Exotoxic Virulence Factors of *Streptococcus pyogenes*

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Abstract

Streptococcus pyogenes is a human pathogen that has been causing life-threatening diseases throughout history. From the 1980s the incidence of severe invasive infections, such as Necrotising fasciitis and Streptococcal toxic shock syndrome, increased substantially.

Streptococcus pyogenes can secrete a wide range of toxins that contribute to its virulence. One of these toxins is the streptococcal pyrogenic exotoxin B (SpeB), which is an important enzymatic toxin. By cleaving host and bacterial molecules SpeB contributes to inflammation and tissue damage. In addition, it facilitates invasion of the bacteria and helps evading the immune system. Beside enzymatic toxins, *Streptococcus pyogenes* secretes toxins that elicit an immense immune reaction, called superantigens. Via activation of the transcription factor NFκB, transcription is altered in many cell types, which eventually can induce a state of shock.

This review will address the pathogenesis of *Streptococcus pyogenes.* In particular, the role of SpeB and superantigenic toxins will be addressed. Furthermore, herein the leading hypothesis of the sudden rise in invasive infections will be discussed.

Keywords: Streptococcus pyogenes, Streptococcal toxic shock syndrome, Necrotising fasciitis, Streptococcal pyrogenic exotoxin B, Superantigens

Contents

1. Introduction

Streptococcus pyogenes is an infectious toxin-producing bacterium that can cause several life-threatening diseases (Box 1). This bacterium is a human specific pathogen that nowadays is better known as the 'flesh-eating bacterium'. However, this pathogen is not a novel discovery; its infections have been agonising mankind throughout history. The eldest report dealing with *Streptococcus pyogenes* infections has been written by Hippocrates (460–379 BC) nearly 2500 years ago. He described an outbreak of the skin infection 'Erysipelas' that at times had grave complications when the infection became invasive:

"Many were attacked by the erysipelas all over the body when the exciting cause was a trivial accident or a very small wound … Many even while undergoing treatment suffered from severe inflammations, and the erysipelas would quickly spread widely in all directions. Flesh, sinews and bones fell away in large quantities … The bones were bared and fell away, and there were copious fluxes. Fever was sometimes present and sometimes absent… There were many deaths. The course of the disease was the same to whatever part of the body it spread. Many lost the arm and the entire forearm." Hippocrates (460-379 BC) (Descamps et al, 1994).

In the following centuries *Streptococcus pyogenes* caused several unpleasant infections in (war)wounds and later in post operational wounds. During the eighteenth and nineteenth century these infections were known as hospital gangrene, a name given by British naval surgeons, for the disease was primarily present in hospitals (Olsen et al., 2010). A confederate medical officer wrote during the American Civil War (1861-1865)*:*

"I have seen the skin in the affected spot melt away in twenty-four hours into a grayish and greenish slough, whilst the skin and tissue within, over which it had just passed, changed rapidly to the ash gray, and green and bluish hue characteristic of this form of gangrene." (Meleney et al., 1948)

Old surgical textbooks report it as an unpreventable disease that sometimes arises spontaneously after surgery. Hygiene and the existence of bacteria still had to be discovered.

In mid-19th-century a second infection was common in hospitals, which mainly affected women. The incidence of childbed fever, also known as puerperal fever, increased dramatically after the rise of maternity hospitals (Box 2). In general, women showed symptoms within three days after delivery. Rapidly, the disease progressed and caused severe abdominal pain, fever and eventually lead to death in the majority of the cases (Lane et al, 2010).

The incidence of infectious diseases shrunk after the discovery of penicillin (1928). However, *Streptococcus pyogenes* infections are still a present-day concern and cause several life-threatening infections in Western countries, including the Netherlands (Olsen et al., 2010). In addition, over the past twenty years there appears to be a global yet unexplained revival of several forms of severe invasive *Streptococcus pyogenes* diseases. Two of the most severe forms of these diseases are Streptococcal toxic shock syndrome (severe septic shock caused by toxins of *Streptococcus pyogenes*) and Necrotising fasciitis (the so-called 'the flesh-eating disease').

The latter is a rapidly progressive infection affecting skin, soft tissue (all tissue except for bone and organs, e.g. adipose tissue), and muscle. The name Necrotising fasciitis was proposed by Wilson in 1952, referring to the inflammation (-itis) of the fascia (sheets of connective tissue separating or binding together muscles and organs) and the predominant necrosis (cell death). Generally, the disease begins with a benign-appearing skin lesion, often mistaken for a spider or

Box 1. *Streptococcus pyogenes*
The name 'Streptococcus' comes from the Greek words 'streptos', which means 'chain', and 'kokkus', which means berry. The German surgeon Theodor Billroth who introduced the term, referred to its appearance under the microscope (figure A) (Vlaminckx et al., 2007).

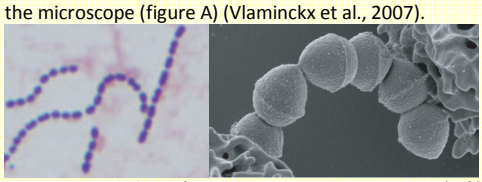


Figure A: Morphology of group A *Streptococcus pyogenes*. (Left) Micrograph of *Streptococcus pyogenes* isolated from the blood of a human patient. *Streptococcus pyogenes* are gram-positive cocci that grow in pairs and chains. (Right) Scanning electron micrograph of *Streptococcus pyogenes* interacting with human neutrophils.

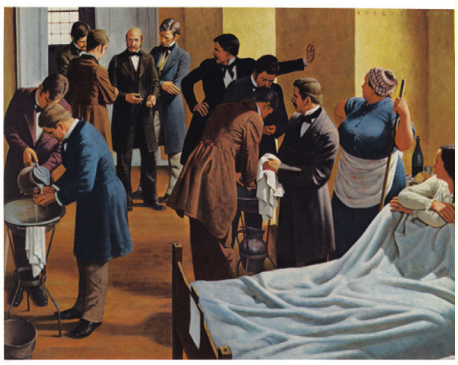
insect bite. At first, the infection spreads along the connective tissue that separate adjacent muscle groups. Later, it can invade other tissues, such as muscle tissue or the bloodstream, which leads to myonecrosis and bacteraemia (bacteria in the blood), respectively. Colonisation of normally sterile sites induces a rapid influx of acute inflammatory cells that produce damaging enzymes. In addition, *Streptococcus pyogenes* expresses degradative virulence factors, such as highly potent exotoxins. Together with the inflammatory enzymes these virulence factors inflict severe tissue damage (Olsen et al., 2010). Even with all the high quality healthcare of today, Necrotising fasciitis has a case fatality rate of 32% in Europe (Lamagni et al., 2008).

Streptococcal toxic shock syndrome is an exotoxin-mediated disease, which complicates up to 50% of Necrotising fasciitis cases (Lamagni et al., 2008). On the other hand, toxic shock syndrome can lead to Necrotising fasciitis. Streptococcal toxic shock syndrome was first described only 25 years ago. The most common initial symptom is localised severe pain, very abrupt in onset. Subsequently, the patient can suffer from flu-like symptoms, such as fever, myalgia, nausea, vomiting, and diarrhoea (Stevens, 2001). In Streptococcal toxic shock syndrome, exotoxins of *Streptococcus pyogenes* act as superantigens, inducing an immense inflammatory response. The overactivated immune system gives rise to mediators that dilate the blood vessels, resulting in shock and multi-organ failure (Lappin et al., 2009). Streptococcal toxic shock syndrome has a 7-day mortality of 44% (Lamagni et al., 2008).

Exotoxins, as mentioned above, are major virulence factors of *Streptococcus pyogenes*. It has not only been reported that streptococcal exotoxins play a role in Streptococcal toxic shock syndrome, but it has also been described that they play a considerable part in the pathogenesis of Necrotising fasciitis. Alongside these severe infections, *Streptococcus pyogenes* causes common clinical illnesses as pharyngitis (Olsen et al., 2009). Expression of virulence factors, e.g. exotoxins, seems to be crucial for invasive properties, but depend on environmental stimuli (Tart et al., 2007).

This review will give an overview of important virulence factors, but will focus on the function of exotoxins in the pathogenesis of severe invasive infections caused by *Streptococcus pyogenes*. In addition, herein alterations in virulence over time will be discussed.

Box 2. In 1846 a young physician named Ignaz Semmelweis, who worked in the Vienna General Hospital, was struck by the difference in mortality rate between the two divisions of the maternity department. One division was used for training midwives and had a mortality rate of 2.7%, which is excessive compared to modern day obstetrics. In the other division medical students were trained, and in contrast to the midwives the medical students preformed autopsies. The mortality rate in the latter division was substantially higher (11.4%) (Charles et al., 1986). Semmelweis postulated that puerperal fever was a contagious disease. By washing hands in a chloride solution after a post-mortem exam, "cadaverous particles" would not be carried out the autopsy rooms by the students and physicians, which according to his hypothesis caused the disease in women after childbirth. Due to these measures the maternal mortality rate in the hospital immediately dropped significantly. Despite these results, Semmelweis' theories were accepted only years after his death (Lane et al, 2010).



SEMMELWEIS: DEFENDER OF MOTHERHOOD

2. Pathogenesis

Streptococcus pyogenes has a wide range of virulence factors (Table 1) of which the most relevant will be addressed in this section. Virulence can be defined as the ability to: (1) avoid opsonisation and phagocytosis, (2) adhere to the host cells, (3) invade into epithelium, and (4) produce toxins and enzymes (Murray et al., 2009).

2.1 Immune evasion

The human immune system comprises a spectrum of complex defence mechanisms that has to be overcome by an infecting agent, such as *Streptococcus pyogenes*. An important defence mechanism against bacteria is opsonisation, where bacteria are labelled for ingestion and destruction by a phagocyte, a

process called phagocytosis. These labels are represented by antibodies, like Immunoglobulin G, or components of the complement system, like C5a. *Streptococcus pyogenes* has several virulence factors to its disposal to avoid being detected and destructed. First of all, it can express a capsule, which is poorly detected by the hosts defence, because the compounds of this capsule are chemically quite similar to components of human connective tissue (Bisno et al., 2003). In addition, this capsule shields the bacterial surface antigens from opsonisation molecules.

Another well described mechanism for evading the immune response is the M protein. This is a surface protein with antiphagocytic activity. By binding two essential molecules of the complement system (Factor H and Fibrinogen), the M protein greatly reduces the disposition and the amount of C3b (Cunningham, 2000).

Finally, the surface molecule C5a peptidase cleaves and inactivates complement C5a; this reduces the recruitment of leukocytes (Fig. 1; Table 1). By shutting down the detection machinery (i.e. the complement opsonisation) *Streptococcus pyogenes* becomes invisible to the hosts immune system.

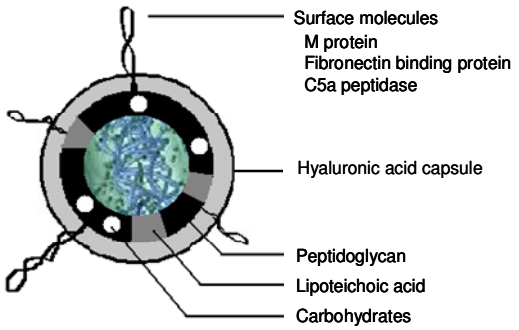


Figure 1: Cell surface compounds of *Streptococcus pyogenes.* (Adapted from Sriskandan et al., 2006).

2.2 Adherence

Effective immune evasion is essential to bacterial survival in a human host. However, before the bacteria can initiate human disease, they need to attach to the surface of the host cells. Strong attachment is necessary to withstand mucous and salivary fluid flow mechanisms and other mechanical stressors. In general, the initial step of *Streptococcus pyogenes* infection is adherence to dermal or pharyngeal epithelium. However, a lesion or injury makes it possible for bacteria to adhere directly to deeper tissue (Cunningham, 2000).

The most extensively studied adhesion molecules of *Streptococcus pyogenes* are pili-like cell surface structures, called M protein and fibronectin binding protein (Fig. 1) (Olsen et al., 2009). Fibronectin binding protein is important for adherence to skin and respiratory epithelium. M protein seems to mediate attachment to skin keratinocytes (predominant cell type of the skin) (Table 1). The expression of these adherins is regulated by environmental stimuli, such as pH, temperature or partial pressure of O_2 and CO_2. Fibronectin binding protein expression is augmented in an O_2 rich environment, whereas expression of M protein increases under conditions of high CO_2. Bisno et al. (2003) postulate that during initial pathogen-host contact, when oxygen is high, *Streptococcus pyogenes* needs to adhere to the host and expresses fibronectin-binding protein. In a later stage, after invading the host, it is more crucial for the pathogen to defend against phagocytes, and here oxygen is low and CO_2 is high which initiates the expression of the antiphagocytic M protein (Bisno et al., 2003).

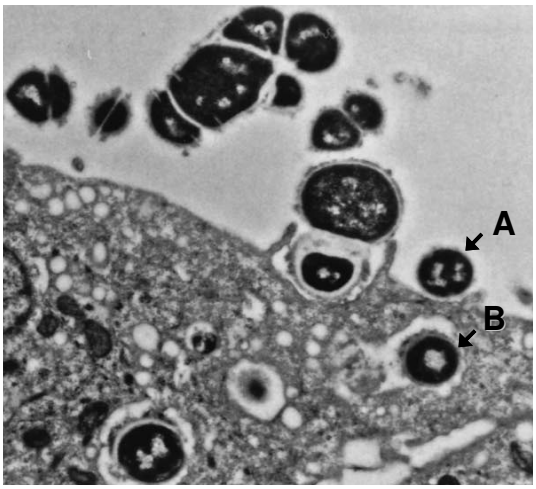


Figure 2: Electron micrograph of *Streptococcus pyogenes* (A) attachment and (B) internalisation (human cultured pharyngeal cells) (From Fluckiger et al., 1998).

2.3 Internalisation and invasion

Streptococcus pyogenes has the ability to penetrate epithelium, using M protein and fibronectin binding protein to force an internalisation mechanism of the cell to ingest the pathogen (Fig. 2 and 3) (Cunningham, 2000). Being intracellular has a considerable advantage for *Streptococcus pyogenes,* because it is protected against antibiotics. This

is consistent with the failure of penicillin treatment and recurrent infections (Olsen et al., 2010).

The most severe infections of *Streptococcus pyogenes* are invasive infections, where the bacteria have breached the epithelium and invaded a normally sterile site. The bacterial capsule appears to facilitate invasion by two mechanisms: (1) disrupting intercellular junctions, and (2) preventing the bacteria to get trapped within endothelial cells by hindering internalisation (Cywes et al., 2001). In contrast, it has been suggested that internalisation also can contribute to infiltrating into deeper tissue (Bisno et al., 2003). In addition, *Streptococcus pyogenes* expresses enzymes causing tissue destruction (proteases and DNases), which makes it easier to invade underlying tissue (Olsen et al., 2010) (Table 1).

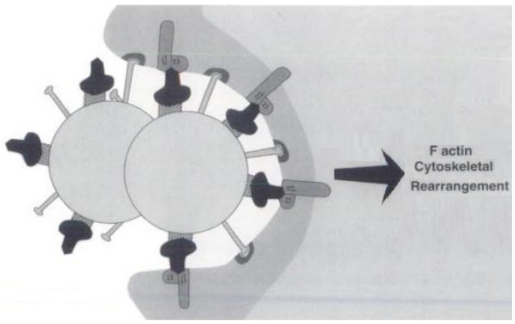


Figure 3: Internalisation of *Streptococcus pyogenes*. M protein and fibronectin binding protein interact with host surface proteins to force internalisation by rearrangement of the cytoskeleton. (Adapted from Fischetti et al. 2000).

2.4 Enzymes and toxins

Streptococcus pyogenes produces two haemolytic enzymes (enzymes that brake down erythrocytes): the pore-forming cytolysin Streptolysin O, and Streptolysin S, the most potent cytotoxin known (Table 1). Both enzymes are toxic to a number of blood elements, including erythrocytes, leukocytes and platelets, as well as for subcellular organelles.

Several extracellular products of *Streptococcus pyogenes* inflict tissue damage, enabling the bacteria to spread through the tissue. To name a few: DNases, which degrade DNA; hyaluronidase, which brakes down a ground substance of connective tissue; streptokinase, which dissolves blood clots; and streptococcal pyrogenic exotoxin B, which is a very potent cysteine protease. *Streptococcus pyogenes* has several streptococcal pyrogenic exotoxins, where exotoxin B is an exception, for the other members of this family are superantigens playing a crucial role in the pathology of Streptococcal toxic shock syndrome (Bisno et al., 2003). The following section will discuss exotoxin B and streptococcal superantigens in more detail.

Table 1: *Streptococcus pyogenes* uses a range of virulence factors to achieve certain goals. (Tart et al., 2007; Bisno et al., 2003).

Aim	Virulence factor
Adherence:	○ M protein (to skin keratinocytes); ○ Capsule (to skin keratinocytes); ○ Fibronectin- and collagen-binding proteins (to oral epithelium, cutaneous langerhans cells); ○ Lipoteichoic acid (to oral epithelial cells).
Internalisation:	○ Fibronectin- and collagen-binding proteins; ○ M protein.
Invasion:	○ M protein; ○ Capsule; ○ Proteases; ○ DNases.
Immune system avoidance:	○ M protein; ○ M-protein-like (e.g. Mrp, Enn, and others); ○ Capsule; ○ C5a peptidase.
Dissemination:	○ Streptokinase; ○ DNases A-D; ○ SpeB (cysteine protease); ○ Hyaluronidase.
Systemic toxicity:	○ Exotoxins; ○ Pyrogenic toxins; ○ Superantigenic exotoxins; ○ Streptolysin O; ○ Streptolysin S.

3. Streptococcal exotoxins

Many bacteria are known to produce toxins, which seem to be responsible for the major symptoms of the illness. Moreover, in a variety of bacteria toxins have been recognised as the main virulence factor. For example, *Vibrio cholerae* produces cholera toxin, which causes the profuse watery diarrhoea and vomiting, the primary symptoms of cholera disease. Another example is the neurotoxin produced by *Clostridium tetani,* which causes severe contraction of skeletal muscle, characteristic for tetanus. *Streptococcus pyogenes* is an exception in that it secretes a whole battery of toxins with different functions. Two kinds of bacterial toxins are known:

exotoxins and endotoxins. Endotoxins are harmful molecules within a bacterium that are released solely when the bacterium dies and falls apart. Generally, toxins refer to the active compound secreted by living bacteria, known as exotoxins.

In this section the exotoxins of *Streptococcus pyogenes* are described. Superantigens and the most studied enzymatic exotoxin will be discussed. Finally, the possible cause of the rise in severe invasive infections will be addressed.

3.1 Enzymatic toxin: Streptococcal pyrogenic exotoxin B (SpeB)

SpeB is a super-potent extracellular cysteine protease, making it a key virulence factor. It is believed that this toxin may contribute to the massive tissue destruction and the accompanied spread of infection (Chaussee et al., 2001). SpeB is secreted in an inactive form by *Streptococcus pyogenes*, and is converted to the active form by SpeB itself. This process is called autocatalysis, as the reaction is catalysed by its own product (Kagawa et al., 2000). SpeB is a protease which cleaves several host molecules as well as bacterial molecules. Cleavage can result in degradation of an active protein (e.g. C3b), activation of a precursor protein (e.g. interleukin-1β), separation of molecules from a surface (e.g. bacterial C5a peptidase) or conversion from one substance to another (e.g. kininogen to bradykinin) (Chiang-Ni et al., 2008).

Box 3. Case report:
Day 1: A previously healthy 25-year old student was admitted to the hospital with pain and oedema of his left foot and shoulder, which started 12 hours earlier. His body temperature was slightly elevated. His foot and especially his arm showed redness, warmth and tenderness. The patient was prescribed analgesic and strong antibiotic medication.
Day 3: Blisters appeared on the left arm and pain increased. Furthermore, swelling and redness spread to the forearm and hand. Over the last two days his body temperature had risen to 38.8 °C. Despite increased medication the symptoms worsened.
Day 4: The pain in the left arm was unbearable. New blisters and skin necrosis emerged. Necrotising fasciitis was suspected and pharmacotherapy was changed. Additionally, necrotic tissue was surgically removed form the arm, as well as from the foot and the patient was transferred to the intensive care unit. He had signs of sepsis and respiratory failure and had to be ventilated mechanically.
Day 6: Again, treatment proved unsuccessful when the inflammation spread toward his thorax. Surgical debridement was performed.
Day 10: The patient presented signs of secondary soft tissue infection, including muscle necrosis.
Day 12: Respiratory distress and multiple organ dysfunction arose. The upper left limb was amputated.
Day 14: After surgery the clinical condition improved significantly.
Day 21: The patient had stable breathing and circulation and was able to leave the intensive care unit.
(Musialkowska et al., 2010)

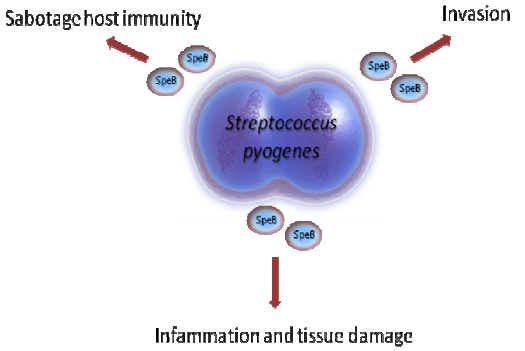


Figure 4: Three main functions of Streptococcal pyrogenic exotoxin B (SpeB).

3.1.1 Cleavage of host molecules

SpeB converts the precursor of interleukin-1β (IL-1β) to the active form (Karur et al., 1993a), thus increasing the activity of IL-1β. IL-1β is a cytokine, released by macrophages, and is involved in cell proliferation, differentiation and apoptosis. Additionally, this inflammatory mediator augments pain sensitivity.

Host matrix proteins, such as fibronectin and vitronectin, are degraded by SpeB, which enhances tissue damage (Kapur et al., 1993b). Tissue destruction is a functional property of bacteria, for it enables dissemination and invasion of novel nutrient-rich tissues.

Kininogen is converted into bradykinin due to SpeB cleavage (Herwald et al., 1996). Bradykinin dilates blood vessels and makes them more permeable for plasma and leukocytes. This results in oedema (swelling) and redness of the skin, which are characteristic for an inflammation site. Furthermore, bradykinin is involved in pain and fever that commonly accompany an inflammation. Some results indicate that SpeB also increases histamine release from mast cells (Nagamune et al., 2005), which corresponds to the elevated plasma histamine found in patients with Streptococcal toxic shock syndrome (Ohkuni et al., 2004).

Finally, SpeB protects *Streptococcus pyogenes* using various mechanisms: it inactivates antibacterial peptides that can

damage or kill *Streptococcus pyogenes* (Schmidtchen et al., 2002) and it helps the bacteria to escape phagocytosis by cleaving complements (C3b) and antibodies (Terao et al., 2008; Collin et al., 2002).

Thus, by cleaving host molecules SpeB promotes inflammation, protects *Streptococcus pyogenes* from the host and facilitates invasion (Fig. 4). The induction of vascular dilation and permeability, through bradykinin and histamine, may play a significant part in the pathology of toxic shock. On the other hand, SpeB-induced tissue destruction may contribute to the pathology of Necrotising fasciitis (Table 2A) (Box 3).

3.1.2 Cleavage of Streptococcus pyogenes molecules

SpeB inhibits adherence of the bacteria to the host cells by cleaving the M protein and fibronectin-binding protein (important adherence proteins) and thereby promotes spreading of *Streptococcus pyogenes* (Kansal et al., 2003; Nyberg et al., 2004). Additionally, bacterial internalisation is reduced, which again promotes spreading. In early growth phase *Streptococcus pyogenes* produces very low levels of SpeB, which allow surface molecules to interact with human host cells. This contributes to cell adhesion. Eventually, when a large bacterial population has grown, nutrients will get scarce and migration becomes more favourable than cell adhesion. This is supported by the fact that stimuli such as the pH and nutritional stress upregulate SpeB expression. SpeB proteolytically inactivates *Streptococcus pyogenes*' surface proteins, terminating the adhesion and promoting spreading. Additionally, tissue dissemination is facilitated by degradation of host cell molecules (Chaussee et al., 2001). When the bacteria reach the bloodstream, SpeB is downregulated, increasing the activity of other virulence factors that are advantageous to survival in blood (Walker et al., 2007).

Two proteins are released from the bacterial cell surface due to SpeB cleavage. One of them is the earlier mentioned virulence factor C5a peptidase. By releasing this enzyme from the bacterial surface, chemotactic complement factors can be degraded even when not in direct contact with *Streptococcus pyogenes* (Ji et al., 1997). The second one is protein H; when released from the bacteria it promotes spreading (Berge et al., 1995).

SpeB was initially believed to be a superantigen, but now it is thought that its contribution to disease results solely from the protease activity (Bisno et al., 2003). Findings show that a streptococcal superantigen (Smez)

Table 2: (A) Host and (B) bacterial proteins cleaved by Streptococcal pyrogenic exotoxin B (SpeB). (Adapted from Chiang-Ni et al., 2008).

A. Host proteins

Before SpeB cleavage	After SpeB cleavage	Potential effects
Interleukin-1β precursor	Active interleukin-1β	Induce inflammation
Pro-matrix metalloprotease	Active MMP	Enhance tissue damage and bacterial invasion
Fibronectin	Fragmented	Participate in bacterial colonization and invasive infection
Vitronectin	Degraded	Enhance tissue damage
Kininogen	Bradykinin	Increase vascular permeability; induce fever and pain
Immunoglobulin	Cleavage into Fc and (IgA, IgM, IgD, IgE, IgG) Fab fragments	Inhibit immunoglobulin-mediated opsonophagocytosis
C3b	Degraded	Escape phagocytosis
Plasminogen	Degraded	Reduce plasmin activity on Streptococcus pyogenes surface

B. Bacterial proteins

Before SpeB cleavage	After SpeB cleavage	Potential effects
SpeB precursor	Active SpeB	Degrade or cleavage of bacterial and host proteins
M protein	Remove 24 amino acids from N-terminus from bacterial surface	Alter immunoglobulin binding properties; release promote bacterial dissemination
Protein F1	Degraded	Reduce bacterial internalization
EndoS	Degraded	Lost IgG glycan-hydrolyzing activity
SmeZ	Degraded	Abolish immune stimulatory activity
Fba	Degraded	Inhibit binding of factor H and factor H-like protein-1
C5a peptidase	Release from bacterial surface	Degrade chemotactic complement factor C5a
Streptokinase	Degraded	Unknown
Protein H	Release from bacterial surface	Promote bacterial dissemination
Sda1	Degraded	Decrease neutrophil extracellular trap clearance

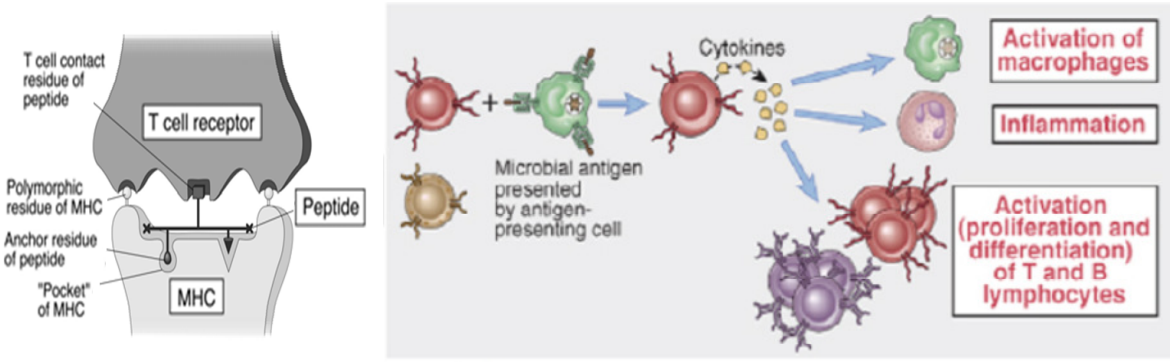


Figure 5: Conventional antigen presentation and T cell activation. (Left) The T cell receptor recognises the antigen (peptide) presented by the antigen presenting cell, upon which (right) the T cell gets activated and secretes cytokines. MHC II= major histocompatibility complex class II. (From Abbas et al., 2007).

is even degraded by SpeB (Nooh et al., 2006).

In general, the effects mentioned above imply that SpeB facilitates dissemination by several mechanisms, which suggests a major role in invasive forms of *Streptococcus pyogenes* infections. This hypothesis is supported by the finding that inactivated SpeB reduced virulence in animal models (Lukomski et al., 1998). Unfortunately, there are a number of conflicting results as well (Ashbaugh et al., 2001). A convincing relation between severity of infection and SpeB production is yet to be discovered (Chiang-Ni et al., 2008) (Table 2B).

3.2 Superantigens

A superantigen is a highly potent immune stimulatory substance that directly activates T-lymphocytes of the adaptive immune system. *Streptococcus pyogenes* expresses at least nine exotoxins which are believed to have superantigenic properties. These superantigens play a crucial role in the pathogenesis of Streptococcal toxic shock syndrome.

3.2.1 Antigen versus superantigen

An antigen is defined as a molecule recognised by the immune system, e.g. bacterial fragments or surface structures. Specialised cells are able to absorb and process antigens, and to present them at their cell surface firmly bound to a receptor (the major histocompatibility complex class II or MHC II, also known as HLA II). These cells are called antigen presenting cells for obvious reasons. The antigens are presented to the immune system to initiate an inflammatory response which can eradicate the potential threat. However, recognition can only take place when T-lymphocytes expressing the appropriate T-cell receptor bind to both antigen and MHC II (presenting receptor). Especially the association between antigen and T-cell is highly specific; merely a small fraction of T cells is able to recognise one specific antigen (Fig. 5) (Sriskandan et al., 2007).

Superantigen binding is a different thing all together. In contrast to the highly specific antigen-T-cell association, superantigens activate T cells in a non-specific manner. Consequently, a minimal amount of superantigen (nano- to picogram) is sufficient to activate up to 30% of the T cells, whereas a conventional antigen activates only 1 in 10^5-10^6 T cells (Sundberg et al., 2002). Although superantigens are not intercellular processed and presented like antigens, they do interact with the MHC II (receptor of the antigen presenting cell). Superantigens bind outside the binding pocket of the receptor (Fig. 6). This interaction alone is sufficient to activate the

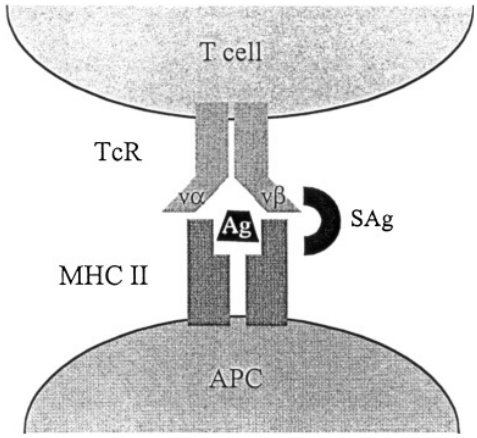


Figure 6: Antigen binding versus superantigen binding. TcR=T cell receptor. MHC II= major histocompatibility complex class II. Ag=antigen. Sag= superantigen. APC= antigen presenting cell (Adapted from Alouf et al. 2002).

antigen presenting cell, which secretes pro-inflammatory cytokines, such as tumour necrosis factor, interleukin-1β, and interleukin-6 (Sriskandan et al., 2007). The superantigen-MHC II complex is readily recognised by large families of T cells (Sriskandan et al., 2007). Once bound by a T cell, a signalling cascade within the T cell initiates activation and proliferation, resulting in an overwhelming cytokine release, including tumour necrosis factor β, interleukin-2, and interferon γ (Llewelyn el al., 2002).

3.2.2 Consequences of superantigenic stimulation

Cytokines are an essential part of our immune system and effectively assist in killing pathogens. However, the overwhelming release of cytokines due to superantigenic stimulation is harmful for the human host (Llewelyn et al., 2002). In Addition to T-cell cytokine release, T-cell activation leads to downstream activation of other cell types, plus their cytokine and chemokine release (White et al., 1989).

Superantigens signal the cell to alter DNA transcription through the well known transcription factor NFκB (nuclear factor κB) (Fig. 7). Overstimulation of NFκB leads to: (1) the expression of inflammatory mediators that initiate systemic inflammation, (2) endothelial injury and increased permeability due to activated neutrophils, (3) microvascular thrombosis, (4) hypotension and reduced cardiac output, and (5) increased nitric oxide, which contributes to hypotension (Lappin et al., 2009). NFκB plays a central role in the adverse effects of superantigens. Moreover, mortality risk correlates with the level of NFκB stimulation.

Taken together superantigens induce a devastating inflammatory response, leading to a reduced vascular tone, organ hypoperfusion and eventually multi organ failure (Faulkner et al., 2005), a so-called state of shock. Shock refers to a condition wherein very low blood pressure causes an insufficient blood flow through vital organs, resulting in oxygen deficiency that ultimately damages the organs. A state of shock can be initiated by several

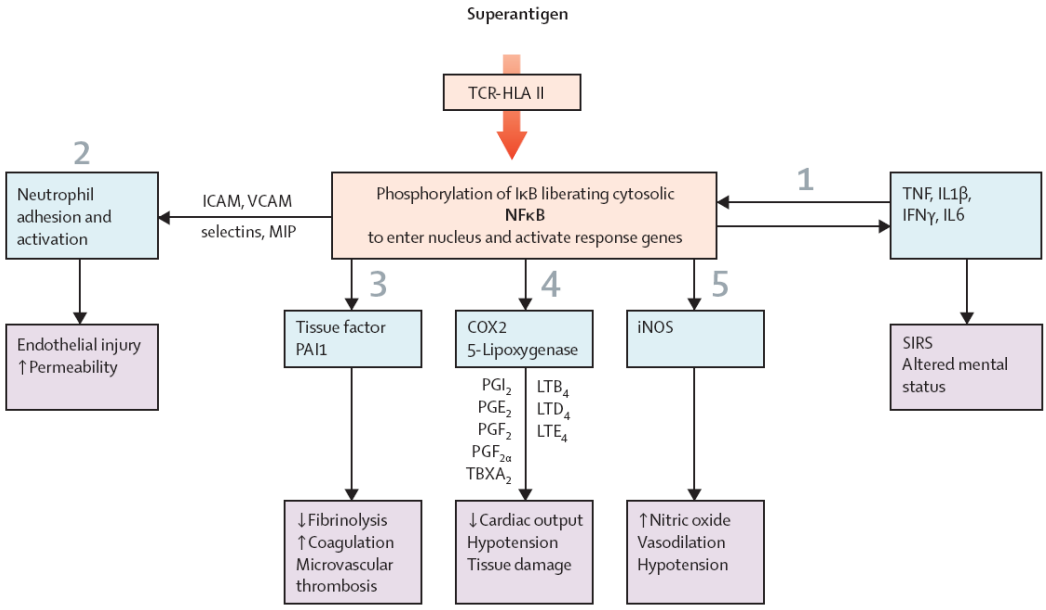


Figure 7: **Superantigenic stimulation of NFκB**. Superantigens bring the T cell receptor (TCR) and the major histocompatibility complex class II (HLA II) together, which stimulates intracellular NFκB. (1) Expression of inflammatory mediators: TNF=tumour necrosis factor, interferon γ (IFNγ), interleukin 1β (IL1β), interleukin (IL6), leading to systemic inflammatory response syndrome (SIRS) and an altered mental status. (2) Enhanced neutrophil adhesion and activation, via expression of intercellular adhesion molecule (ICAM), vascular cell adhesion molecule (VCAM), selectins and macrophage inflammatory protein (MIP). (3) Induction of tissue factor and plasminogen activator inhibitor 1 (PAI1). (4) Activation of cyclo-oxygenase 2 (COX2) and 5-lipoxygenase systems leading to secretion of pro-inflammatory prostanoids (prostaglandin I2, prostaglandin E2, prostaglandin F2, prostaglandin F2α), leukotrienes (leukotriene B4, leukotriene D4, leukotriene E4), and thromboxane A2 (TBXA2). (5) Inducible nitric oxide synthase (iNOS) elevation induces vasodilatation and hypotension. IκB=inhibitor of NFκB. (Adapted from Lappin, 2009).

things, including severe blood loss, severe allergic reaction or a weakened heart. When caused by toxins of *Streptococcus pyogenes* it is known as Streptococcal toxic shock syndrome. Many studies support the idea that superantigens play a critical part in the pathogenesis of Streptococcal toxic shock syndrome. Norrby-Teglund et al. found a direct correlation between the intensity of inflammatory cytokine responses and the development of toxic shock (Norrby-Teglund et al., 2000).

The benefit of superantigen production to *Streptococcus pyogenes* is not fully understood. It is hypothesised that the devastating secretion of cytokines subverts the normal immune response, and thereby contributes to immune evasion (Sundberg et al., 2002).

3.2.3 Differences in susceptibility

Only a minority of patients infected with a *Streptococcus pyogenes* strain, capable of initiating Streptococcal toxic shock syndrome, actually develop toxic shock. It turns out that regulation of superantigens expression is influenced by interaction with the host. Thus, host factors play a significant role in infection outcome. For instance, patients with invasive disease have lower concentrations of superantigen-neutralising antibodies then do controls (Basma et al., 1999). Moreover, there seems to be a direct correlation between specific MHC II types (virtually all individuals have a distinct MHC II type) and the tendency to develop this shock syndrome. Susceptible MHC II types show a higher bindings strength of the superantigen-MHC II interaction. Moreover, the binding- strength has a direct influence on the degree of cytokine production (Llewelyn et al., 2004). Some evidence support the idea that toxic shock susceptibility is gender related, partly related to oestrogen (Faulkner et al., 2007). However, this relation is still under debate and requires further investigation.

Box 4. Case report:
A previously healthy 52 year old woman came to her general practitioner with acute pharyngitis. Although he prescribed her glucocorticosteroids, her condition worsened in the next two days. Her body temperature and heart rate were elevated, she was hypotensive, and suffered from nausea, diarrhoea, and increasing stomach pains. The following day het general practitioner found her at her home in shock and she was immediately admitted to the hospital.
The patient made a very ill impression. Her abdomen seemed swollen and her pulse rate and blood pressure were unstable. A CT-scan revealed a considerable amount of fluid in the abdomen. The medical staff believed to deal with an inflammation of the peritoneum (membrane lining part of the abdominal cavity) or an acute appendicitis, which could explain the shock and abdominal fluid. An operation was performed to check the digestive and internal genital organs, but all looked well. However, a culture of the abdominal fluid and blood showed the presence of the bacteria *Streptococcus pyogenes.* The final diagnosis read streptococcal toxic shock syndrome, and antibiotic treatment was started immediately.
The patient was transferred to the intensive care unit, where she developed red rash on the face, chest and extremities. Additionally, the patient had developed multi organ failure and had to be carefully monitored. During the following weeks she recovered gradually. Eventually, ten weeks after admission, the patient left the hospital in a fairly good condition. (Van den Bossche et al., 2008)

3.3 What caused the rise in severe invasive infections?

In 1924 streptococcal gangrene (Necrotising fasciitis) was treated with surgical removal of all dead and affected tissue. A reduction of mortalities to 20% were achieved using this approach, even though this was well before the discovery of antibiotics (Meleney et al., 1924). In contrast, a recent study in Europe reports a fatality rate of 32% (Lamagni et al., 2008), even with antibiotics and surgery. Additionally, modern day streptococcal infections seem to progress more rapidly, spreading faster to various tissues (destroying skin, fascia, muscle and fat), and often patients die within two or three days (Stevens et al., 1989). These findings suggest that *Streptococcus pyogenes* strains have become more virulent in the past 30 years.

Streptococcus pyogenes can be divided in several strains, classified according to genetic variation, which is a common method to characterise bacterial pathogens (Beres et al., 2002). *Streptococcus pyogenes* is classified on the basis of differences in the M protein (a cell-surface molecule with anti-phagocytic properties), resulting in more than 130 M type strains (Cunningham, 2000). The strains all express a different set of virulence factors, which may contribute to the observed variability in virulence (Banks et al., 2004). Furthermore, there appears to be an association between certain M types and types of infection. For example, invasive diseases, such as Necrotising fasciitis, have been associated with M1 strains (Beres et al., 2002). A point of concern is that the prevalence of

this strain is increasing in parts of Europe (Stevens, 2001).

The variability in virulence among the different strains results from bacterial viruses, so-called bacteriophages (Banks et al., 2004). When bacteriophages inject their genetic material into a bacterium, the genetic material can integrate with the bacterial genome. Thus, the viral genes stay in the bacterial population, as every bacterial replication replicates the bacteriophage genes as well. Up to 10% of the streptococcal genome may consist of viral genes (Banks et al., 2002). Interestingly, most superantigens are encoded by bacteriophage genes integrated in the bacterial chromosome. Moreover, bacteriophages can transfer virulence factors, such as superantigens, from one strain to another, transforming non-virulent strains in highly virulent strains (Vojtek et al., 2008). It is hypothesised that bacteriophages are responsible for the rise in severe invasive infections caused by *Streptococcus pyogenes* during the late 20th century (Beres et al., 2002).

4. Discussion

Streptococcal toxins are potent virulence factors and contribute to devastating illnesses such as Necrotising fasciitis and Streptococcal toxic shock syndrome. However, toxins have shown to be useful in scientific as well as in medical respect. Toxins have improved our basic knowledge of cell biology. For instance, cholera toxin and pertussin toxin provided a lot of information about crucial molecular mechanisms within the cell. In addition, several potent cytotoxins are under investigation as potential cancer therapies, e.g. the plant toxin ricin (Kreitman et al., 2006). The powerful bacterial botulinum toxin is already clinically used against muscle spasms (Wissel et al., 2001).

Even a toxin of *Streptococcus pyogenes* has proven to be medically useful. This toxin, streptokinase, gives *Streptococcus pyogenes* the ability of penetrating barriers (such as extracellular matrix, basement membranes and endothelial cell layers) (Lähteenmäki et al., 1995), as it dissolves fibrous proteins. Streptokinase forms a complex with the inactive plasminogen and catalyses the conversion to plasmin, which is the active form (Ringdahl et al., 1998). In the blood, streptokinase-plasmin complex promotes the solution of blood clots, which makes it pharmacologically useful (Bisno et al., 2003). Interestingly, plasminogen activated by streptokinase cannot be inhibited by its normal inhibitor, making it a powerful therapy against blood clots (Cederholm-Williams et al., 1979). Streptokinase is prescribed to patients suffering pulmonary embolism, vein thrombosis or other thromboembolisms.

In conclusion, *Streptococcus pyogenes* is a dangerous bacterium and the fact that its virulence increases is disturbing. Because the infection progresses rapidly and the symptoms are often unspecific, it is difficult to identify the disease in time. Knowledge about the pathogenesis and toxins may contribute to an early and correct diagnosis. Although streptococcal toxins play a considerable part in invasive diseases, scientific knowledge about the toxins can also contribute to drug development and aid in the struggle against other diseases.

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