The streptococcal IgG degrading enzyme IdeS
- studies on host-pathogen interactions

Jenny Johansson Söderberg
Vi er alle oppdagelsesreisende i livet, hvilken vei vi så enn følger.

Fridtjof Nansen
ABSTRACT

The important human pathogen *Streptococcus pyogenes* causes both mild infections such as pharyngitis and impetigo but also severe life threatening invasive infections. Specific antibodies (IgG) recognize pathogens and are important mediators for pathogen clearance by the immune defence. *S. pyogenes* expresses a highly effective and specific IgG endopeptidase called IdeS (immunoglobulin degrading enzyme of *S. pyogenes*). IdeS rescues bacteria from opsonising IgG by cleavage of IgG generating two fragments F(ab')2 and ½Fc. Moreover, IdeS block ROS production by neutrophils. In this thesis I have studied (i) allelic variants of IdeS and their biological potential, (ii) consequences of ½Fc production for host-pathogen interactions and (iii) IdeS processing by streptococcal and neutrophil proteases.

When investigating the allelic variants of IdeS we could show that in respect to IgG degradation and inhibition of ROS production the allelic variants where indistinguishable, however the allelic variant of serotype M28 appears to be an unique exception as this protein was deficient in IgG cleavage but still inhibited ROS production. Further, the ½Fc fragments produced when IgG is cleaved by IdeS were shown to prime human neutrophils and under ex vivo experimental conditions this increased the bactericidal activity of the neutrophils. Finally, we made the interesting finding that IdeS is N-terminally processed by neutrophil proteases and by the streptococcal protease SpeB, but retain enzymatic activity and was less immunogenic compared to the full length protein.
MANUSCRIPTS AND ORIGINAL PUBLICATIONS


<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARF</td>
<td>Acute rheumatic fever</td>
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<tr>
<td>CR</td>
<td>Complement receptor</td>
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<tr>
<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay</td>
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<tr>
<td>EndoS</td>
<td>Endoglycosidase of streptococci</td>
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<tr>
<td>FcR</td>
<td>Fc-receptor</td>
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<tr>
<td>fMLP</td>
<td>N-Formyl- L -methionyl- L -leucyl- L -phenylalanine</td>
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<tr>
<td>GAS</td>
<td>Group A streptococcus</td>
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<tr>
<td>GST</td>
<td>Glutathione S-transferase</td>
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<td>IdeS</td>
<td>Immunoglobulin G-degrading enzyme of S.pyogenes</td>
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<tr>
<td>Ig</td>
<td>Immunoglobulin</td>
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<tr>
<td>LPS</td>
<td>Lipopolysaccharide</td>
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<tr>
<td>Mga</td>
<td>Multiple gene regulator</td>
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<td>MMPs</td>
<td>Metalloproteinases</td>
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<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
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<tr>
<td>PMA</td>
<td>Phorbol 12-myristate 13-acetate</td>
</tr>
<tr>
<td>PMN</td>
<td>Polymorphonuclear leukocyte</td>
</tr>
<tr>
<td>PRRs</td>
<td>Pattern recognition receptors</td>
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<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
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<tr>
<td>SIC</td>
<td>Streptococcal inhibitor of complement</td>
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<td>SKA</td>
<td>Streptokinase</td>
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<tr>
<td>SLO</td>
<td>Streptolysin O</td>
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<tr>
<td>SpeB</td>
<td>Streptococcal exotoxin B</td>
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<td>STSS</td>
<td>Streptococcal toxic shock syndrome</td>
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<tr>
<td>TCR</td>
<td>Two component regulatory system</td>
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**Aims of the thesis**

The aim of this thesis is elucidate how the IgG cleaving enzyme IdeS influences the interaction between *S. pyogenes* and the human host.

**Specific aims**

- Compare allelic variants of IdeS regarding enzymatic activity and biological functions.
- Examine the biological effects of ½Fc fragments generated by IdeS mediated proteolysis of IgG.
- Assess IdeS in the proteolytic environment of infection.
Introduction

Infection biology and host-pathogen interactions
The word “infection” comes from the Latin word *infiere* with the meaning to dye, stain, or to poison (Lindskog, 1997). Today the word is commonly used to describe disease caused by microorganisms. At start, the studies of infection biology was only directed towards the microorganism but it was soon clear that infection did not only depend upon the microorganism but also dependent upon the host. Today it is well know that some bacterial species will cause disease in some humans, but not in all humans. A microorganism that has the ability to colonize and cause disease can be referred to as a pathogen and the human (or another organism) that is colonized is referred to as host. If the colonization will cause disease depends upon the interaction between the pathogen and the host. The disease causing factors of the pathogen are often called virulence factors, and can easily be described as the pathogens armour. The host on the other side has a defence against pathogens and their virulence factors, called the immune system, or immune defence. However, the damage caused by infection, might be caused by the humans own immune defence as well as by the pathogen. Host-pathogen interactions are deeply intriguing and immensely important in both health and disease.

Human immune defence
The human body is equipped with a defence against invading pathogens, called the immune system. The human immune system can be dived into adaptive immunity and innate immunity. Adaptive immunity covers the humoral immune defence with specific antibodies that recognise pathogens and in which T- and B-cells are important cells. Adapted immunity provides “memory” so that the immune system, by immunoglobulins, can rapidly recognize and kill invading microorganisms. Innate immunity includes cells and effector molecules that are encoded by germlines genes. The core of innate immunity are phagocytic cells (neutrophils and macrophages), antimicrobial peptides and cell surface receptors that recognize common features of microorganisms. The innate and the adaptive immune systems are not working as two separate units but are linked together by several pathways (Chaplin, 2010).

Immunoglobulins
Immunoglobulins (Ig) are a central player in the adaptive immune defence by mediating the clearance of invading pathogens. Immunoglobulin, also called antibody, recognises epitopes on pathogens and then mediates signals to other parts of the immune system. The immunoglobulins are produced by
differentiated B-cells called plasma cells. The circulating B-cell has the intrinsic ability to become a producer of antibodies of a certain specificity, if the circulating B-cell encounters the right antigen a process starts that ends either with a plasma cell that produce highly specific antibodies, or with a memory B-cell. There are several different classes of immunoglobulins with differences in structure and main functions. Immunoglobulin G (IgG) is the immunoglobulin found with the highest level in plasma, the average concentration of IgG is about 10 mg/ml. IgG can both fix complement and interact with immune cells by the Fc-receptors (these properties varies between different subclasses of IgG). (Janeway, 2005)

The IgG molecule is built of four protein chains, two heavy chains and two light chains. The different protein chains are connected by disulphide bonds. The variable regions recognize antigens and the constant regions mediate signals to immune cells. By using proteases the IgG molecule can be digested to stable fragments, fab (Fraction Antigen Binding) and fc (Fraction crystallisable), where the fab fragment consists of the variable regions and the fc fragment consists of the constant regions. Commonly used proteases to produce stable fragments are papain that produces two Fab

![Figure 1. Schematic presentation of Immunoglobulin G. V, variable region; C, constant region; L, light chain; H, heavy chain. CH2 domains are connected by carbohydrates. The amino acid sequence of the hinge region with indicated cleavage sites of selected IgG degrading enzymes is depicted at the bottom. HNE (human neutrophil elastase), MMP, (metaloproteases). To the left are designations of the stable fragments generated by proteolytic cleavage.](image)
fragments and one Fc fragment, pepsin is used two produce one F(\text{ab}')_2
fragment, however there is no \frac{1}{2}Fc fragment produced as pepsin also
degrades the heavy chain (see figure 1).

Ig degrading proteases
Immunoglobulins are important in the defence against microorganisms, but to
counteract the functions of IgG microorganisms have evolved proteins with
the ability to degrade and inactivate immunoglobulins. IgG proteases from
pathogenic microorganisms have been reported including \textit{S.pyogenes} (Collin
\& Olsen, 2001a, von Pawel-Rammingen et al., 2002), \textit{Pseudomonas
aeruginosa} (Holder \& Wheeler, 1984), \textit{Staphylococcus aureus} (Prokesova et
al., 1992), \textit{Prevotella intermedia} and \textit{Prevotella nigrescens} (Jansen et al.,
1995), \textit{Pasteurella multocida} (Pouedras et al., 1992), \textit{Candida albicans}
(Kaminishi et al., 1995), \textit{Trichomonas vaginalis} (Min et al., 1998), \textit{Paragonimus
westermani} (Shin et al., 2001) and \textit{Proteus mirabilis} (Loomes et al., 1993) Also human proteases such as matrix metalloproteases can
cleave human IgG (Brezski \& Jordan, 2010).

Neutrophils
Neutrophils (polymorphonuclear leucocytes) are commonly referred to as
the “first line of defence” against microorganisms and are a part of the innate
immune defence. Neutrophils are produced in the bone marrow from
hematopoietic stem cells and are then released to the blood stream where
they circulate for a few hours (half-life of 6-8h) (Kobayashi et al., 2005).
Upon infection the neutrophils leave the blood stream by transendothelial
migration into the surrounding tissue where the neutrophil chemotax by
following gradients of different compounds, such as the bacterial peptide
fMLP or compounds produced by the immune system like C5a and IL-8.
After encountering the microorganism the neutrophils kill the
microorganisms by phagocytosis, release of antimicrobial peptides and the
formation and release of reactive oxygen species (Borregaard, 2010).

Phagocytosis
The initial step in phagocytosis is recognition and interaction with the
microorganism. The recognition can be direct or indirect. Direct recognition
is mediated by pattern recognition receptors (PRRs) on the neutrophil that
recognize carbohydrates, peptidoglycans or lipoprotein of microbial origin.
Indirect recognition is mediated by opsonins (immunoglobulins and
components of the complement system) that bind to pathogen surfaces. By
binding to the pathogen surface the opsonin change conformation and is
recognized by neutrophil receptors (Fcγ receptors (FcγRs) and complement
receptor 3). Interaction between the pathogen and neutrophil receptors leads
to intra cellular signalling downstream of the receptors and results in
engulfment of the pathogen. Following the engulfment of the bacteria the phagosome mature stepwise where the phagosome fuses with subcompartments of the endocytic pathway, resulting in a mature phagolysosome and microbial killing (Flannagan et al., 2009).

**Neutrophil granules**
Neutrophils contain granules in which important receptors and secreted proteins are stored. The proteins can be stored inside the granules as well as in the granule membranes. Granule proteins and receptors includes endothelial adhesion molecules, components of the NADPH oxidase, membrane bound receptors, antimicrobial proteins and proteases. The granules are divided into four sub-groups: azurophil granules, specific granules, gelatinase granules and secretory vesicles. The granules are formed during maturation of the neutrophil. When the neutrophil is activated the granules are released in a controlled manner (Faurschou & Borregaard, 2003).

**ROS production and priming**
Besides the antimicrobial effect of proteins and enzymes the generation of reactive oxygen species (ROS) are vital for effective killing of microorganisms by neutrophils. The generation of ROS is commonly referred to as oxidative burst. The ROS are produced by the NADPH complex. The importance of ROS production is demonstrated by the fact that patients with mutations in the NADPH complex have reduced killing of microorganisms. The generation of ROS is activated by phagocytosis but

<table>
<thead>
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<th>Priming</th>
<th>Activation</th>
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<tr>
<td><strong>Cytokines</strong></td>
<td></td>
<td></td>
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<tr>
<td>TNF-alpha</td>
<td>+++</td>
<td>+?</td>
</tr>
<tr>
<td>IL-8</td>
<td>++</td>
<td>+?</td>
</tr>
<tr>
<td><strong>TLR agonists</strong></td>
<td></td>
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<tr>
<td>LPS</td>
<td>+++</td>
<td>-?</td>
</tr>
<tr>
<td>Zymosan</td>
<td>++</td>
<td>+?</td>
</tr>
<tr>
<td><strong>Chemoattractants</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>fMLP</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>C5a</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td><strong>Chemical agents</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PMA</td>
<td>+</td>
<td>+++</td>
</tr>
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</table>

Table 1. Selected activating and priming agents for neutrophils. (?) indicates that there are conflicting data in the literature. Table adopted from El-Benna et al 2008.
The NADPH oxidase is built up by several subunits present in the cell membrane (or in the membrane of specific granules and secretory vesicles) and in the cytoplasm. Upon activation the cytosolic subunits gets phosphorylated and translocates to the cell membrane where they interact with the membrane bound subunits. The fully assembled NADPH oxidase then reduces cytosolic NADPH to NADP generating two electrons that cross the membrane and then generate $O_2^-$ in the phagolysosome (or extra cellularly) (El-Benna et al., 2008, Sheppard et al., 2005).

The NADPH oxidase can be as described above, in a resting state with the membrane and cytosolic components separated, or in an active form with all of the components in one complex in the membrane. However, there is also a stage of “priming”. Priming of the neutrophil results in a more powerful ROS production upon activation. Along to the factors that activate the neutrophil there are also factors that only prime the neutrophil, however, some molecules can both prime and activate the neutrophil. The effect on the NADPH oxidase in priming might differ between different priming agents but some proposed mechanisms of priming includes phosphorylation of subunits, or translocation of subunits to the membrane (El-Benna et al., 2008, Sheppard et al., 2005). A number of priming agents are included in table 1.
**Streptococcus pyogenes**

*Streptococcus pyogenes*, also known as Group A Streptococcus (GAS), is a gram positive bacterial pathogen that causes a wide variety of diseases ranging from mild infections to severe life threatening infections. Mild infections caused by *S. pyogenes* include pharyngitis and impetigo, and severe diseases include sepsis and necrotizing fasciitis.

**Groups and serotypes**

*S. pyogenes* belongs to a group of streptococci that is beta-hemolytic on bloodagar plates. In 1933 Rebecca Lancefield differentiated this group further depending on carbohydrates on the bacterial surface (Lancefield, 1933). This classification is referred to as Lancefield group antigens. *S. pyogenes* only carries the group A antigen (hence, *S. pyogenes* is also referred to as Group A streptococcus (GAS)), however, other streptococcal species may carry several (or none) different group specific carbohydrates (Facklam, 2002). *S. pyogenes* (Group A) is a strict human pathogen, but several of the other streptococci can infect animals as well as humans.

There are many different serotypes of *S. pyogenes*, so called M-types (after the surface exposed M protein) also known as *emm*-types (after the *emm*-gene encoding the M protein), currently there are more than 200 known *emm*-types (Steer et al., 2009). Classically the M-types was classified by the M protein reacting with specific antisera (Todd & Lancefield, 1928). Also the surface exposed T-antigen is used to classify streptococcus.

**Clinical manifestations and epidemiology**

*S. pyogenes* is a strict human pathogen and is able to colonize several sites in the human body including skin and mucous cutaneous surfaces, this is reflected by the wide range of infections caused by this bacterium. *S. pyogenes* mostly causes mild infections such as pharyngitis and impetigo, but can also cause severe infections such as cellulites, bacteraemia, necrotizing fasciitis and streptococcal toxic shock syndrome (STSS). Severe infections caused by *S. pyogenes* are associated with a heavy inflammatory response from the immune system induced by the bacterial virulence factors. *S. pyogenes* also causes post-infectious sequelae such as rheumatic heart disease and glomeronephritis (Cunningham, 2000).

Infections conform to *S. pyogenes* has been described since Hippocrates (400 B.C) (Olsen & Musser, 2010). During the 19th and early 20th century invasive infections caused by *S. pyogenes* were common, but then the invasive diseases caused by *S. pyogenes* almost vanished. Streptococcal infections were even mostly regarded as paediatric infections causing pharyngitis and impetigo. But in the mid 1980th there were several cases of acute rheumatic fever. Shortly after several cases of STSS was reported (Kaplan, 1991, Efstratiou, 2000, Salyers & Whitt, 2002).
Today there are an estimated number of 616 million new cases of pharyngitis annually resulting in considerable economic consequences. There are 1.78 million new cases of severe streptococcal disease with at fatality rate of 15-25% (Carapetis et al., 2005). In Sweden invasive streptococcal diseases has to be reported to the Swedish Institute for Communicable Disease Control (Smittskyddsinstitutet) and in 2011 4.24 cases per 100,000 citizens was reported. (http://www.smi.se/statistik/betahemolyserande-streptokocker-grupp-a-gas/)

Different serotypes of *S. pyogenes* are epidemiologic distinct from each other. In a clinical study (Strep-EURO) performed during 2003 and 2004 in ten European countries the most common isolates causing severe disease were *emm* 1, *emm* 28, *emm* 3, *emm* 89 and *emm* 87 (Luca-Harari et al., 2009). As well as that the different serotypes are diverse regarding the disease severity, there are also regional differences in the spread of the different serotypes (Steer et al., 2009). *S. pyogenes* remains sensitive to penicillin, but resistance to macrolides and also tetracycline resistance is emerging. This is a problem when treating people hypersensitivity to penicillin (Bessen, 2009). Today there is no vaccine available.

**The genome and gene regulation**

The circular genome of *S. pyogenes* is about 1.9Mb in size. Approximately 10% of the genome consists of variable genetic elements such as integrated conjugative elements and prophages and even within the same M-type there might be considerable differences in gene content (Musser & Shelburne, 2009). Regulatory systems important for virulence include both two-component regulatory systems (TCR) and stand-alone regulators. The expression of M protein is regulated by the stand-alone regulator Mga. Mga also regulates a big set of other genes many of importance for metabolism (Hondorp & McIver, 2007).

A regulatory system with great importance for virulence is the CovRS two-component regulatory system (Cole et al., 2011) CovS is responsible for sensing environmental factors and controls the phosphorylation stage of CovR, that is the transcription regulator (Cole et al., 2011). The two-component regulator CovRS is a negative regulator of virulence factors. The CovRS TCR regulates by direct or indirect mechanism about 10% of the genes in the streptococcal genome, including several genes of important virulence factors (Sumby et al., 2006). Strains with a deficient CovRS have been shown to have an increased ability of causing systemic infections (Sumby et al., 2006, Walker et al., 2007). Mutations in the CovRS occur spontaneously during infection. The increased ability of Cov mutants to cause invasive disease is a consequence of both an increased production of virulence factors and a decreased production of SpeB. SpeB does not only degrade host proteins but also streptococcal proteins including virulence
The less production of SpeB will then also result in less degradation of other virulence factors (Aziz et al., 2004, Kansal et al., 2000, Raeder et al., 1998). However, the Cov mutants have reduced ability to initializing colonization, explaining why the Cov mutants not have outnumbered the wild type strains (Hollands et al., 2010, Cole et al., 2011).

### Virulence mechanisms of S. pyogenes

The successful human pathogen *S. pyogenes* has a broad repertoire of virulence factors that aid bacterial evasion of the human immune system. One unusual feature of *S. pyogenes* is the serum resistance that enable the bacterium to survive and multiply in human blood. This feature is due to the capsule and the M protein. The capsule of *S. pyogenes* consists of hyaluronic acid polymer containing repeats of glucuronic acid and N-acetylglucosamine. The capsule has been shown to be required for resistance to phagocytosis (Wessels et al., 1991). Some of *S. pyogenes* broad repertoire of virulence factors is included in table 2.

<table>
<thead>
<tr>
<th>Virulence factors</th>
<th>Description</th>
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<tbody>
<tr>
<td>M protein and M-like proteins</td>
<td>Bind plasma proteins</td>
</tr>
<tr>
<td>SpeB</td>
<td>Degrades host- and bacterial proteins</td>
</tr>
<tr>
<td>IdeS</td>
<td>Cleaves IgG</td>
</tr>
<tr>
<td>EndoS</td>
<td>Deglycosylates IgG</td>
</tr>
<tr>
<td>Streptokinase</td>
<td>Activates plasminogen to plasmin and dissolves fibrin clots</td>
</tr>
<tr>
<td>SLO</td>
<td>Lysis of erythrocytes</td>
</tr>
<tr>
<td>SLS</td>
<td>Lysis of erythrocytes</td>
</tr>
<tr>
<td>SpeA</td>
<td>Superantigen mediates unspecific activation of T cells</td>
</tr>
<tr>
<td>C5a pepidase</td>
<td>Degrades C5a</td>
</tr>
<tr>
<td>Sda1</td>
<td>DNase degrades nucleaci acid found in NETs</td>
</tr>
<tr>
<td>SIC</td>
<td>Inhibits complement and degrades antimicrobial peptides</td>
</tr>
<tr>
<td>Hyaluronic acid capsule</td>
<td>Resistance to phagocytosis</td>
</tr>
<tr>
<td>SsvCEP</td>
<td>Degrades IL-8</td>
</tr>
</tbody>
</table>

Table 2. Short summary of a subset of the virulence factors produced by *S. pyogenes*. Adopted from Cole et al 2011.
protein is encoded by the \textit{emm} gene, denoting the different \textit{emm} types that correlate with the M type (Cunningham, 2000).

The N-terminal is highly variable and is the part of the M protein that gives serum specificity (different M types) the carboxy-terminal is conserved and located within the cell wall and contains the anchoring motif LPXTG. The M protein contains regions with different number of repeats where the number of repeats in the A and B region leads to different sizes of M proteins in different strains of \textit{S. pyogenes} (Fischetti, 1989) (also see figure 2). The M protein has high degree of variation and not only the size of the M protein can differ, but also the ability to bind different plasma proteins.

The antiphagocytic effect of M protein is due to its ability to interfere with the complement cascade. The ability to bind one or several of the complement factors; factor H, factor H-like1, C4-binding protein and CD46 complement regulatory protein are a shared feature between many M proteins. Further, M protein can be able to bind fibrinogen, there by decrease deposition of the opsonin C3b on the bacterial surface (Oehmcke et al., 2010). In 2006 it was reported that soluble M protein by binding fibrinogen activated neutrophils to release Heparin binding protein (HBP) that induce

Figure 2. The cell wall anchored and surface exposed M protein consists of regions with different numbers of repeats.
vascular leakage and explains the leakage of plasma proteins into tissues during streptococcal toxic shock syndrome (Herwald et al., 2004).

In addition to the M protein *S. pyogenes* also express M-like proteins that are structurally related to the M protein. M-like proteins bind plasma proteins such as albumin, factor H, fibrinogen, plasminogen, IgG and IgA (Cunningham, 2000). In 1973 it was shown that *S. pyogenes* bind IgG in a non-immune fashion by binding the Fc portion of the IgG molecule (Kronvall, 1973). Since then IgG binding has been shown to be accomplished by different proteins. Protein H (Gomi 1990) found in a M1 strain accounts for the majority of that strains ability to bind IgG (Kihlberg et al., 1999).

The ability to bind immunoglobulins is not exclusive to *S. pyogenes*. Group C and G streptococci express protein G and *Staphylococcus aureus* express protein A, *Finegoldia magna* express protein L (that bind to the Fab-region and not to the Fc-region). There are also reports of Ig binding proteins in gram-negative bacteria (Sidorin & Solov’eva, 2011).

**IdeS**

The first description of IdeS was done in 2000, when the protein was identified as protein spot number 22 in a D-2 gel analyzing culture supernatants of streptococci (Lei et al., 2000). The protein in spot 22 was expressed by the two strains tested (one M1 and one M3 serotype) and patient sera from pharyngitis, acute rheumatic fever and STSS all recognized the protein. The same group continued to characterise the protein and denoted the protein Streptococcal Mac-1 as the protein showed a low homology to human Mac-1. They also described binding of the protein to neutrophils and inhibition of phagocytosis (Lei et al., 2001).

Independently, another group described the finding of a novel IgG cleaving enzyme of streptococci that they denoted “Immunoglobulin degrading enzyme of *S. pyogenes*” (IdeS). They also showed that degradation of specific antibodies aided the survival of *S. pyogenes* in human immune blood (von Pawel-Rammingen et al., 2002).

Further, at the same time a group described a protein with IgG binding properties that they called Sib35 (Kawabata et al., 2002). Interestingly, the proteins are identical and today the enzyme is most often referred to as IdeS due to the IgG cleaving activity.

IdeS has a molecular weight of 34 kDa and is expressed in its active form. IdeS is a secreted protein and the expression is controlled by the two component regulatory system CovRS (Lei et al., 2001). As of today, the only known substrate of IdeS is IgG. A list of IgG cleaving activity is presented in table 3. IdeS hydrolyzses IgG in a site located in the hinge region. Full hydrolysis of IgG results in one F(ab’)_2 fragment connected by disulphide bonds, and one ½Fc fragment. The ½Fc fragment is stable in its dimeric
form by the CH2 domains that interacts weakly via the carbohydrate and the
two CH3 domains that interacts more strongly with each other (Deisenhofer,

Several groups have reported the presence of specific antibodies towards
IdeS in sera from patients with streptococcal infections, implying that IdeS is
expressed in vivo during infection (Åkesson et al., 2006, Su et al., 2011, Lei
et al., 2000).

By comparing DNA sequences of *ides-*genes in different strains allelic
variants were found, and these allelic variants could be grouped into two
complexes, complex I and complex II. The two complexes has an average
difference of one-seventh of the 340 amino acid sites, with the most of the
differences in the middle one-third of the proteins. It has been suggested that
the differences between the complexes is too large to be the result of
accumulation of neutral nucleotide substitutions, but likely to be the results
of recombinations. Interestingly it was shown that complex II is more similar
to the IdeS homologue present in *S.equi* than complex I is, hence it was
concluded that complex I has evolved from complex II (Lei et al., 2002).

**IdeS homologues present in group C streptococcus.**
The gene homologue for IdeS was found in the genome of *Streptococcus
equi* ssp. *equi* (Lei et al., 2002) and the protein homologues have now been
described. The protein homologue of *S.equi* ssp. *equi* was denoted IgG-
degrading enzyme of *S.equi* ssp. *equi* (IdeE) and the protein from the closely
related *Streptococcus equi* ssp. *zooepidemicus* was denoted IdeZ. The

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Activity</th>
<th>Reference</th>
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<tbody>
<tr>
<td>IgG1 human</td>
<td>+++</td>
<td>Agniswamy 2004</td>
</tr>
<tr>
<td>IgG2 human</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>IgG3 human</td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td>IgG4 human</td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td>IgG monkey</td>
<td>yes</td>
<td>Agniswamy 2004</td>
</tr>
<tr>
<td>IgG pig</td>
<td>no</td>
<td></td>
</tr>
<tr>
<td>IgG rabbit</td>
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<td></td>
</tr>
<tr>
<td>IgG sheep</td>
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</tr>
<tr>
<td>IgG1 mouse</td>
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<td>Nandakumar 2007</td>
</tr>
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<tr>
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<td>no</td>
<td></td>
</tr>
<tr>
<td>IgG3 mouse</td>
<td>yes</td>
<td></td>
</tr>
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</table>

*Table 3. IdeS activity Data summarized from (Agniswamy et al., 2004)
and (Nandakumar et al., 2007). IdeS activity against human IgG subclasses is not
constant as indicated by plus symbols. * Regarding IdeS activity against sheep IgG,
no cleavage was detected in the publication of Agniswamy, but the
commercial available IdeS protein variant Fabricator® degrades Sheep IgG
(www.genovis.com)*
recombinant proteins did cleave both human IgG and horse IgG, however quite weakly, and horse IgG to a lesser extent than human IgG (Lannergard & Guss, 2006). Later two other homologues IdeE2 and IdeZ2 were found in *S. equi* ssp. *equi* and *S. equi* ssp. *zooepidemicus* respectively. Compared to IdeE and IdeZ, the two novel proteins exhibited higher cleavage activity towards horse IgG (Hulting et al., 2009).

*SpeB*

The streptococcal pyrotoxin SpeB is a long know important virulence factor of *S. pyogenes* and has been extensively investigated. In 1945 Elliot published the first report about SpeB. During that time it was impossible to determine the M type of some streptococcal strains, and a study was undertaken with these untypable strains. It was shown that these strains expressed an enzyme that degraded M protein. Further, it was shown that this enzyme degraded several other proteins including casein, milk and gelatin. Interestingly, Elliot also showed that strains producing the enzyme when passaged in mice lost the ability to produce the enzyme and gained the M protein, this also increased the virulence of the strains (Elliott, 1945).

SpeB is expressed as zymogen of 40 kDa and is processed by autocatalysis in reducing environment to its active form of 28 kDa (Elliott, 1945, Liu & Elliott, 1965). In addition to degradation of host proteins SpeB also degrades several streptococcal proteins including know virulence factors such as Streptolysin O, Streptokinase, M1 protein etc. (Aziz et al., 2004, Nelson et al., 2011). Interestingly, SpeB also degrades IgG (Collin & Olsen, 2001b), at the same site as IdeS (Collin & Olsen, 2001a, von Pawel-Rammingen et al., 2002). As reviewed by Carroll and Musser the regulation of SpeB expression is complicated, but the regulators include CovRS and Mga, as well as many other regulators (Carroll & Musser, 2011).
Methods

In the original publications and manuscripts complete Material and methods sections are included. Here I make some comments and further explanations of a subset of the used methods.

IgG cleaving assay
IgG cleavage by IdeS is easily detected on a SDS-PAGE protein gel. Visible on the gel is the IgG cleavage product as an additional band situated just above the light chain. To be able to quantify the IgG cleaving activity IgG was labelled with radioactive iodine ($^{125}$I). $^{125}$I-IgG and IdeS was incubated and the reaction was then run on a denaturing and reducing protein gel. The different protein bands were excised from the gel and the radioactivity of the single bands were determined. The percentage of the cleavage was calculated. IgG viewed on protein gel is included in figure 3.

Detection of ROS production
In two of the manuscripts we have employed an assay that detects reactive oxygen species in whole blood. Purification of neutrophils affects the cells in various ways (Haslett et al., 1985, Freitas et al., 2008). By using the whole blood assay purification of neutrophils in not needed and thus, the neutrophils are in a more native stage. We used ABEL® cell activation kit (Knight Scientific Ltd., United Kingdom). The kit includes a photoprotein, Pholasin® that originates from a mollusc. The protein starts to chemiluminescence when it encounters singlet oxygen, hydroxyl radicals and superoxide anion. The chemiluminescence was recorded in a luminometer.

Figure 3. Reducing SDS-PAGE protein gel showing IgG and IdeS cleavage products. H indicates IgG heavy chain, L light chain. * is the IgG cleavage product. When all IgG is proteolysed no heavy chain is present.
**S. pyogenes survival assays**

To examine the survival of *S. pyogenes* we employed a variant of Lancefield classical bactericidal assay (Lancefield, 1957). Important to remember is that *S. pyogenes* not only survivals in human blood, but also multiply. Thus, most of the experiments were carried out in immune blood. Either the blood donor had opsonising antibodies or we supplied the blood with specific anti-bodies raised in rabbit. All the blood was donated by healthy volunteers.

The survival assay was performed as follows; *S. pyogenes* in exponential growth phase was collected, centrifuged and washed and appropriated diluted. To 900µl of fresh blood 100µl of diluted *S. pyogenes* was added. The blood was incubated at 37°C with end-over-end rotation for 30 minutes and samples were plated on agar plates. Agar plates were incubated over night in 37°C 5%CO₂. The following day colonies were counted and colony forming units were determined.

**Purification of ½Fc fragments**

½Fc fragments were purified by expressing GST-taged IdeS in *E.coli* and the GST-IdeS were purified on Glutathione Sepharose. To the pure GST-IdeS still bound to the sepharose polyclonal IgG was added, and incubated until all IgG was totally hydrolyzed. The IgG was drained from the column and further purified on protein A sepharose that specifically binds to the Fc part of the IgG. Finally the ½Fc fragments were put on polymyxine sepharose to remove any LPS that might have contaminated the final ½Fc preparation.

**Binding of IgG to bacterial surface**

To study the binding of IgG and ½Fc fragments to the bacterial surface we used radiolabeled protein. To avoid unwanted expression of proteases we used killed *S. pyogenes*. The bacteria were incubated, washed and then was the amount of ¹²⁵I measured. Initial binding of radiolabeled IgG was challenged with 10 mg/ml unlabeled IgG. 10 mg/ml is about the IgG concentration in human blood.
Results and discussion

IdeS allelic variants.

-Comparison of biological functions in respect of IgG cleavage and inhibition of ROS production.

Paper I

Two allelic variants of IdeS, complex I and complex II, has been described (Lei et al., 2002). Complex II differ from complex I in the middle one-third of the protein. One of Lei and co-workers main findings was that the complex II had deficient IgG endopeptidase activity. They argued that this was because autophosphorylation of an additional cysteine (in amino acid position 257) in complex II. They supported their statement by carrying out analysis with reduced proteins and by demonstrating that the cysteine C94 (in the active site of the protein) was important for IgG cleaving activity. Their conclusion was that the complex II protein was deficient of IgG endopeptidase activity due to the additional cysteine. Further, they also showed that complex II has lower binding to human integrins and does not block ROS production or inhibits killing by human neutrophils, differences that they ascribe the differences in the middle one third of the protein. However, they only studied complex II from serotype M28 in detail. In a later study by Åkesson and co-workers (Åkesson et al., 2006) clinical isolates was studied and of the strains included in that study only complex II from the M28 serotype carried the additional cysteine in the position 275. They also suggested that the impairment of IgG cleaving activity was due to formation of a disulphide-bond between the C94 in the active site and the additional C275. In Paper I we studied the importance of the cysteine at position 275, together with examining the ability of complex I and complex II proteins to interfere with human antimicrobial mechanisms.

Consequences of cysteine at position 275 for IgG cleaving activity

IgG cleaving activity was assessed in six complex II expressing strains. Only complex II from M28 serotype strains were deficient in IgG endopeptidase activity. For further studies and site-directed mutagenesis an inactive complex II from a M28 serotype strain and an enzymatically active complex II from M8 serotype strain were chosen. To begin with the amino acid at position 275 was exchanged, in the M28 a cysteine was exchanged to tyrosine, and respectively in the M8 the tyrosine was exchanged to a cysteine. The proteins with the tyrosine exhibited IgG cleaving activity whereas the proteins with cysteine only had minor IgG endopeptidase activity. This results supports the suggestion that the lack of enzymatic
activity most likely is due to a disulphide bond formation blocking the active site.

**Analysis of ROS production and survival in immune blood**
The ability to interfere with human antimicrobial mechanisms was further studied. The complex I and II (wild-type and mutated) recombinant proteins were studied regarding (i) ability to block ROS production and (ii) promote bacterial survival in immune blood. The different proteins were equally good in inhibiting ROS production in human whole blood, clearly showing that the ability to block ROS production is independent of the enzymatic activity. In the whole blood assay we were not able to detect that complex II was deprived in the ability of blocking ROS production compared the complex I, as shown before in an assay using purified neutrophils (Lei et al., 2002). This divergence might be explained by the different assays that were employed.

However, regarding promoting bacterial survival in immune blood a clear difference between the enzymatically active and the inactive proteins was shown. Only the enzymatic active proteins promoted the bacterial survival. The survival of bacteria incubated with inactive protein was not increased compared to the PBS control. This also suggest that even thou this protein inhibits ROS production this is not sufficient to promote bacterial survival in immune blood.

**Enzymatic activity in IdeS homolouges of S. equi**
IdeS homologues found in *S. equi* sspp. are more similar to complex II than complex I, however these homologues do not cleave horse IgG. One group showed that IdeE reduced the killing of *S. equi* ssp. *equi* mediated by binding to equine neutrophil surface (suggested by binding Fc receptor), however the experimental execution was somewhat unclear in respect to the cleaving activity towards the rabbit antibody used to opsonise the *S. equi* bacteria (Timoney et al., 2008). In a later publication it was shown that IdeE cleaves rabbit IgG (Hulting et al., 2009). Another publication showed that IdeE does not protect *S. equi* ssp. *equi* from being phagocytosed by horse neutrophils, when opsonised with horse IgG, but that IdeE rescued the bacteria when opsonised with human IgG, because IdeE cleaves human IgG more efficiently than horse IgG (Liu & Lei, 2010). These findings are also in concordance with our findings, that it is the enzymatic activity of IdeS that is important for an increased bacterial survival in ex vivo assays. Interestingly, *S. equi* ssp. *equi* and *S. equi* ssp. *zooepidimicus* also have two other IdeS homologues (IdeE2 and IdeZ2) that have higher activity towards horse IgG, but whether the activity of these proteins is high enough to rescue opsonised bacteria remains to be investigated.
**Uniqueness of M28 complex II protein**

Our results that only complex II from serotype M28 is enzymatically deficient raise interesting questions. So far the only IdeS of either complex I or complex II that harbours the additional cysteine is the protein from the M28 serotype strain. *S. pyogenes* serotype M28 is the 3rd most commonly serotype in high income countries (Steer et al., 2009), and in Europe M28 was the second most commonly serotype causing severe disease (Luca-Harari et al., 2009), M28 has thus to be accounted as a highly virulent serotype. As implied in the introduction *S. pyogenes* has a broad repertoire of virulence factors with the capsule and the M protein being the main virulence factors as these two proteins aid the survival in blood. One might draw the conclusion that since serotype M28 is highly virulent but has an inactive IdeS, the IdeS protein is of low biological relevance. However, this statement is complicated to test experimentally, (*i*) it is difficult to properly assess the impact of IdeS in vivo since *S. pyogenes* is a strict human pathogen, and (*ii*) IdeS does only poorly cleave IgG in mice. The argument that IdeS is not important because serotype M28 has an inactive form, must be put in the light of how many virulence factors *S. pyogenes* has, of with both IdeS and EndoS (a secreted protein that deglycosylates IgG) inactivates IgGs. The virulence of *S. pyogenes* will probably not stand or fall with one virulence factor, but rather be the sum of virulence factors expressed at a certain time point. But, why is the M28 serotype continuing to express a protein, which at least in our assays, do not promote bacterial survival? Of course not all possible survival situations that the bacteria will encounter in vivo are tested in the whole blood survival assay. In certain situations the ability to block ROS production might well be of great benefit, enough benefit for keeping the protein in the genome and to express it. However, the fact that the *S. equi* strains have two variants of IdeS homologues, one that degrades horse IgG and one that do not, also indicates that the enzymatically inactive variants still have an important biological role. It remains to elucidate if the enzymatically inactive complex II of strain M28 has any properties that are beneficial for the M28 serotype, that the other proteins of complex I and complex II lack.
IgG cleavage products
- Binding to bacterial surface and consequences for host response
Paper II

Total proteolysis of IgG by IdeS results in the production of $\frac{1}{2}$Fc and F(ab’)$_2$ fragments. This study was undertaken to examine the biological properties of the $\frac{1}{2}$Fc fragment.

Non-immune binding of intact IgG to the bacterial surface

The ability of *S. pyogenes* to bind IgG in a non-immune fashion is long known (Kronvall, 1973), and the M protein, M-like proteins and protein H have all been characterised (Heath & Cleary, 1989, Åkesson *et al.*, 1990, Gomi *et al.*, 1990). However, it remains to be demonstrated how non-immune binding of IgG contributes to the evasion of the host immune defence (Smeesters *et al.*, 2010). It has however been shown that by binding IgG protein H inhibits complement activation by the classical pathway (Berge *et al.*, 1997). In order to evaluate the binding of $\frac{1}{2}$Fc fragments to the surface of *S. pyogenes* we started out with evaluating the binding of IgG to the surface of *S. pyogenes* M1 strain AP1. *S. pyogenes* was incubated with radiolabeled IgG and then the initial binding was challenged with unlabeled IgG. Already after 15 minutes almost 50% of the initially bound radioactive IgG had been released from the bacterial surface. Indicating that the binding of IgG to the bacterial surface by the IgG binding proteins is not static by dynamic reaction where the IgG is bound and un-bound. This also implies, that specific IgG, that has the ability to opsonise the bacterium, will time-dependently be released from the IgG binding proteins, and hence have the ability to opsonise the bacterium and aid the immune defence in killing the bacterium. These findings suggest that non-immune binding of IgG to the bacterial surface does give a long lasting protection against the actions of specific IgG.

Interestingly, in 2011 it was shown that IdeS have a preference to cleave Fab-bound IgG (opsonising IgG) rather than IgG bound by the Fc-region to M protein (Su *et al.*, 2011). This means that IdeS will disarm opsonising IgG before cleavage of non-opsonising IgG. Hence, IgG binding and IgG hydrolysis work together to minimize the amount of IgG able to opsonise the bacterium.

Binding of $\frac{1}{2}$Fc fragments to the bacterial surface

We then continued with examining the ability of $\frac{1}{2}$Fc fragments to bind to the bacterial surface and to compete with intact IgG to bind to the surface. $\frac{1}{2}$Fc did bind to the surface, however with low efficiency. But $\frac{1}{2}$Fc could not compete in binding with intact IgG, suggesting that $\frac{1}{2}$Fc fragments will not be attached to the bacterial surface but be a part of the soluble IgG pool.
**Effect of ½Fc fragments in human blood**

Using a survival assay with human immune blood we determined the effect of soluble ½Fc fragments. The blood was either pre-incubated 40 min with ½Fc fragments before addition of bacteria, or the ½Fc fragments and bacteria were added simultaneously. In the blood pre-incubated with ½Fc fragments the survival of *S. pyogenes* decreased. When further evaluating the effect of ½Fc fragments we could detect a priming response in neutrophils. The priming effect was primarily assayed in the whole blood assay (see comments is Methodology section), priming was detected when the neutrophils were activated with fMLP, PMA as well as with IgG opsonised latex beads. Priming is defined as an enhanced response to an activating stimulus. To rule out that the priming was due to LPS the priming was also preformed with purified neutrophils without plasma. LPS needs the LPS binding protein, that is present in plasma, to act as priming agent (Aida & Pabst, 1990). The priming also explains the increased killing of *S. pyogenes* in immune blood. But note, we could not detect any increased killing in non-immune blood.

However, what is the evolutionary benefit for a virulent bacterium to produce an enzyme which hydrolysis products increases the killing of the bacterium itself? When attempting to answer this question there are several aspects that must be considered. First of all, IdeS is a very efficient enzyme that rapidly cleaves IgG and rescue *S. pyogenes* from being killed. That positive effect of this very specific and efficient enzyme is most likely out ruling any possible negative effects that might be caused by ½Fc fragments. This view is confirmed by the findings that we could not detect any increased killing in non-immune blood after treatment with ½Fc. Further, the effect of IdeS generated ½Fc fragments is also depending on when the enzyme is produced by the bacterium. IdeS expression is controlled by the CovRS two component regulatory system and we have detected the highest production of IdeS in strains with mutations in the CovRS, results consistent with publications from other groups (Lei et al., 2001, Gryllos *et al.*, 2008, Trevino *et al.*, 2009). Mutations in CovRS arises spontaneously in *S. pyogenes* and have been described as a way for the bacterium to increase its virulence in the host after establishment (Cole *et al.*, 2011).

However, that *S. pyogenes* causes inflammation is a known fact, in this point of view, the priming of neutrophils by ½Fc fragments quit well fits with the current picture of *S. pyogenes* infection and the heavy inflammatory response.

Recent investigations in our group of the kinetics and molecular events of the two step process, in which IgG is cleaved (Vincents *et al.*, 2004, Ryan *et al.*, 2008), have revealed that the first cut is significantly rapider than the second cut (unpublished data U. von Pawel-Rammingen and R. Vindebro). Consequently, all possibly “first” cuts will take place before
the possible “second” cuts. This indicates that one of the heavy chains of the IgG is firstly cleaved. The single cleavage is enough to inhibit the functions of IgG (Brezski et al., 2009). Accordingly, there is not likely to be produced so much ½Fc as long as there is un-cleaved IgG present. It is currently not known if single cut IgG affect neutrophils. Although, in an infective focus with large amounts of bacteria producing IdeS it might well be that all IgG is cleaved and thus produce ½Fc fragments.

Even though it remains to determine how ½Fc fragments prime neutrophils it is intriguing that neutrophils seem to respond to ½Fc fragments. Possibly ½Fc might be a “danger signal”. Not only *S. pyogenes* and other microorganism degrade IgG but also several host proteins degrade IgG. MMP-3 cleaves IgG in a fashion resembling of IdeS (however much slower), the neutrophil elastase is also able to degrade IgG but results in Fc-dimers (Ryan et al., 2008). An indirect evidence of IgG fragmentation in vivo is the presence of anti-bodies directed towards IgG-hinge region (Brezski et al., 2008). In the early 1990s the group of Havemann published a number of papers about the effect on neutrophils caused by elastase generated Fc fragments. They could detect binding to neutrophils by Fc fragments (Eckle et al., 1990c), and that these fragments inhibited the oxidative burst (Eckle et al., 1990a) and inhibited chemotaxis (Eckle et al., 1991), further they also described that Fc fragments could stimulate release of elastase and myeloperoxidase (Eckle et al., 1990b). Elastase generated Fc fragments includes the disulphide bonds in the hinge region, in contrast to the ½Fc fragments generated by IdeS that not contains the disulphide bonds.

It remains to be established how neutrophils are primed by ½Fc fragments and which receptors are involved. Our preliminary data have not been able to show any interaction between Fc-receptors and ½Fc fragments, or been able to inhibit priming with soluble Fc-receptors, or by blocking Fc-receptors with antibodies (unpublished data). That IdeS generated ½Fc-fragments do not bind to FcγRIII has also been shown by others (Agniswamy et al., 2004).
Processing of IdeS
- Truncation of IdeS by SpeB and neutrophil proteases, consequences for enzymatic activity and immunorecognition.

Paper III

IdeS is present in an environment rich of proteases, both with origin from the host, and from the bacterium itself. The streptococcal cysteine protease SpeB is a promiscuous enzyme that degrades both host proteins as well as streptococcal proteins (Nelson et al., 2011) and neutrophils are rich of proteases stored in granules (Pham, 2006). In Paper III we investigate the molecular events of IdeS processing with proposed consequences for IdeS activity in vivo.

Processing by SpeB and neutrophil proteases
Initially, the effect of neutrophil proteases on IdeS and SpeB was assessed. SpeB was under the conditions tested resistant to degradation. IdeS however, was partially degraded. We continued to assess the ability of SpeB to degrade IdeS. Surprisingly, a similar partial degradation was observed. Interestingly, the partial degradation was stable and further processing of IdeS did not occur when incubation was prolonged. The partially degraded IdeS was examined by NH2-terminal sequence analysis and revealed putative cleavage sites within the 63 first amino acids.

Biological properties of NH2-processed IdeS
To further investigate the partially degraded IdeS, NH2-terminal truncated versions were recombinantly expressed. The IgG degrading capabilities of the truncated versions were assessed. Interestingly, the truncated IdeS proteins were enzymatically active (albeit lower than the full length protein in our assays).

Further, immunorecognition of truncated IdeS was investigated. B-cell epitope mapping has shown that the NH2-terminal of IdeS is highly immunogenic (Lei et al., 2002). This result was confirmed by the presence of specific antibodies in patient sera directed to the NH2-terminal of IdeS. When these specific antibodies to the NH2-terminal were removed, the detection of full length IdeS by ELISA was reduced. Consequently, truncated IdeS is less prone to be recognized by specific antibodies generated during infection.

It is known that SpeB degrades streptococcal virulence factors (reviewed in (Nelson et al., 2011)) However, SpeB only degrades IdeS in the NH2-terminal and the truncated IdeS is still enzymatically active, this finding is in comparison with the finding that SpeB processes SLO without inactivation (Pinkney et al., 1995). Thus, in contrast to most other streptococcal virulence factors that are inactivated by SpeB, IdeS remains
relatively active, and also, the truncated protein is less immunogenic. SpeB might be considered as a post-translational regulator of virulence factors, but SpeB and IdeS rather act in concert to minimise antibody-recognition of IdeS.

Intriguingly, our data suggest that IdeS is not degraded by neutrophil proteases but only are truncated in the NH2-terminal, which can be of benefit for the bacterium by less immunogenicity. It is earlier described how IdeS is activated by cystatin C (Vincents et al., 2008), a cysteine protease inhibitor that thus should inhibit and not activate IdeS. Here we describe how IdeS is processed and remains enzymatically active but probably less immunogenic. IdeS is thus a good example how bacterial virulence factors can evolve to take advantage of the host response against them.
Non-immune binding of IgG does not give long-lasting protection against specific IgG. 1/2Fc fragments do not bind to the streptococcal surface.
Concluding remarks

- Currently, the only known IdeS variant of *S. pyogenes* lacking IgG endopeptidase activity is the protein expressed by the M28 serotype strain. This protein is deficient in enzymatic activity due to an additional cysteine that forms a disulphide bond with the cysteine in the active site, and hence blocks the enzymatic function.

- In ex vivo ROS assays both complex I and complex II variants inhibit ROS production. However in immune blood assays, the IgG cleaving activity is required for increased bacterial survival.

- Non-immune binding of IgG to the streptococcal surface is a constant association and dissociation, but ½Fc fragments cannot compete with IgG for binding.

- Soluble ½Fc fragments can prime neutrophils and increases the ability of neutrophils to kill bacteria.

- IdeS is NH2-terminally processed by neutrophil serine proteinases and by streptococcal SpeB.

- Processed IdeS remains enzymatically active and is less immunogenic than the full length protein.

Figure 4. Summary of main findings in the thesis. (on opposite page)
I den här avhandlingen har jag studerat den sjukdomorsakande bakterien *Streptococcus pyogenes* och hur den interagerar med vårt immunförsvar. *S. pyogenes* orsakar många olika typer av sjukdomar, de vanligaste är milda infektioner som halsfluss och svinkoppor, men samma bakterie kan även orsaka invasiva sjukdomar som blodförgiftning och svåra hud- och mjukdels infektioner.

Som en del till sitt försvar mot bakterier har immunförsvaret antikroppar som känner igen bakterierna. Dessa så kallade IgG molekyler kan binda till bakteriens yta och därigenom signalera till övriga immunförsvaret. En av de celltyper som kan känna igen IgG molekyler som har bundit till ytan av bakterier är neutrofilerna. Neutrofiler är viktiga celler i vårt immunförsvar och kallas i dagligt tal för ”vita blodkroppar”. I normala fall finns neutrofilerna i blodet men vid infektion lämnar neutrofilerna blodet för att kunna bekämpa de inkräktande bakterierna. Om bakterierna då har antikroppar på sin yta är de extra lätt att känna igen och oskadliggöra.

*S. pyogenes* har emellertid ett enzym, kallat IdeS, som gör IgG molekylen verkningslös genom att klippa den i två bitar. När IdeS klipper specifika antikroppar i immunblod överlever bakterien. I avhandlingens delarbeten har olika aspekter av proteinet IdeS studerats.

Alla proteiner med samma namn behöver inte vara exakt likadana utan ofta finns det små skillnader, alleliska variationer, IdeS proteinets olika alleliska variationer kan delas in i två grupper (komplex I och komplex II), de första rapporterna om dessa alleliska variationer indikerade att det skulle vara tämligen stora biologiska skillnader mellan dessa alleliska variationer. Vi utökade våra studier med IdeS proteiner från streptokocker av flera olika serotyper och utförde även egna punktmutationer i proteinet. Våra resultat visade att de olika alleliska varianterna var oskiljaktiga gällande de biologiska funktionerna som vi testade, men med ett undantag, IdeS proteinet från *S. pyogenes* av serotyp M28 som hör till komplex II hade låg enzymatisk aktivitet och kan därigenom inte klippa IgG.

När IdeS klipper IgG bildas två delar av IgG molekylen kallade F(ab’)_2 och ½Fc. Vi studerade ½Fc fragmentet och kunde visa att ½Fc fragment påverkar neutrofiler genom att ”primera” denna celltyp. När neutrofilen är primad reagerar den kraftigare på stimuli och till exempel dödar primade neutrofiler bakterier mer effektivt. Detta skulle kunna vara till nackdel för streptokockerna men de positiva aspekterna av att klippa IgG överväger troligen nackdelarna. Dessutom är det inte helt klarlagt exakt hur neutrofilerna påverkas av priming med ½Fc fragment.
References


