar to the TCR, B cell receptors are unique to each B cell due to random re-arrangements of gene elements, so-called V(D)J recombination [67]. In addition, B cell receptor heterogeneity is further increased through somatic hypermutation and class switch recombination, which occur after antigen activation. Somatic hypermutation generates high-affinity antibodies via point mutations in the antigen-binding part of the Ig molecule. In some B cell receptors, the mutations lead to an increased antigen-affinity, and these cells will be selected through continuous antigen stimulation. Class switch recombination refers to the change of antibody classes from IgM or IgD on naïve B cells, to IgG, IgE, or IgA on activated cells. This is achieved via DNA deletions that join the gene segment coding for the antigen-binding part with a new constant region, thereby changing the antibody class without altering the antigen-specificity [68].

When the infection is cleared, some plasma cells remain resting as memory B cells. Upon re-challenge with the same antigen, memory B cells rapidly become plasma cells that secrete high-affinity antibodies. Moreover, some B cells develop into so-called long-lived plasma cells, which reside in the bone marrow and constantly secrete low levels of antigen-specific immunoglobulins. Both mechanisms are important for maintaining a long-term immunologic memory [69].

3 Coagulation

Coagulation is vital to prevent blood loss and maintain blood pressure in case of injury, but it is also intimately connected with inflammation. The following chapter describes the molecular mechanisms behind blood coagulation, and its role in immunity.

3.1 The coagulation cascade

The coagulation system consists of a number of serine proteases, or coagulation factors, that circulate in plasma in their inactive forms. Clotting is initiated via the intrinsic or the extrinsic pathway of coagulation, both of which lead to activation of the common pathway (Fig. 2). The common pathway is triggered by the activation of factor X, that subsequently catalyses the conversion of pro-thrombin to thrombin. Next, thrombin activates fibrinogen to fibrin, which forms the clot [2].

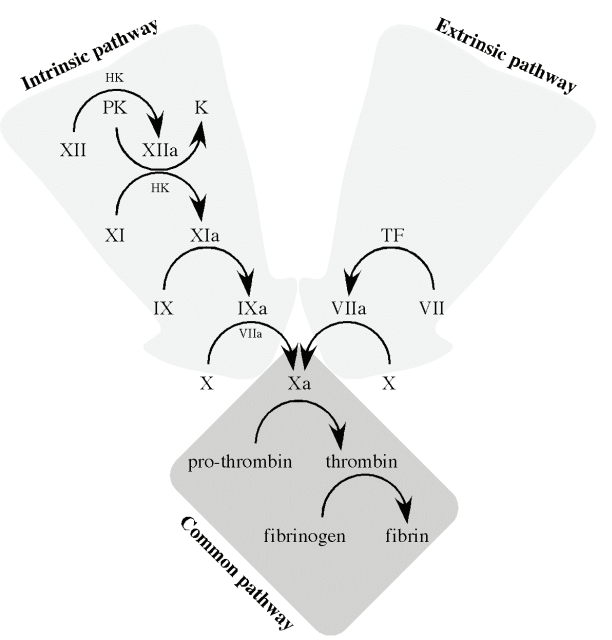

Figure 2. The coagulation cascade. Induction of the intrinsic or extrinsic pathway triggers a cascade of coagulation factor activation. Both pathways converge at the activation of factor X, which initiates the common pathway. (HK, H-kininogen; K, kallikrein; PK, prekallikrein. Active (a) and inactive clotting factors are represented by their roman numerals.)

3.1.1 Extrinsic pathway

The extrinsic pathway is the most important initiator of coagulation *in vivo*. It is activated by the glycoprotein tissue factor (TF), which is constitutively expressed at high concentrations by fibroblasts surrounding the blood vessels [70]. Upon vessel damage, TF is exposed to plasma and forms a catalytic complex with coagulation factor VII, leading to the activation of factor X (Fig. 2). The coagulation cascade thereafter propagates along the common pathway until a blood clot is formed [71]. Although blood cells do not express TF under normal conditions, monocytes can be induced to up-regulate TF in response to pro-inflammatory cytokines or bacterial compounds such as LPS, peptidoglycan, LTA and superantigens [72-75]. Platelets and neutrophils have also been suggested to express TF, but whether the protein is produced by these cells or taken up from monocytes is still under debate [76, 77]. Systemic TF-driven activation of the coagulation cascade is believed to explain the disturbed blood coagulation often seen in septic patients. Severe cases may progress into disseminated intravascular coagulation (DIC), a serious condition characterized by a massive formation of microthrombi. As a result, platelets and coagulation factors are consumed, leading to localized bleeding [78]. Moreover, activation of blood clotting leads to enhanced inflammation in a positive feedback loop. The coagulation factors of the extrinsic pathway – factor VII, factor X and thrombin - all have pro-inflammatory properties when activated, as they induce the release of inflammatory cytokines and growth factors through protease-activated receptor (PAR) activation (reviewed in [79]). Consequently, mice expressing low levels of TF or factor VII display reduced coagulation, inflammation, and mortality compared to wildtype mice upon LPS challenge [80, 81]. The role of coagulation in severe infectious diseases is also exemplified by the fact that the anticoagulant agent active protein C is successfully used in the treatment of sepsis [82].

3.1.2 Intrinsic pathway

The intrinsic pathway is initiated by the so-called contact system, consisting of factor XII, prekallikrein and high molecular weight (H-)kininogen. The contact system assembles on negatively charged surfaces, leading to the reciprocal activation of factor XII and prekallikrein. Active factor XII triggers the sequential activation of factors XI, IX, and X, and subsequent induction of the common pathway (Fig. 2)[83].

In addition to the haemostatic effects, activation results in the release of the potent pro-inflammatory and vasoactive mediator bradykinin from its precursor H-kininogen [84]. Several bacterial species, including *Escherichia coli*, *Salmonella*, *Staphylococcus aureus*, and *S. pyogenes*, can trigger the contact system by assembling the participating proteins at their surfaces [85-88]. Activation of the intrinsic pathway is believed to contribute to the hypotension seen in sepsis [89, 90].

3.2 Fibrinolysis

Blood coagulation is balanced by the fibrinolytic system, serving to dissolve fibrin clots. Fibrinolysis is mainly mediated by plasmin, which is generated from its precursor plasminogen upon activation by tissue plasminogen activator (tPA) or urokinase plasminogen activator (uPA). Activation is enhanced in the presence of fibrin, ensuring that plasmin is mostly generated during blood clotting [91]. Fibrinolysis is controlled by the plasminogen activator inhibitor-1 (PAI-1), or by inhibitors of plasmin itself, such as α2-antiplasmin and α2-macroglobulin. It is also attenuated by thrombin activatable fibrinolysis inhibitor (TAFI), as described in more detail below.

3.2.1 TAFI

TAFI prevents fibrinolysis by removing carboxy-terminal lysine and arginine residues from the surface of fibrin clots. As a consequence, the binding and activation of plasminogen is prevented, resulting in a prolonged lysis time. TAFI is a zinc-dependent procarboxypeptidase that is synthesized in the liver [92]. It is thereafter released into the circulation, where it has a plasma concentration of 70-275 nM [93]. TAFI activation occurs by cleavage at Arg^{92}, generating a 19 kDa activation peptide and an enzymatically active 35 kDa fragment, TAFIa (Fig. 3). After only a few minutes, TAFIa is inactivated via a conformational change, followed by further processing into fragments of 25 and 11 kDa [94]. The most potent TAFI activator is the thrombin/thrombomodulin complex [95]. Plasmin, trypsin and neutrophil elastase can also activate TAFI, albeit less efficiently [96-98].

Interestingly, TAFI knock out mice have a normal hemostasis, but display impaired wound healing [99], increased recruitment of inflammatory cells to the infectious site, and an enhanced inflammatory response to *E. coli*-induced sepsis

[100]. TAFI deficiency also leads to increased complement-mediated inflammation and death in LPS-primed mice [101], suggesting that TAFI plays an important role in inflammatory conditions.

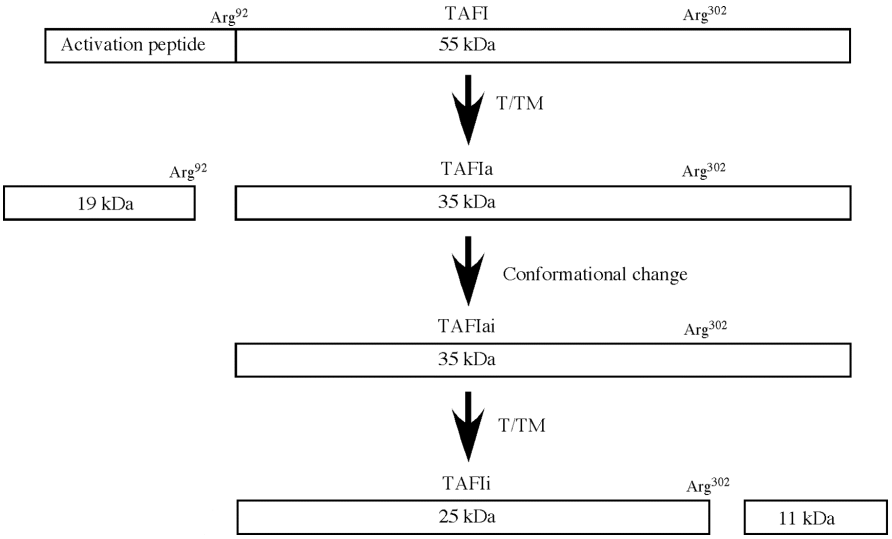

Figure 3. Activation of TAFI. Active TAFI (TAFIa) is generated via the enzymatic removal of a 19 kDa activation peptide. TAFIa is rapidly inactivated via a conformational change, which is followed by further processing into smaller fragments.

4 *Streptococcus pyogenes*

Streptococci are Gram-positive bacteria that grow in chains or pairs. They are classified into α, β and γ-hemolytic bacteria according to their ability to lyse red blood cells. α-hemolytic bacteria reduce the iron in hemoglobin, resulting in a greenish color on blood agar plates, whereas β-hemolytic bacteria cause complete lysis of erythrocytes. In contrast, γ-hemolytic streptococci are incapable of lysing host cells. β-hemolytic bacteria are then subdivided into groups A-H, K-M and O-V, based on group-specific cell wall carbohydrates. *Streptococcus pyogenes*, which was used throughout this work, is a β-hemolytic group A streptococcus. These bacteria are further divided into more than 100 different serotypes based on the type of M protein and T antigen expressed on the bacterial surface [102].

S. pyogenes is a strictly human pathogen that mainly causes throat- and skin infections, such as pharyngitis, scarlet fever, impetigo, erysipelas and cellulitis. Although the vast majority of streptococcal infections are uncomplicated and self-limiting, some cases may progress into sepsis, necrotizing fasciitis and streptococcal toxic shock syndrome (STSS), all of which display an extremely rapid progression and high mortality rates [103]. STSS is characterized by systemic toxicity, hypotension, and multiple organ failure, and around 70% of STSS cases are accompanied by necrotizing fasciitis or myositis [104]. Necrotizing fasciitis is an infection of the deep subcutaneous tissue that spreads along the muscle fascia. It leads to massive tissue destruction, often requiring surgical intervention in addition to intravenous antibiotic therapy [105]. Streptococcal infections can also lead to non-suppurative complications including rheumatic fever and acute glomerulonephritis [103], the former being the leading cause of cardiovascular morbidity in the third world. Taken together, *S. pyogenes* is responsible for over 500.000 deaths world wide every year, placing the bacterium among the 10 most mortality-causing human pathogens [106].

In order to infect the human host, *S. pyogenes* expresses a number of virulence factors that mediate adhesion to host tissues, enable dissemination of bacteria, or that modulate the immune response. Some of the virulence factors of *S. pyogenes*, with emphasis on those important for this thesis, are described below.

4.1 Capsule and cell wall

Gram-positive bacteria, including *S. pyogenes*, are surrounded by a thick cell wall composed of peptidoglycans and LTA. LTA mediates adhesion of the streptococcus to fibronectin on oral epithelial cells [107, 108], and both peptidoglycan and LTA trigger innate immunity via TLR2 [109]. Outside the cell wall, streptococci may form a hyaluronic acid capsule. The capsule is an important anti-phagocytic factor [110], but it also enables adhesion to CD44-positive keratinocytes [111] and facilitates throat colonization *in vivo* [112]. The ability to encapsulate varies between streptococcal strains, and virulence differs accordingly. For example, clinical isolates from severe streptococcal infections are more commonly encapsulated than strains causing uncomplicated pharyngitis [113], suggesting that the capsule renders the bacterium more virulent.

4.2 Surface attached virulence factors

4.2.1 M proteins

The importance of M proteins was demonstrated already in the 1960s, when Rebecca Lancefield observed that streptococci survive in human blood in the absence of M type-specific antibodies [114]. M proteins are expressed by all streptococcal strains and can be seen at high magnification as hair-like structures protruding from the cell surface. As depicted in figure 4, the protein is organized into an α-helical coiled-coil dimer [115] with a conserved carboxy-terminal domain, a semi-variable central part, and a hyper-variable amino-terminal end [116]. Similar to other surface proteins of Gram-positive cocci, the carboxy-terminal is anchored to the cell wall via an LPxTG motif [117]. The far amino-terminal sequence is highly variable among streptococci but is stable within a strain, and is therefore used to define the serotype. Antibodies towards the amino-terminus allow effective killing of streptococci and render the host immune against this particular serotype. The gene encoding M protein, called *emm*, is regulated by the Mga regulon (multiple gene regulator of group A streptococcus), which is maximally expressed during the logarithmic growth phase [118], and *in vivo* during the acute phase of infection [119]

M proteins interact with a large number of host proteins, such as fibrinogen [120], fibronectin [121], IgG [122], albumin [122], kininogens [123], factor H [124],

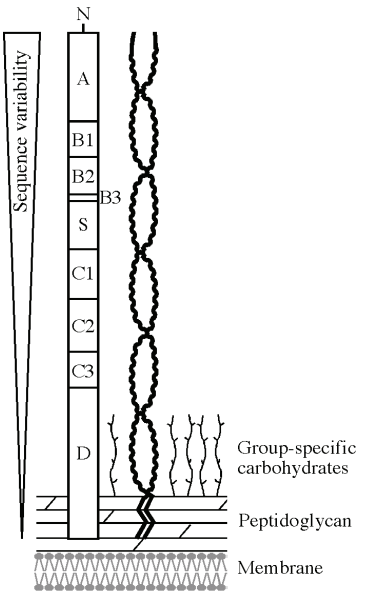

Figure 4. Streptococcal M1 protein. M1 protein forms an α-helical coiled-coil dimer at the cell surface (right). The mature protein has a hypervariable amino-terminal end (A), which is followed by B- and C-repeats. The D domain contains the wall-spanning region and LPxTG motif (left).

factor H-like protein-1 (FLH-1) [125], and C4b-binding protein (C4BP) [126]. The binding of fibrinogen is crucial for streptococcal virulence [127] and for the anti-phagocytic properties of M protein [128, 129]. It inhibits complement activation and subsequent opsonization via C3b deposition, thereby limiting phagocytosis [130, 131]. M proteins lacking affinity for fibrinogen bind the complement regulatory protein C4b binding protein instead, which inhibits phagocytosis by the same mechanism [130, 132]. However, the anti-phagocytic properties of M protein have been questioned by several recent studies showing that streptococci are in fact taken up by neutrophils, but are able to survive inside the cell [133, 134]. The intracellular survival seems to be M protein-dependent, as M-negative mutants are efficiently killed [135]. Another function of M protein is to mediate adhesion to skin keratinocytes [136] and Hep-2 tissue culture cells [137]. The protein has also been demonstrated to promote intracellular invasion of *S. pyogenes* bacteria into epithelial cells [138], and to allow throat colonization in a baboon model of group A streptococcal pharyngitis [112]. Moreover, M proteins have been implicated in the pathogenesis of rheumatic heart fever, as the streptococcal protein generates cross-reactive auto antibodies against human myosin and collagen [139, 140].

Of importance for this thesis, M proteins can be released from the bacterial surface by proteinases from the host or from the bacterium itself [122, 141, 142]. The soluble protein forms complexes with fibrinogen, thereby triggering the activation of neutrophils [142].

In addition to the M protein, some streptococcal strains express one or two structurally related proteins, collectively referred to as M-like proteins. The corresponding genes (*mrp*, *enn*, *protH* and others) are clustered together with the *emm* gene on the bacterial chromosome, and are controlled by the Mga regulon [103]. Similar to the M protein, M-like proteins bind a wide array of host proteins, and they seem to cooperate with M proteins in rendering the bacteria resistant to phagocytic killing [143-145].

4.2.2 Streptococcal collagen-like surface protein

Streptococcal collagen-like surface protein A and B (SclA and SclB), also known as Scl1 and Scl2, are two related proteins with a structure similar to that of human collagen. They contain a collagen-like region with a glycin at every third amino acid position, and an amino-terminal non-collagenous variable region. The carboxy-terminus contains an LPxTG motif that anchors the proteins to the cell wall. Like human collagen, Scl proteins adopt a "lollipop-like" structure with the variable region forming a globular head, and at least some of the Scls can form triple helices [146]. Both SclA and SclB have conserved signal peptides and carboxy-terminal cell wall regions, whereas the collagen-like and variable regions differ in size and primary sequence. The genes encoding SclA and B are located at different sites of the bacterial chromosome and are differently regulated. While *SclA* is up-regulated by the Mga regulon [147], the transcription of *SclB* is down-regulated by the same protein [148]. Moreover, SclB expression is controlled in an ON-OFF fashion at the translational level due to CAAAA repeats in the signal peptide sequence of the SclB gene. The number of repeats determines if the gene is in frame, and as a result, only some strains express a functional SclB protein.

Although the physiological role of the Scls is still largely unknown, SclA has been implicated in the adhesion of streptococci to human epithelial cells [149] and SclB to fibroblasts [148]. In addition, SclA from an M41 serotype binds low density lipoprotein (LDL) in human plasma [150], and induces adhesion and spreading of human lung fibroblasts through interactions with the collagen-binding $\alpha_2\beta_1$ integrin [151].

4.2.3 Other surface proteins

S. pyogenes expresses a number of plasmin/ogen- and fibronectin-binding proteins at the cell surface. The recruitment of plasmin/ogen is mediated by M and M-like proteins [152, 153], α-enolase [154] and glyceraldehyde-3-phosphate dehydrogenase [155], and is central to streptococcal virulence [156, 157]. Binding of fibronectin to the bacterial surface involves protein F1/Sfb1 [158, 159], streptococcal opacity factor [160] and glyceraldehyde-3-phosphate dehydrogenase [161], as well as M and M-like proteins [121, 145]. Bound fibronectin mediates adhesion and internalization of the streptococcus into host epithelial cells [162-164], rendering the bacterium inaccessible to antibiotic therapy and favoring a carrier state [165]. However, studies addressing the role of fibronectin-binding proteins in virulence have shown conflicting results. Some demonstrate that *S. pyogenes* mutants lacking these proteins are less virulent to mice than the wild type strains [166, 167], whereas others on the contrary implicate that the fibronectin-binding property attenuates bacterial virulence [168]. Another streptococcal surface protein is the C5a peptidase. It specifically cleaves the anaphylatoxin C5a [169], leading to retarded recruitment of inflammatory cells to the site of infection and enhanced bacterial survival in the early phase of infection in a mouse model [170].

4.3 Secreted virulence factors

4.3.1 Superantigens

Superantigens are among the most potent inflammatory mediators known. They by-pass normal T cell activation by crosslinking MHC class II and the variable β (Vβ) chain of the TCR, without prior intracellular processing (Fig. 5). Humans express around 40 different Vβ families, and superantigens therefore induce the preferential expansion of T cells bearing Vβ chains to which the superantigen has affinity. It gives each superantigen an unique Vβ profile, and causes a massive Th1 response in up to 30% of the T cell population. In contrast, a normal antigen activates less than 0,01% of the T lymphocytes [171]. 11 different superantigens have been described so far in *S. pyogenes*, namely the streptococcal pyrogenic exotoxins (Spe) A, C, and G-M, the streptococcal superantigen (SSA), and the streptococcal mitogenic exotoxin (SmeZ) [172]. In addition, pepsin-cleaved M protein fragments (pepM) from

rheumatogenic strains such as the M5 serotype, have been reported to function as superantigens [173, 174], although this has been a controversial issue [175]. Superantigens are believed to play a role in the pathogenesis of STSS, based on the findings that (i) high frequencies of SpeA-expressing strains have been isolated from STSS patients [176-178], (ii) lack of protective antibodies against superantigens is associated with a higher risk for STSS [179, 180], (iii) SpeA induces toxic shock-like symptoms and death in transgenic mice expressing HLA class II and human CD4 [181], and (iv) circulating superantigens have been found in patients suffering from STSS [182].

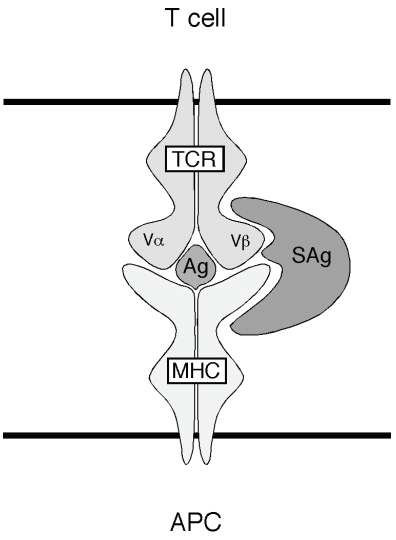

Figure 5. Function of superantigens. Normal antigens are processed and presented to T cell as peptide fragments on MHC class II. In contrast, superantigens bind directly to both MHC class II and the TCR, thereby cross-linking the two receptors and causing a massive but non-specific T cell response. (Ag, antigen; APC, antigen presenting cell; SAg, superantigen).

4.3.2 Other secreted proteins

Streptococcal pyrogenic-erythrogenic exotoxin B (SpeB), the perhaps most well-characterized secreted protein of *S. pyogenes*, is a cystein proteinase with broad substrate specificity. Among other things, SpeB releases the potent inflammatory mediator bradykinin from H-kininogen [183], it converts pro-IL-1β into its active form [184], it degrades the antibacterial peptide LL-37 and releases dermatan sulfate that inhibits antimicrobial peptides [185, 186], and it degrades immunoglobulins [187]. SpeB can also release endogenous proteins, including M protein, from the bacterial surface [141]. Apart from SpeB, *S. pyogenes* secretes two additional enzymes targeting immunoglobulins, namely immunoglobulin degrading enzyme of *S. pyogenes* (IdeS) that specifically cleaves IgG in the hinge region [188], and endoglycosidase of streptococci (EndoS) that hydrolyzes glycans on IgG [187]. The

cleavage of immunoglobulins is believed to be an important mechanism to avoid immune recognition.

The streptococcal inhibitor of complement (protein SIC) was first reported to prevent complement-mediated lysis by interfering with the formation of the membrane attack complex [189], but it was later suggested that its most physiologically relevant function is to neutralize antibacterial peptides, including lysozyme [190], human β-defensins hBD-1, hBD-2, and hBD-3 [191], and neutrophil-derived α-defensin and LL-37 [192]. Streptolysin O and S (SLO and SLS, respectively) are two streptococcal proteins that induce lysis of host cells. SLO forms large pores in eukaryotic membranes through the binding to cholesterol [193], whereas the less well-characterized SLS is responsible for the β-hemolytic properties of *S. pyogenes* [194]. Both proteins have been demonstrated to contribute to virulence and to the induction of necrotic lesions in murine models of streptococcal infection [194, 195]. Streptococci also secrete DNases. These enzymes protect bacteria from being killed by DNA-containing neutrophil extracellular traps (NETs), and have been demonstrated to promote the development of skin lesions in a mouse model of necrotizing fasciitis [196].

Present investigation

Paper I: M1 protein activates monocytes via TLR2

M proteins are regarded as classical streptococcal virulence factors by virtue of their anti-phagocytic properties. In addition, it was recently reported that soluble M1 protein triggers innate immunity by stimulating neutrophils to release HBP [142]. To further study the effects of the streptococcal protein on innate immunity, the aim of the present study was to explore the effects of M1 protein on monocytes in the absence or presence of HBP.

Treatment of human peripheral blood mononuclear cells (PBMCs) with M1 protein induced a potent inflammatory response, with secretion of the pro-inflammatory cytokines IL-6, IL-1β, and TNFα. In the presence of HBP, the inflammatory response was potentiated, whereas HBP alone did not trigger cytokine release. This finding was also confirmed in whole blood, where an antibody against HBP reduced M1 protein-induced cytokine secretion.

TLR2 and TLR4 are important sensors of Gram-positive and Gram-negative bacteria. Using Chinese Hamster Ovary cells transfected with TLR2 or TLR4, we found that TLR2-transfected cells, but not cells expressing TLR4, were activated by M1 protein. Moreover, immunohistochemical stainings, followed by electron microscopy, demonstrated that M1 protein and TLR2 co-localized at the bacterial surface.

Flow cytometry studies revealed that HBP rapidly evoked elevated intracellular calcium levels in cells of the monocytic cell line Mono Mac 6. The response was blocked in the presence of an antibody against the β_2 integrin CD11/CD18, suggesting that this receptor is involved in HBP signaling in monocytes. Taken together, we propose a model where M1 proteins are shed from the streptococcal surface, form complexes with human fibrinogen, and trigger neutrophils to release HBP. The soluble protein can also interact with TLR2 on monocytes. As a consequence, monocytes mount an inflammatory response, which is further enhanced by the presence of HBP. The exact mechanism by which HBP potentiates cytokine secretion is unknown. However, a recent study demonstrated that TLR signaling is controlled by phosphatidylinositol 4,5-bisphosphate (PIP2), and that CD11b signaling in macrophages triggers local PIP2 production and enhances LPS-induced IL-6

production [197]. These findings may explain the effects of HBP on M1 protein-evoked inflammation.

Paper II: Monocytes up-regulate TF expression in response to M1 protein

Since monocytes can up-regulate TF on the cell surface, the aim of paper II was to study if M1 protein could induce pro-coagulant activity. To that end, PBMCs or human whole blood were incubated with M1 protein, and their ability to induce coagulation of human plasma was thereafter analyzed with clotting assays. M1 protein induced dose-dependent pro-coagulant activity in both PBMCs and human whole blood, and flow cytometry analysis confirmed that monocytes up-regulate TF in response to M1 protein. Electron microscopy of a blood clot, induced by M1 protein-treated PBMCs, demonstrated that the fibrin network was connected with the cell surface, indicating that fibrin formation emanates from surface-bound TF. The findings may help explain the disturbed blood coagulation often seen in sepsis.

Paper III: M1 protein is a potent inducer of T cell activation

After having investigated the effects of M1 protein on innate immunity, we next wished to study whether the streptococcal protein interferes with T cells of the adaptive immune system. ^{3}H-Thymidine-incorporation assays showed that M1 protein evoked massive T cell proliferation, similar to the response triggered by a superantigen-containing streptococcal supernatant. This finding led us to investigate whether M1 protein displayed the characteristics of a superantigen (see chapter 4.3.1), and the results from a series of experiments suggested that this was the case. Firstly, incubation of human T cells with M1 protein in the presence of inactivated Bare Lymphocyte Syndrome (BLS) cells lacking MHC class II, or BLS cells transfected with different HLA alleles, demonstrated that M1 protein required the presence of MHC class II in order to trigger T cell activation, and that no intracellular processing of the streptococcal protein was necessary. Secondly, flow cytometry analyses implied that M1 protein-induced T cell proliferation was Vβ restricted, with a preferential expansion of T cells bearing Vβ2 and Vβ4 chains. Thirdly, immunohistochemical stainings of PBMCs demonstrated that up to 4 % of the T cell population produced the Th1 type cytokines IFNγ and TNFβ in response to M1

protein. Taken together, the results implicate that soluble M1 protein triggers massive T cell activation in a superantigen-like manner.

Superantigens are believed to be involved in the pathogenesis of STSS, a serious streptococcal infection associated with high mortality rates. Lately, administration of polyspecific immunoglobulins (IVIG), containing antibodies against superantigens and M proteins, to STSS patient has been proven beneficial for the outcome of disease [198]. Incubation of PBMCs with M1 protein in the presence of IVIG reduced the M1 protein-induced cytokine response to nearly background levels, suggesting that neutralization of M proteins may be an important mechanism of action for IVIG.

Paper IV: TAFI binds to SclA and SclB

Proteolysis is important for many biological processes, including inflammation. The streptococcus expresses several proteolytic enzymes, but it can also recruit host proteases and protease inhibitors in order to create a tightly regulated proteolytic atmosphere at the bacterial surface (reviewed in [199]). In paper IV, we studied the interaction between the human procarboxypeptidase TAFI and *S. pyogenes*. Several streptococcal strains were tested for their ability to bind TAFI, and the results showed that the AP41 strain had the highest TAFI-binding potential. In order to identify the structure involved in the binding, surface proteins from the AP41 strain were solubilized and incubated with TAFI immobilized on sepharose beads. A 35 kDa protein was recovered from the TAFI sepharose, and internal sequencing identified the protein as SclA from *S. pyogenes*. The genes encoding both SclA and SclB in the AP41 strain were thereafter cloned and expressed in *E. coli*, and subsequent binding studies confirmed an interaction between SclA and TAFI. SclB bound TAFI as well, although less efficiently.

TAFI is mainly activated by the thrombin/thrombomodulin complex, but other enzymes, such as plasmin, can also induce activation. It is well known that streptococci bind plasmin/ogen, but the present study shows that also thrombin is recruited by the AP41 strain. In the presence of plasmin or thrombin/thrombomodulin, streptococcal-bound TAFI was processed in a pattern resembling TAFI activation. Moreover, clot lysis assays confirmed that activation and enzymatic properties of TAFI were still intact when the enzyme was bound to AP41 bacteria, or in the

presence of soluble SclA or B. We speculate that recruitment and subsequent activation of TAFI may be a way for *S. pyogenes* to modulate the host response.

Discussion

For bacterial pathogens such as *S. pyogenes*, interactions with the human host are pivotal for survival and spread. Colonization of normally sterile sites gives streptococci the advantage of not having to compete for nutrients with other bacterial species. Instead, they will encounter the human immune defense that immediately will try to eliminate the invading pathogen. The present thesis demonstrates several mechanisms by which *S. pyogenes* interacts with the human immune system. In papers I-III, M1 protein is identified as a potent inducer of inflammation. Given that the immune response is harmful to bacteria, it may seem contradictory that the bacterium expresses pro-inflammatory virulence factors. M1 protein activates monocytes via TLR2 (paper I), and one characteristic feature of TLRs is that they recognize pathogen-associated molecular patterns that are vital to the bacterium and cannot be changed [20]. For example, TLR2 also senses LTA and peptidoglycan, both of which are necessary building blocks of the Gram-positive bacterial cell wall [109]. M proteins are undoubtedly critical virulence factors of *S. pyogenes*, as they protect the bacterium from being killed by immune cells. It could therefore be speculated that the positive effects of M proteins on streptococcal virulence outweigh their negative sides, thereby preventing their potentially harmful pro-inflammatory properties from being eliminated by evolution.

In paper III, we find that soluble M1 protein triggers a potent T cell response in a superantigen-like manner. Through interactions with MHC class II, superantigens can also trigger monocytes to secrete pro-inflammatory cytokines [200] and to up-regulate TLRs [201], and both observations may contribute to the M1 protein-induced response reported in paper I. Superantigens are extremely potent pro-inflammatory mediators, and the benefits of these proteins to bacteria are still unclear. Interestingly, it has been shown that T cell activation by superantigens is followed by T cell anergy and depletion [202]. For example, administration of the staphylococcal superantigen SEB to mice resulted in anergy of T cells and failure to raise antibodies against SEB as well as other co-injected antigens, suggesting that superantigens may be a way for the bacterium to inhibit an antibody response via antigen-specific T cells [203]. To

summarize the pro-inflammatory properties of M1 protein, the following model can be suggested: During the early phase of infection, neutrophils are recruited to the infectious site. M1 proteins are shed from the streptococcal surface via neutrophil-derived proteases, and soluble M1 protein forms aggregates with fibrinogen that trigger neutrophils to release for example HBP. Next, monocytes infiltrate the inflamed tissue. Their activation by M1 protein is largely dependent on TLR2, and is enhanced by the presence of HBP. At a later stage, T cells accumulate at the site of infection, and M1 protein induces a massive inflammatory reaction by virtue of its superantigen-properties. The corrupted immune response undermines efficient combating of the infection and is of little threat to the streptococcus. In contrast, inflammation may be beneficial to the bacterium, as it causes vascular leakage that provides nutrients and facilitates bacterial dissemination.

Blood coagulation is another important arm of the human immune system. In the present thesis, two different ways of favoring blood clot formation are demonstrated for the streptococcus; firstly the induction of pro-coagulant activity via TF up-regulation on monocytes (paper II), and secondly the protection of formed blood clots via activated TAFI (paper IV). Coagulation enables the immune defense to wall off invading pathogens and prevent bacterial dissemination, but bacteria may also utilize the clot as a shield in order to evade immune recognition. Recruitment of TAFI to the bacterial surface may have a second role in protecting streptococci from the immune system, as the active enzyme can neutralize inflammatory mediators such as fibrinopeptides and the chemotactic C5a.

Taken together, the present thesis demonstrates several mechanisms by which streptococci interact with the immune defense. The findings may help explain why *S. pyogenes* is one of the most common and successful human pathogens.

Conclusions

- Soluble M1 protein from *Streptococcus pyogenes* triggers monocyte activation via TLR2.

- M1 protein induces pro-coagulant activity in human blood via TF up-regulation on monocytes.

- Streptococcal M1 protein induces T cell activation in a superantigen-like manner.

- *Streptococcus pyogenes* recruits the human plasma protein TAFI to its surface, where it is activated in the presence of plasmin or thrombin/thrombomodulin.

Populärvetenskaplig sammanfattning

Dagligen utsätts våra kroppar för bakterier och andra mikroorganismer, och utan det livsviktiga immunförsvaret hade vi snabbt dukat under för svåra infektioner. Immunförsvaret består huvudsakligen av olika sorters vita blodkroppar och kan indelas i två grupper; det medfödda och det adaptiva immunsystemet. Det medfödda immunförsvaret utgörs av neutrofiler och monocyter, vilka snabbt är på plats vid infektionshärden och bekämpar infektionen genom att bokstavligt talat äta upp de invaderande mikroorganismerna. De utsöndrar också en mängd olika inflammatoriska substanser som i sin tur aktiverar det adaptiva immunförsvaret, bestående av B och T lymfocyter. Aktivering av det adaptiva immunförsvaret leder bl.a. till produktion av antikroppar riktade mot den specifika mikroben. Ironiskt nog kan de substanser som immunförsvaret utsöndrar för att oskadliggöra mikroorganismer även åsamka skador på kroppens egna vävnader. I allvarliga fall kan en bakterieinfektion orsaka en överaktivering av immunförsvaret, som i sin tur leder till multiorgansvikt, chock och i värsta fall döden.

Streptococcus pyogenes är en vanligt förekommande bakterie som i första hand orsakar banala infektioner som halsfluss, svinkoppor och rosfeber. I sällsynta fall kan dock bakterierna bli mycket aggressiva och sprida sig i muskelvävnad och till blodomloppet. Dessa tillstånd kännetecknas av mycket snabba förlopp och hög dödlighet, men den bakomliggande orsaken är fortfarande okänd. Den här avhandlingen studerar hur strukturer på streptokockens yta inverkar på det mänskliga immunförsvaret. I delarbete I-III undersöker vi effekterna av streptokockens M1 protein, ett protein som sedan länge är känt för att skydda streptokocken från att avdödas av det mänskliga immunförsvaret. Tidigare var det också visat att M1 protein får neutrofiler att utsöndra HBP, en viktig inflammatorisk substans. I delarbete I finner vi att M1 protein har förmåga att aktivera monocyter så att dessa frisätter inflammatoriska cytokiner, och i närvaro av HBP förstärks monocytaktiveringen ytterligare. I delarbete II ser vi att M1 protein även får monocyter att visa upp proteinet tissue factor (TF) på sin yta. TF aktiverar koagulationskaskaden och får blodet att levra sig snabbare. Vid allvarliga streptokockinfektioner ses ofta en påverkan på blodets koagulationsförmåga, och resultaten från delarbete II antyder att M1 protein bidrar till dessa symptom. I delarbete III studerar vi slutligen effekten av M1 protein på T lymfocyter, och finner att streptokockproteinet åstadkommer en

massiv aktivering av dessa celler. Ytterligare försök tyder på att M1 protein fungerar som ett s.k. superantigen, vilka kännetecknas av sin förmåga att kortsluta immunförsvaret. Istället för att aktivera de fåtal T celler som känner igen och kan oskadliggöra bakterien, får man en retning av ett stort antal ospecifika T celler. Detta leder till en kraftig retning av immunsystemet, men svaret är dysfunktionellt och inte riktat mot streptokocken.

För att lättare kunna infektera människor har streptokocken förmåga att använda värdens egna system. Genom att ansamla olika mänskliga proteiner på sin yta kan bakterien antingen skydda sig från immunförsvaret eller underlätta spridning i vävnaden. I delarbete IV finner vi att *S. pyogenes* binder det mänskliga plasmaproteinet TAFI till sin yta. TAFI är ett enzym som i första hand hindrar blodkoagel från att lösas upp, men det kan också påverka immunsvaret genom att klippa sönder olika inflammatoriska substanser. TAFI tillverkas dock som ett inaktivt s.k. pro-enzym som först måste aktiveras för att kunna utföra sina effekter. Intressant nog finner vi att två aktivatorer, trombin och plasmin, kan ansamlas på streptokockytan, där de har förmåga att aktivera bakterie-bundet TAFI. Vi spekulerar i att detta kan ha betydelse för att skydda bakterien mot immunförsvaret genom att kapsla in bakterien i ett skyddande blodkoagel och genom att dämpa det inflammatoriska svaret vid infektionshärden.

Sammantaget demonstrerar avhandlingen olika sätt som streptokocker utnyttjar för att påverka vårt immunförsvar. Fynden kan hjälpa till att förklara varför *S. pyogenes* är en så pass vanlig och framgångsrik sjukdomsframkallande bakterie hos människor.

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