Platelets and their immunomodulatory roles during Streptococcus pyogenes infection

Hurley, Sinead

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SINÉAD M. HURLEY
DEPARTMENT OF CLINICAL SCIENCES | FACULTY OF MEDICINE | LUND UNIVERSITY

Sepsis and invasive bacterial infection is a major cause of human disease and death worldwide. *Streptococcus pyogenes* is one of the major pathogens responsible for bacterial infections, which range from mild to severe and life threatening conditions. *S. pyogenes* contains a cell wall anchored M protein, which is an important virulence factor that can interact with many cells of the immune system.

One of the smallest and most rapidly responding cells in our bloodstream is platelets. There are hundreds of millions of platelets in our blood system, which demonstrate multifaceted roles. They mend and seal our blood vessels at the site of vasculature damage and recruit, alert and activate other majorly important immune cells some of which have been investigated herein.

Collectively, this thesis has revealed multiple strategies for *S. pyogenes* to modulate the host immune response during invasive bacterial infection by interacting directly and indirectly with cells of our immune system: platelets, neutrophils, monocytes and endothelial cells. These interactions may have far reaching consequences during the pathogenesis of sepsis.

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Faculty opponent

Dr. Steven W. Kerrigan

Royal College of Surgeons, Dublin, Ireland

Organization
LUND UNIVERSITY
Department of Clinical Sciences
Division of Infection Medicine
BMC B14
22184 Lund, Sweden

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Abstract

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Platelets can bind to neutrophils, resulting in the formation of heterotypic complexes, however the function of neutrophils within these complexes has not been described. In *paper I,* we investigated platelet-neutrophil complexes (PNCs) generated in response to thrombin, a key factor involved in coagulation and compared these to complexes formed in response to *S. pyogenes* M1 protein. We determined that platelet dependent neutrophil activation occurs in response to thrombin, while *S. pyogenes* M1 protein compromised neutrophil functions by a platelet-dependent mechanism. In addition, this was dependent on donor specific IgG against M1 protein. This paper highlights the modulation of neutrophil function by platelets during inflammation and infection.
In *paper II,* platelet aggregation and platelet-leukocyte complex formation was investigated in whole blood in response to *S. pyogenes* bacteria. Platelet aggregation occurred and heterotypic complexes were formed between activated platelets and neutrophils and monocytes. Platelet dependent activation of these leukocytes was observed and bacteria were associated with the platelet and leukocyte complexes. The platelet aggregates remained stable over time, were viable and did not disaggregate. Taken together this study provides new insights into the role of platelets and heterotypic complex formation for bacterial survival in blood.
In *papers III and IV,* the contribution of platelets and the kinetics of platelet activation during invasive *S. pyogenes* infection were investigated *in vivo* in a mouse model. We demonstrated that during a bacterial infection thrombocytopenia, neutrophil activation and platelet-neutrophil complex formation occurs. The acute pro-inflammatory response to the infection and diminished bacterial dissemination in platelet-depleted animals indicated that platelets contribute to the immune response. In *paper IV* we determined that monitoring platelet activation, particularly PNC formation might provide prognostic information during the progression of sepsis. Platelet activation occurred and aggregated platelets accumulated in the liver at the late stages of sepsis.
In *paper V,* we investigated the effect of the *S. pyogenes* M1 protein on endothelial cells *in vitro* using two cell lines. We have demonstrated that M1 protein binds to endothelial cells, increases endothelial cell vascular permeably in a TLR-2 and Rho kinase dependent manner and generates limited cytokine release. This may reflect an innate immune recognition of the bacterial protein, however increased vascular permeability and vascular leakage has also been reported to contribute to the pathogenesis of *streptococcal* infection.

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Sinéad Hurley

LUND
UNIVERSITY

Sinéad M Hurley
Division of Infection Medicine,
Department of Clinical Sciences,
Lund University,
Sweden

The cover represents a drawing of a Platelet-Neutrophil complex containing S.pyogenes and its surface
associated M-protein and an image of platelets (red) in complex with neutrophils (blue), embedded in a
fibrinogen network (green) generated in response to M1 protein.

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For my family, myself and precious ones =)

Sláinte

PREFACE

“ A book is not a book unless it is an experience”

This PhD for the last number of years has been a complex mix of challenge and commitment but it has also been rewarding and fulfilling. As scientists we always question “why”? We constantly search to explore, to invent, to change, to create and to inspire. It is our willingness to seek and strive, to rediscover the known and discover the unknown that earns us the title of “scientist”. My journey throughout these last years has certainly been a journey of discovery.

My interest in the human body and its ability to protect and defend us from all harm absolutely fascinates me and this interest has developed through working on the studies that are presented within this thesis today. When I initially embarked on this PhD one could say that I was assailed by the “flight or fight syndrome”, however with the endless support, enthusiasm, knowledge and friendship from those around me it has been a very fulfilling and rewarding Journey.

I have invested much time in conducting this research, realising that it was not a sprint but a marathon. Still, time spent doing what you desire is time well spent.

The starting point of all achievement is desire. Desire was the key to all my motivation but it was my unrelenting determination and commitment to the pursuit of my goals - a commitment to excellence - that enabled me to attain the success, which I sought and present herein. It has been a true learning experience.

Dear friends & scientists, here’s to you, I hope you enjoy reading it.

Beatha agus sláinte daoibh go léir

Sinéad M Hurley.

Sinéad M Hurley

Lund, 23th of February 2016

CONTENTS

ABSTRACT

Sinéad M. Hurley

Sepsis and invasive bacterial infection is a major cause of human disease and death worldwide. *Streptococcus pyogenes* is one of the major pathogens responsible for bacterial infections, which range from mild to severe and life threatening conditions. *S. pyogenes* contains a cell wall anchored M protein, which is an important virulence factor that can interact with many cells of the immune system. One of the smallest and most rapidly responding cells in our bloodstream are platelets and they are a main focus for the studies presented in this thesis and have been investigated.

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In *papers III and IV,* the contribution of platelets and the kinetics of platelet activation during invasive *S. pyogenes* infection were investigated *in vivo* in a mouse model. We demonstrated that during a bacterial infection thrombocytopenia, neutrophil activation and platelet-neutrophil complex formation occurs.

The acute pro-inflammatory response to the infection and diminished bacterial dissemination in platelet-depleted animals indicated that platelets contribute to the immune response. In *paper IV* we determined that monitoring platelet activation, particularly PNC formation might provide prognostic information during the progression of sepsis. Platelet activation occurred and aggregated platelets accumulated in the liver at the late stages of sepsis.

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ORIGINAL PAPERS

Paper I

Platelet-dependent neutrophil function is dysregulated by M protein from *Streptococcus pyogenes*.
Sinéad M Hurley, Fredrik Kahn, Pontus Nordenfelt, Matthias Mörgelin, Ole E Sørensen, Oonagh Shannon.

Infection and Immunity. 2015 Sep; 83 (9): 3515-25.

Paper II

Streptococcus pyogenes induces platelet activation in human blood and modulates leukocyte function in a platelet dependent manner.
Lisbeth Svensson#, **Sinéad M Hurley#,** Oonagh Shannon.

Manuscript (# equal contribution).

Paper III

Platelets promote bacterial dissemination in a mouse model of streptococcal sepsis.
Fredrik Kahn, **Sinéad M Hurley**, Oonagh Shannon.

Microbes and Infection. 2013; 15 (10-11): 669-76.

Paper IV

The dynamics of platelet activation during the progression of streptococcal sepsis.
Sinéad M Hurley, Nataliya Lubuy, Bo Holmqvist, Oonagh Shannon.

Manuscript (submitted)

Paper V

M1-protein from *Streptococcus pyogenes* increases endothelial vascular permeability and mediates cytokine release.
Sinéad M Hurley, Jana Fisher, Pontus Nordenfelt, Oonagh Shannon.

Manuscript

ADDITIONAL ARTICLES
not included in this thesis

Active but inoperable thrombin is accumulated in a plasma protein layer surrounding *Streptococcus pyogenes*.
Clement Naudin, **Sinéad M Hurley**, Erik Malmström, T Plug, Oonagh Shannon, J C M Meijers, Matthias Mörgelin, Lars Björck, Heiko Herwald.

Thromb Haemost. 2015 May 21; 114 (3)

ABBREVIATIONS

PNC, Platelet-neutrophil complex

S. pyogenes, Streptococcus pyogenes

PRR, Pattern recognition receptor

PAMP, Pathogen associated molecular pattern

DAMP, Damage associated molecular pattern

TLR, Toll-like receptor

fMLF, Formyl-methionyl-leucyl-phenylalanine

Fab, Fragment antigen binding

Fc, Fragment crystallizible

G-CSF, Granulocyte colony stimulating factor

Spe B, *Streptococcal* pyrogenic exotoxin B

SLO, Streptolysin O

SIC, *Streptococcal* inhibitor of complement

ECM, Extracellular matrix

MHC, Major histocompatibility complex

SIRS, Systemic inflammatory response syndrome

DIC, Disseminated intravascular coagulation

TNF-α, Tumor necrosis factor alpha

IL-Interleukin

HBP, Heparin binding protein

ICAM-1, Intercellular adhesion molecule-1

TPO, Thrombopoietin

TGFβ, Transforming growth factor β

PF4, Platelet factor 4

Groα, Growth-regulating oncogene-alpha

RANTES, Regulated on activation, normal T cell expressed and secreted

OCS, Open canalicular system

CAM, Cell adhesion molecule

ITAM, Immunoreceptor tyrosine-based activation motifs

ADP, Adenosine diphosphate

ATP, Adenosine triphosphate

vWF, von Willebrand Factor

PAR, Protease activated receptor

GP-, Glycoprotein

PSGL-1, P-selectin glycoprotein ligand-1

TF, Tissue factor

Mac-1, Macrophage-1 antigen

Ig, Immunoglobulin

SAg, Superantigen

LPS, Lipopolysaccharide

ClfA, Clumping factor A

HIV, Human immunodeficiency virus

ICAM-2, Intracellular adhesion molecule-2

JAM-3, Junctional adhesion molecule 3

CD40L, CD40 ligand

NETs, Neutrophil extracellular traps

MPO, Myeloperoxidase

NGAL, Neutrophil gelatinase associated lipocalin

GTP, Guanosine triphosphate

LAD, Leukocyte adhesion deficiency

PAF, Platelet activating factor

FPR, Formyl peptide receptor

CR3, Complement receptor 3

ROS, Reactive oxygen species

CGD, Chronic granulomatous disease

HSC, Hematopoietic stem cell

WPB, Weibel-Palade body.

MCP-1, Monocyte chemoattractant protein-1

LFA-1, Leukocyte-function associated antigen-1

PECAM-1, Platelet endothelial cell adhesion molecule-1

VE-cadherin, Vascular endothelial-cadherin

VCAM-1, Vascular cell adhesion molecule-1

IAP- Integrin associated protein

VLA-4, Very late antigen 4

IAP, Integrin associated protein

APC, Activated protein C

TFPI, Tissue factor pathway inhibitor

AT, Antithrombin

PAI-1, Plasminogen activator inhibitor-1

"Life can be much broader once you discover one simple fact, and that is;

- *Everything around you that you call life, was made up by people that were no smarter than you. And you can change it, you can influence it, you can build your own things that other people can use." Steve Jobs*

-

1. THE IMMUNE SYSTEM- OUR PROTECTOR AND DEFENDER

Host-Pathogen Interaction

The host is constantly exposed to bacteria on a daily basis and our bodies are continually being colonised by bacteria, including non-pathogenic commensals within the human micro-biome, such as those found on the skin and mucosal surfaces in particular the gastrointestinal tract. All individuals are unique in their micro-biome content (1). The human body possesses a very potent immune system to defend and protect from bacterial infection. However, despite this very efficient immune system, which is amply attempting to eradiate invading bacteria, pathogens have developed ways to overcome our defence systems and invade and damage the bodies' tissues. This thesis addresses aspects of host-pathogen interaction within the bloodstream.

The cells of our bloodstream- Transporters, Defenders, Preventers!

Erythrocytes or red blood cells (RBCs) are the most abundant cells in the blood, essential transporters that carry oxygen around the body and carbon dioxide away to be eliminated out from the body. The leukocytes in the blood consist of five main types; neutrophils, eosinophils, basophils, monocytes and lymphocytes which together account for between 4-11 x 10^9 cells/L of blood (2). They are defenders and protect us from harmful threats, including bacteria. Neutrophils, monocytes and macrophages are some of the key professional phagocytic cells that play central roles in preventing and resolving a bacterial invasion or inflammation. These cells originate from the myeloid progenitor cell in the bone marrow.

Platelets, the smallest of all the blood cells range from 150-400 x 10^9/L in healthy individuals (3). These cells have a small discoid shape. Together with the coagulation system platelets form blood clots and prevent blood loss during vascular damage allowing maintenance of vasculature integrity. These cells circulate in an inactive state in the blood but rapidly become activated in response to vessel damage and this activation is attributed to their very specialised structure.

The Innate & Adaptive immune response

The human body protects us from infection by two interlinked defence systems, the innate and the acquired immune system. The innate immune system is the first defence mechanism against an invading pathogen or in response to tissue injury or damage. This response is rapid and consists of physical barriers such as the skin and mucosal membranes and chemical barriers such as host derived chemicals and our innate immune cells. The innate immune system is comprised of the following; phagocytes such as neutrophils and macrophages, dendritic cells, the coagulation system, the complement system and other cells that participate in the innate immune response such as epithelial and endothelial cells.

The complement system forms part of the immune response and consists of three main pathways, the classical, the alternative and the lectin pathway. The final step results in the generation of the membrane attack complex (MAC) that may mediate cell lysis. The central step for all pathways is the generation of C3 convertase (4), which cleaves C3 into C3a and C3b and can attach to the surfaces of microbes, coating and opsonising the microbe.

Host pattern recognition receptors (PRRs) form the backbone of the innate immune response by distinguishing self from non-self by recognition of pathogenic associated features and patterns called pathogen associated molecular patterns (PAMPs) (5) (6). The innate immune system also recognises molecules released as a result of tissue damage, known as damage associated molecular patterns (DAMPs) (7,8). Different types of PRRs exist including, the Toll-like receptors (TLRs), Nod-like receptors, C-type lectin receptors and retinoic acid-induced gene (RIG)-1-like receptors (9). There is also the f-Met-Leu-Phe receptor (fMLP) (10). This receptor recognises bacterial peptides that are released when combating an infection.

Inflammation

Inflammation is a protective innate host response that is composed of four key components which are written in Latin as they were first described by the roman scholar Celsus; calor (heat), rubor (redness), dolor (pain) and tumor (swelling, oedema) (11). When the host receives signals, generated externally from pathogens or internally from tissue damage, an inflammatory response is initiated. An inappropriate host response that results in too little or too much inflammation will lead to progressive tissue damage.

The calor is derived from vasodilation, where a rise in blood volume results in heat and then redness. Initial responses involve changes in the vasculature, whereby blood flow is increased so that cells and plasma proteins can be transported to the local area and so the healing process can begin (Figure 1). The blood vessel walls become more permeable so that solutes can pass leading to fluid leakage (edema). Solutes can exudate to the tissues and within a few hours the process of extravasation takes place whereby granulocytes, particularly neutrophils can enter the tissue and facilitate the repair process. Leukocyte transmigration is a key event in the accumulation of effector cells at the site of damage (12).

The coagulation system plays a role in prevention of blood loss and consists of primary and secondary hemostasis (13). In primary hemostasis the goal is to prevent blood loss as a result of vascular damage. The coagulation system does this through platelet activation and platelet plug formation. In secondary hemostasis, clotting factors are initiated which results in fibrin deposition and further contribute to the cessation of blood loss (14). The coagulation system consists of a cascade of serine proteases, which become activated during the cascade process. The coagulation system is also involved in inflammation and results in fibrin deposition (15). Fibrinogen and fibronectin are deposited and dying cells contribute to pus formation during inflammation. Finally the process of resolution occurs and normal tissue architecture is resolved (16). In response to tissue damage, due to prolonged inflammation a more severe chronic cellular response occurs whereby macrophages and lymphocytes infiltrate the damaged area.

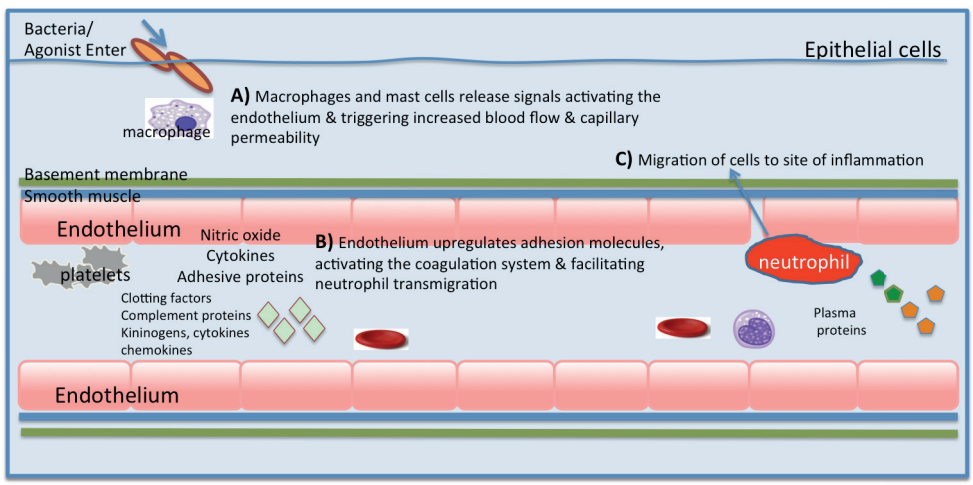

Figure 1: Stages of inflammation.

The innate immune system responds immediately to eliminate invading pathogens. In contrast, a second branch of our immune system, the adaptive immune system responds more slowly on first encountering a pathogen. However, this response is more specific and consists of an immunological memory, which means a more finely tuned and heightened pathogen specific response can be mobilised when the same pathogen is encountered again (17). The primary cells involved are the lymphocytes in communication with antigen-presenting cells. B- and T-lymphocytes respond to microbial invaders by production of antibodies or generation of cell-mediated responses, respectively (18).

Antibodies produced by activated B cells bind microbes and facilitate their elimination via phagocytosis or complement activation. Antibodies exist in five different classes of which the IgG class is the most abundant throughout the body. The IgG antibody is composed of two regions, the Fab fragment (Fragment antigen binding), which recognises and binds to antigens and the Fc region (Fragment crystallizible) whereby the molecule binds receptors (Fc receptors) and interacts with cells of the immune system.

The Fc receptors expressed by phagocytes are responsible for binding to the IgG class of immunoglobulins are known as the Fcγ receptors (19). Fc receptors bind to antibodies at their Fc region, which may be coating the surface of microbes, and this process induces phagocytosis. An important mechanism of phagocytosis is FcγR- mediated phagocytosis. Human cells express six Fcγ receptors (Fcγ-RI, -RIIA, -RIIB, -RIIC, -RIIIA and FcγRIIIB). Neutrophils constitutively express the Fc receptors, FcγRIIa (CD32), FcγRIIIb (CD16) and FcγRIV (20,21) and they express FcγRI (CD64) upon stimulation with granulocyte colony stimulating factor (G-CSF) (22).

The ability of the host to distinguish self from nonself is an important phenomenon of our immune system. Generally, host PRRs do this flawlessly because they recognise a molecular pattern that is only produced by the pathogen and not by the host. In contrast, receptors of the adaptive immune system occasionally recognise self-antigens, which may lead to autoimmune disease. The immune system must respond adequately to protect us but it must not over-respond and result in over activation and inflammation, which would be deleterious to the host. *S.pyogenes* can cause mild infections but can also lead to severe infectious diseases such as sepsis. In sepsis our immune system responds in an uncontrolled manner and it is this over-activation of the host response itself that leads to the severity of the disease.

In the following sections I will discuss these host systems and host-pathogen interactions in greater detail.

2. *STREPTOCOCCUS PYOGENES*

Streptococcus comes from the Greek words (strɛptəʊˈkɒkə); streptos meaning "chain" and kokkos meaning "grain/berry". The name is derived from the fact that streptococci are linked together in cocci chains like a pearled necklace. The streptococcal bacterium was isolated in 1879 by Louis Pasteur, from blood cultures from a woman with puerperal sepsis. It wasn't until 1884 that Friedrich Rosenbach described the bacteria, which he isolated from wounds and skin infections, and coined the actual term *Streptococcus pyogenes* for the first time (23).

Approximately 10% of the adult population are asymptomatic carriers of this pathogen (24,25). *S. pyogenes* infections range from mild superficial skin infections such as pharyngitis and impetigo to life-threatening diseases such as necrotizing fasciitis, sepsis and streptococcal toxic shock syndrome. There are approximately 1.78 million new severe GAS infections worldwide each year for example due to rheumatic fever, rheumatic heart disease and invasive disease (26).

S. pyogenes is a Gram-positive facultative anaerobic bacterium which was classified as Group A streptococci by Rebecca Lancefield based on the carbohydrate composition of the antigens found on the bacterial cell wall (27). Another mechanism of classification was introduced in 1928 based on variations of M-protein on the surface of *S. pyogenes* (28). This forms the basis of serotyping of the bacteria, however nowadays this is based on sequencing of the emm gene that encodes for the M protein.

Group A *streptococcus* (GAS) stimulates the innate immune system, causing local inflammation and damage, resulting in the initiation of an immune response. *S. pyogenes* has developed many mechanisms that enable it to penetrate the constitutive host defence systems and in some cases cause invasive disease. The *S. pyogenes* genome has been sequenced and multiple genes encode for virulence factors (29). *S. pyogenes* produces a wide range of virulence factors which allow the pathogen to attach to host tissue, degrade tissue proteins, multiply and spread in the host (Figure 2). The bacteria can be surrounded by a carbohydrate capsule composed of hyaluronic acid, a peptidoglycan cell wall and proteins are embedded in the cell wall such as protein H, F and M protein that contribute to bacterial attachment and invasion of host cells (30,31). The capsule has been shown to prevent phagocytosis (32) and may alter the response of antibodies to bacterial products or the M protein. The bacteria can secrete proteases for example streptococcal pyrogenic exotoxin B (Spe B), streptokinase and streptolysins that facilitate tissue invasion (33) (34). Streptolysin O (SLO) is a pore forming toxin that can destroy red blood cells, leukocytes and other cell types.

Secreted IdeS cleaves IgG at the hinge reason thereby inactivating Fc effector functions (35) (36,37). *Streptococcal* inhibitor of complement (SIC) described in 1996 was initially reported to inhibit formation of the membrane attack complex during complement activation (38), however it has more recently been reported to bind to and neutralise antimicrobial peptides.

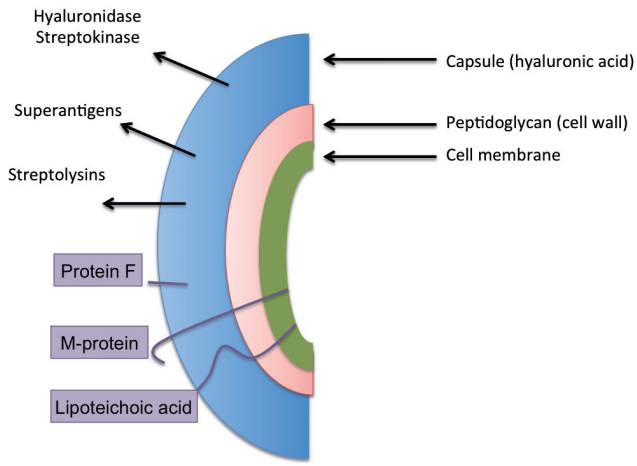

Figure 2: *Streptococcus pyogenes* stucture & virulance factors.

Plasminogen

Following activation, plasminogen is converted to plasmin, a protease that can breakdown fibrin clots, adhesion molecules, the extracellular matrix (ECM) and connective tissue (39,40). *S pyogenes* secretes streptokinase which binds and activates plasminogen (41). In addition to degrading fibrin, plasmin can also bind fibrinogen (42) and this increases the streptokinase induced plasminogen activation (43). This is reported to play a significant role in the pathogenesis of invasive *S. pyogenes* infection (44) and in enhancing the dissemination of the bacterium from the local infection.

Superantigens

There are five classes of superantigens (I-V). They are toxic substances that bind to major histocompatibility complex class II and rapidly trigger an uncontrolled proinflammatory response (45,46) and toxic shock syndrome (47). These antigens activate T cells non-specifically i.e. they do not undergo the normal mechanism of antigen processing and presentation but directly bind to the T cell receptor. These bacterial toxins can stimulate an inflammatory and cytokine storm and very low concentrations of bacterial SAgs (< 0.1 pg/ml) are sufficient to induce toxic levels of cytokines in the bloodstream and an uncontrolled adaptive immune response (48). *S. pyogenes* can release a number of different superantigens. In addition, the M protein that is released from the *S. pyogenes* surface exhibits superantigenic properties (49).

M Protein

M-protein is one of the main virulence factors that cover the surface of *S. pyogenes*. M-protein is normally anchored on the bacterial surface but can be released by the action of cysteine proteases secreted from the bacteria (50), neutrophil proteases (51) and small quantities of M protein can be shed from the bacteria during growth *in-vitro* (52). M protein has a α-helical fibrillar coiled-coil structure and each homodimer chain of M-protein is composed of four repeating domains (Figure 3 A-D), each with various host interacting partners and functions during infection. A structural model of M protein was first proposed by Fischetti in 1989 and 1991 (53) (54). The M protein was later confirmed to have irregularities within the coiled-coil structure that facilitated interaction with host proteins, including fibrinogen (55). M protein can facilitate attachment to host cells and tissues for example it can bind to extracellular matrix and cell surface glycosaminoglycans (56), it can bind directly to extracellular matrix components such as fibronectin (57) and indirectly these molecules bind to host cells and thereby act as bridges, promoting uptake of bacteria into these cells (58,59).

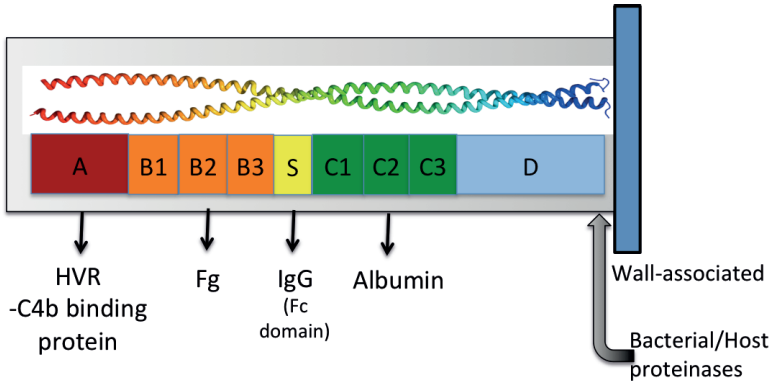

Figure 3: Each chain of the M protein includes different regions (A-D) that have different sizes and amino acid sequences. A - amino-terminal region or hypervariable region (HVR), B- Binds Fibrinogen, S- IgG binding region, C & D- Both the C and D region can bind factor H and serum albumin, D- Contains an LPxTG motif that attaches to the peptidoglycan which is required for correct anchoring of the protein in the cell wall.

The antigenic diversity at the N-terminal hypervariable region (HVR) is the basis of M-protein serotyping. Type specific antibodies are directed against this HVR and have been proposed to confer protection against subsequent infection with the same serotype (60). There are >100 serotypes of the M protein known (60) (61) but only a few are particularly associated with invasive disease where M1 and M3 serotypes in particular have dominated (62).

M protein is strongly anti-phagocytic because it can bind plasma proteins and the Fc region of IgG antibodies thereby preventing activation of complement. Complement regulatory components can bind to the HVR of M protein and limit complement activation on the bacterial surface, for example C4b-binding protein which degrades C3 convertase and decreases opsonisation (63-65). M protein of certain serotypes may bind factor H and prevent C3b binding to the bacteria. Another mechanism that prevents complement activation is the binding of the plasma protein, fibrinogen to M protein. Fibrinogen acquisition by *S. pyogenes* was first described by Kantor, however the importance of this interaction for bacterial pathogenesis was not clarified in this study (66). In 1985, *Whitnack et al* demonstrated that when fibrinogen binds to M protein the binding of C3b is inhibited and this prevents activation of the alternative complement pathway and phagocytosis (67). Fibrinogen binding has also been shown to prevent complement activation by blocking the classical complement pathway (68).

The M protein - fibrinogen complex has been show to protect against phagocytosis of group A streptococci (69). In addition the recruitment of the Fc region of IgG to the bacterial surface is also a mechanism to prevent complement activation and bacterial killing (70). Furthermore, M protein contributes to intracellular survival of *S. pyogenes* that has been phagocytosed by neutrophils (71).

The released M1 protein has potent pro-inflammatory properties that are associated with the formation of a complex between M1 protein and plasma fibrinogen. This complex mediates neutrophil activation and release of vasoactive substances (such as heparin binding protein) and has been shown to induce vascular leakage in animal models of disease (51,72,73). Furthermore, M1 protein stimulates neutrophil and mast extracellular formation and the bacteria can survive inside these extracellular traps (74). M1 protein activates platelets (75), monocytes (76) and epithelial cells (77) and initiate's an inflammatory response. In addition, the M protein can also interact with the coagulation system where it has been shown to cause tissue factor production from monocytes (78) and contact system activation at its surface with the release of bradykinin, a potent mediator of inflammation (79).

Severe bacterial infection: Sepsis

When *S. pyogenes* disseminates into the bloodstream, life-threatening disease and sepsis may result. Sepsis is the result of a systemic inflammatory immune response to a bacterium, characterised by a number of clinical parameters. The definition of sepsis that is in use today arose from the sepsis consensus conference held in the USA in 1991 led by Prof. R.C. Bone and colleagues (80). This was later refined at another sepsis consensus conference in 2001 to enhance the definition to contain additional added criteria (81). Sepsis is a complex and life-threatening condition that has a high mortality rate in those severely affected individuals and despite continuous research and continually emerging antibiotics, sepsis still remains a very complicated condition to detect and treat.

Each year in the US there are approximately 750,000 new cases of severe sepsis (82) and in 2001, sepsis resulted in approximately 215,000 deaths (83). The initial clinical symptoms for diagnosing sepsis include the systemic inflammatory response syndrome (SIRS) criteria; fever, high temperature, increased respiratory rate, elevated heart rate and an altered white blood cell count (Figure 4) (80).

There are three clinical stages of sepsis; sepsis, severe sepsis and septic shock and each have a different definition depending on the severity of the clinical symptoms (Figure 4) (84). One important cardiovascular change in these individuals is hypotension, low blood pressure and if this occurs rapidly, in addition to other symptoms and the patient are irresponsive to fluids, septic shock can be diagnosed which is the phase with the highest mortality rate (70-80%) (85,86).

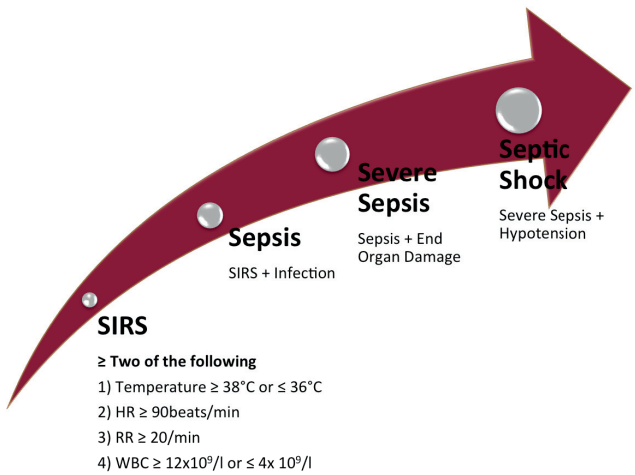

Figure 4: The classification of Sepsis. The initial clinical presentation is the systemic inflammatory response syndrome (SIRS) and then a progession to sepsis, severe sepsis and septic shock.

Sepsis involves a deregulation of both pro- and anti-inflammatory pathways. The inflammatory response generated during sepsis is very often followed by a period of immune suppression or immunoparalysis which can persist for days and involves bacterial endotoxins and superantigens (SAgs) (87,88).

During sepsis there may be a high or low neutrophil count (Figure 4) or the presence of greater than 10% immature blast cells and the neutrophils function is generally altered (89) and impaired for example an impaired neutrophil chemotactic ability was seen in patients with severe sepsis (90). The gene expression of the neutrophil may be increased during sepsis (91) and becomes altered as the condition progresses.

This leads to a gene suppression of proinflammatory molecules and a decreased production of neutrophil reactive oxygen species (ROS). The result may be an abnormal recruitment of neutrophils to sites of inflammation and a subsequent accumulation of these cells, which have deregulated responses.

Some different theories have been proposed about the immune response during sepsis for example according to *Hodchkiss et al.* one theory proposed that both pro- and anti-inflammatory responses occur during sepsis where patient deaths are due to more of an immunosuppression and a second theory proposed that deaths from sepsis are more due to a stronger innate immune response than the adaptive response (87). Sepsis is a very complicated phenomenon and a lot more work is still to be done to better understand the role of the immune system.

Coagulation and Sepsis

Sepsis and septic shock involve a massive dysregulation of the pro- and anti-inflammatory systems, but also the coagulation system. Significant crosstalk occurs between coagulation and inflammation. During sepsis the coagulation system is fully activated and thrombin is generated, which will result in the generation of a fibrin clot, activation of the endothelium and platelet activation. This will further escalate the coagulation system and in advanced sepsis disseminated intravascular coagulation (DIC) may occur. Furthermore, bacterial toxins can directly activate the coagulation system by upregulation of tissue factor (92) and indirectly activate the system through the activation of inflammatory molecules such as tumor necrosis factor alpha (TNF-α) and interleukins (93). The end result is a state of pro-coagulation and generation of fibrin clots. During DIC, systemic activation of the coagulation system occurs with fibrin deposition in small vessels, consumption of coagulation factors and regulators of coagulation. This can culminate in an increased bleeding risk in these patients. Platelets are affected during DIC. Thrombocytopenia occurs during invasive bacterial infection and is an independent marker of mortality (94,95). The activation and aggregation of platelets could be responsible for platelet consumption and the decreased platelet numbers observed in these individuals.

Sepsis Management

Sepsis diagnosis, management and treatment continues to remain a major health challenge due to the disease complexity, therefore identifying a reliable biomarker of sepsis is extremely difficult. Some of the current septic biomarkers include procalcitonin, cytokine biomarkers, such as IL-8 or IL6, mRNA expression in blood and in leukocytes, micro RNAs (miRNA) (96). Some cell receptors or adhesion molecules can also serve as biomarkers including soluble ICAM-1 or E-selectin. Heparin binding protein (HBP), also known as azurocidin is released from activated neutrophils during sepsis and is an inducer of vascular leakage (97). Plasma HBP was shown to be a prognostic marker of the severity of septic progression (98) and HBP was shown to be a possible marker of circulatory failure in septic patients (99).

Management and treatment for sepsis includes fluids, vasopressors, ventilation and antibiotic administration. Some studies are aimed at blocking inflammatory molecules for example TNF-α (100), however, targeting one specific molecule in sepsis usually is inefficient and many have failed in clinical trails (101). Due to the complex nature of sepsis finding a single and effective treatment is rare and a combination of treatments offer more potential.

3. THE POWERS OF PLATELETS

Platelets are small anucleate cells, 3μm in diameter and the human body has between 150-400 x 10^9 platelets per litre of blood, of which one hundred billion are produced daily from the bone marrow (102). Megakaryocytes are precursor cells produced from pluripotent stem cells in the bone marrow and James Homer Wright was the first to note that platelets are produced from megakaryocyte fragmentation in the bone marrow (103).

Within the bone marrow, maturation of the megakaryocyte takes place, which increase in size and content. The production of megakaryocytes in the bone marrow is mainly regulated by the platelet growth factor thrombopoietin (TPO) (104-107) which facilitates the in vitro culture and expansion of megakaryocytes and therefore advanced our understanding with regard to the platelet developmental process (108). Following maturation, the mature megakaryocytes then degrade their basement membrane and the cell cytoplasm is reorganised into beaded extensions called proplatelets (109-112). An intermediate before this step occurs in the sinusoidal blood vessel of the bone marrow and are now defined as preplatelets, which are larger than platelets and can convert into proplatelets (113). As proplatelets continue to develop and mature, platelet specific granules are taken along microtubule bundles to the proplatelets before finally releasing platelets into the blood vessel from their surface (114). Each megakaryocyte produces between 1000-3000 platelets and on average the platelet has a lifespan between 8-10 days in the circulation (115). Mouse platelets circulate in greater numbers than that of human platelets and have shorter lifespans (116,117).

Platelet Cytoskeleton

The small and discoid shape of the platelet allows them to be close to the vessel edge and therefore to quickly respond to vascular damage. Their unique shape is maintained by their cytoskeletal structure. Platelets have an open canalicular system (OCS) which serves as a conduct for granule secreted substances, to facilitate their transport to the platelet surface and to allow substances to be transported into the cell (118,119). Platelets contain small numbers of mitochondria which provide energy to the platelet for processes such as platelet activation and granule secretion (120). Glycolysis and oxidative phosphorylation are the main energy demanding process that take place during platelet activation and secretion (121).

Platelet Granules

Platelets have three main granules, α-granules, dense granules and lysosomes, of which the α-granules are the most abundant (122) (123). Platelet α-granules contain more than 300 proteins per platelet including adhesive proteins, coagulation clotting factors such as fibrinogen, fibrinolytic factors, cellular mitogens, proteases, growth factors and proteins (124). They contain cytokines and chemokines, for example transforming growth factor β (TGFβ) (125), platelet factor 4 (PF4), Growth-regulating oncogene-alpha (Groα) and regulated on activation, normal T cell expressed and secreted (RANTES). P-selectin is an important membrane protein stored in the α-granules of resting unactivated platelets. This cell adhesion molecule (CAM) becomes translocated from the platelet granules and upregulated to the platelet surface following platelet activation (126). Endothelial cells have also been shown (in 1989) to contain p-selectin stored in the Weibel-Palade bodies (WPBs) (127). In addition platelets release antimicrobial peptides from their α-granules for example after activation with thrombin, platelets release peptides called thrombocidins (128). Actin in the cytoskeleton plays a key role in the release of platelet α-granules by the action of actin polymerisation (129) (130). Microparticles are small vesicle structures that are shed from the plasma membrane of cells in the bloodstream. The release of microparticles involves actin and cytoskeleton detachment from the plasma membrane and primarily requires calcium (131).

In contrast, platelet dense granules are smaller and carry other activating factors such as adenosine triphosphate (ATP), adenosine diphosphate (ADP), serotonin and cations such as calcium and magnesium. Platelets have a few lysosomes, which store digestive and acid hydrolase enzymes including cathepsin, β-galactosidase and acid phosphatases. Platelets also have cytoplasm peroxisomes that store the enzyme, catalase.

Platelets express thousands of copies of receptors on their surface and these numbers are continuously expanding as research reveals new receptors with increasingly new functions. Among these, some receptors are present in platelet granules and are only expressed on the platelet surface upon activation. Platelets express TLR's, initially shown to express TLR1, TLR2 and TLR3 on both mouse and human platelets but further studies have demonstrated that platelets express TLR's 1-9 and some are functional (132-134).

Platelets express one receptor class for IgG, FcγRIIA (135). When Fcγ binds the Fc region of IgG a number of downstream signalling events occur including phosphorylation of tyrosine residues in the receptor within immunoreceptor tyrosine-based activation motifs (ITAMs) which begins with the Src family of tyrosine kinases (136). These signalling events have been shown to result in the activation of the platelet integrin GPIIbIIIa (137). Table 1 indicates some of the most important platelet receptors and their ligands.

Receptors	**Ligands**	**Family**	**References**
GPIIbIIIa	Fibrinogen, fibrin, vWF,fibronectin	Integrins	(138)
GPVI	Collagen	Ig Superfamily	
$P2Y_1$, $P2Y_{12}$	ADP	G protein-coupled receptors	(139)
PAR1, PAR4	Thrombin	G protein-coupled receptors	(139)
CD62P	PSGL-1, GPIb, TF	C-type lectin receptor family	
GPIb-IX-V complex	Thrombin, vWF, FXI, FXII, P-selectin, Mac-1	Leucine-rich repeat family	
GPIaIIa	Collagen	Integrin	
FcγR's	IgGs	Ig superfamily	
TPα	Thromboxane	G protein-coupled receptors	(139)

Table 1: Platelet receptors and their coresponding ligands.

Platelets & Haemostasis

Platelets patrol the vasculature in an inactive state, however following vessel injury or inflammation platelets are rapidly activated and responsive. Platelets are activated in response to agonists such as collagen, Adenosine diphosphate (ADP) and thrombin, which bind to their respective receptors on the platelet surface (Figure 5). ADP and thrombin bind to G-protein coupled receptors on the platelet and initiate a downstream signalling event, that results in a calcium influx which can result in further platelet granule release and platelet integrin conformational changes.

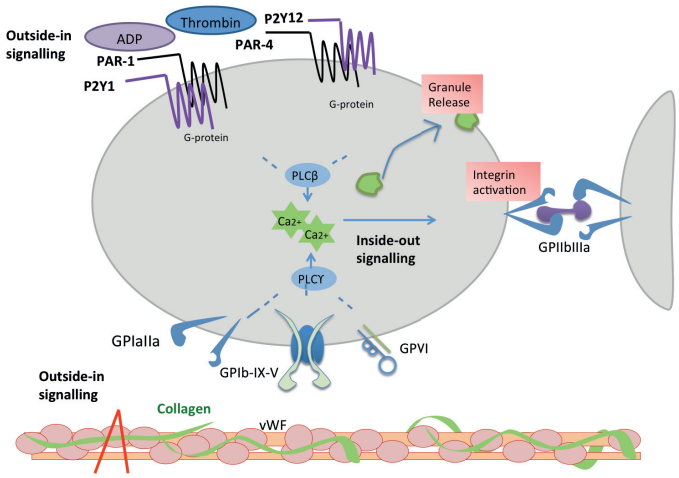

Figure 5: Platelets become activated and release their granules upon stimulation. Upon vascular damage collagen is exposed which can bind to platelet receptors and initiate a process of firm platelet attachment and endothelial transmigration.

During primary haemostasis platelets undergo a series of events that ultimately result in repair or cessation of blood flow (Figure 6). This is one of the primary functions of the platelet. Upon vessel injury, subendothelial extracellular matrix components are exposed such as collagen (140). One of the initial steps in platelet adhesion is the immobilisation of circulating von Willebrand Factor (vWF) on the collagen surface which exposes the vWF binding site and subsequently promotes platelet tethering and attachment (141,142). The A1 domain of collagen bound vWF interacts with GP-Ib-IX-V receptor complex on the platelet surface (143,144). Further interactions of vWF-GPIb are formed and platelet rolling occurs. Other more stable interactions occur that involve integrins including the collagen receptors GPIaIIa and GPVI and the GPIIbIIIa receptor.

The GPIIbIIIa receptor which is activated by inside-out signalling (145-147) binds fibrinogen which bridges adjacent platelets and recruits more circulating platelets to the site of damage resulting in further platelet-platelet interactions and formation of a platelet plug (148). The platelet plug is sufficient to cease blood loss in small vessels, however, if the injury is more extensive in the larger blood vessels, this in insufficient and secondary haemostasis occurs.

Secondary haemostasis is the stage where insoluble fibrin is generated (149), which stabilises the platelet plug. The process requires the regulated control of coagulation factors, cofactors and inhibitors to generate the protease thrombin, which initiates the formation of fibrin.

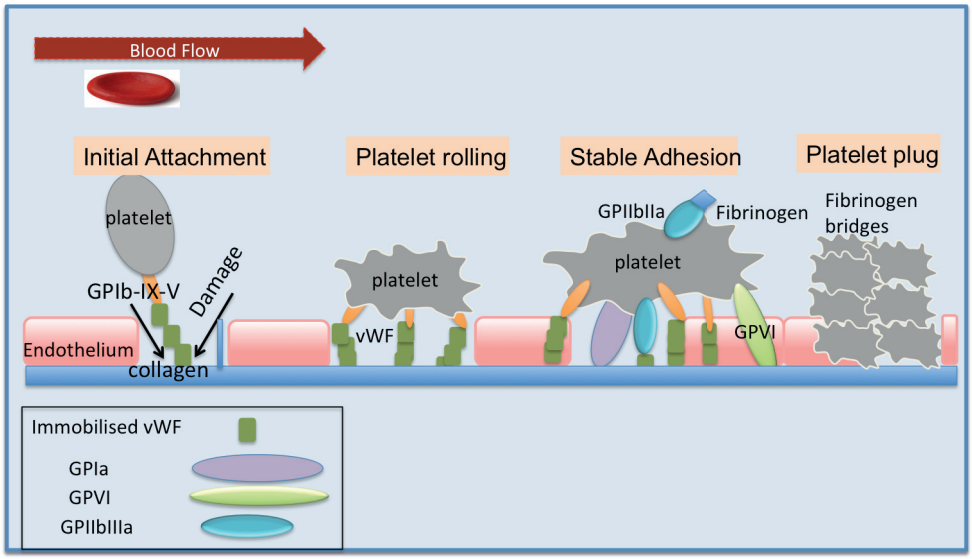

Figure 6: Schematic representation of the events involved in formation of a platelet plug, cessation of blood flow and repair of the damaged vessel.

Fibrinogen

Fibrinogen is a major plasma protein and coagulation factor in blood. The molecule has multifunctional roles due to its complex structure and functions. Fibrinogen has multiple binding sites which are open or which open as a result of proteolysis and/or conformational change. The fibrinogen molecule is a trinodular structure that consists of two sets of three different polypeptide chains Aα-, Bβ- and γ- chains (150).The chains are joined by disulphide bonds to form the N-terminal amino acid 'E' domain of the molecule and the two D domains are connected to this domain by a coiled-coil segment (Figure 7). Fibrinogen is involved in inflammation, repair, fibrinolysis and cellular and matrix interactions (151).

The interaction between fibrinogen and platelet GPIIbIIIa plays an important role in the generation of platelet-platelet aggregates and platelet thrombi. Fibrinogen can bind to the activated platelet integrin GPIIbIIIa receptor and that initiates an outside-in signalling event, which provides bridging between platelets.

Following activation of the plasma coagulation cascade fibrinogen is converted to fibrin by thrombin (Figure 7). The molecule has two recognition sites for thrombin in the E domain (EA and EB). Thrombin cleaves fibrinogen at these sites, first cleaving fibrinopeptide A (FPA) followed by fibrinopeptide B (FPB). The structure is stabilised by FXIII which crosslinks the fibrin molecules. Fibrin combines with other fibrin molecules to form long threads of fibrin polymers that intertwines with the forming thrombus and forms a scaffold i.e. a blood clot.

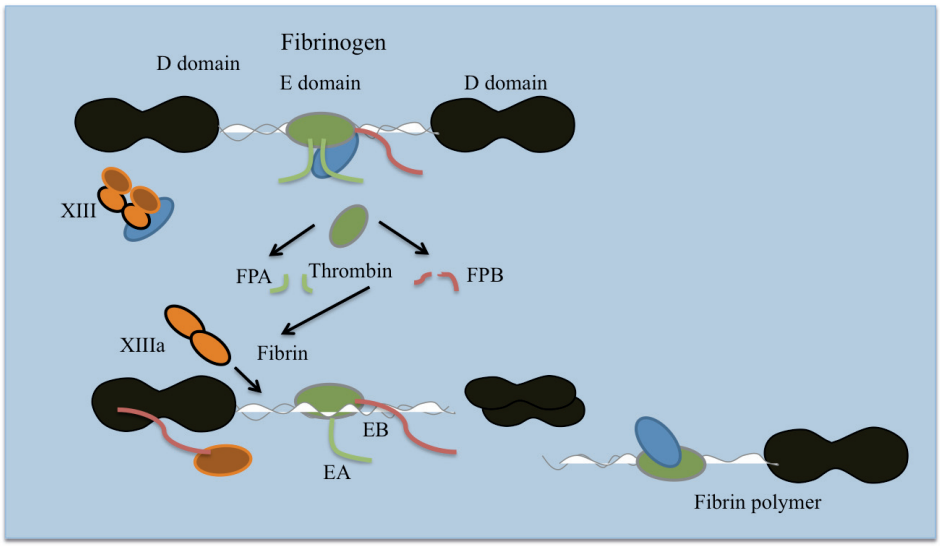

Figure 7: Schematic diagram of the fibrinogen structure and the thrombin cleavage sites.

Bacteria-Platelet interaction

During severe bacterial infection platelets are either directly or indirectly affected and thrombocytopenia can occur. Thrombocytopenia may be due to the increased consumption of clotting factors and the subsequent DIC. It may also be as a result of direct interaction of bacteria with platelets resulting in activation and aggregation. Many *in vitro* studies have shown that bacteria can interact with platelets, however less is know about the role of these interactions *in vivo*.

Mechanism of bacterial-platelet interactions

Bacteria can bind either directly or indirectly to platelet receptors. They can bind directly via bacterial surface proteins or they can associate indirectly via acquisition of plasma proteins including fibrinogen, fibronectin, IgG and the complement component C1q that can bind to both the bacteria and the platelet. In addition bacteria can also release products such as bacterial toxins that can interact with and activate platelets for example α-toxin, lipopolysaccharide (LPS) and *streptococcal* M1-protein (75,152,153).

In the case of *S. pyogenes* and *S. aureus* indirect binding to platelets can occur via bridging molecules that connect the bacteria to the platelet surface (154-156). For example *S. aureus* clumping factor A (ClfA) binds to fibrinogen and interacts with the platelet through the GPIIbIIIa receptor (157-159). These studies of *S. aureus* aggregation are IgG dependent. A similar mechanism of fibrinogen and IgG dependent platelet aggregation has been described for *S. pyogenes* bacteria (160). Furthermore, *S. pyogenes* can release M protein from the bacterial surface, which forms a complex with plasma fibrinogen and engages with the platelet fibrinogen receptor (Figure 8) and in the presence of specific IgG against M1 protein bound to the platelet FcγRIIa receptor, platelet activation can occur (75). In the absence of this IgG, M1 protein binding still occurs but the platelets do not become activated.

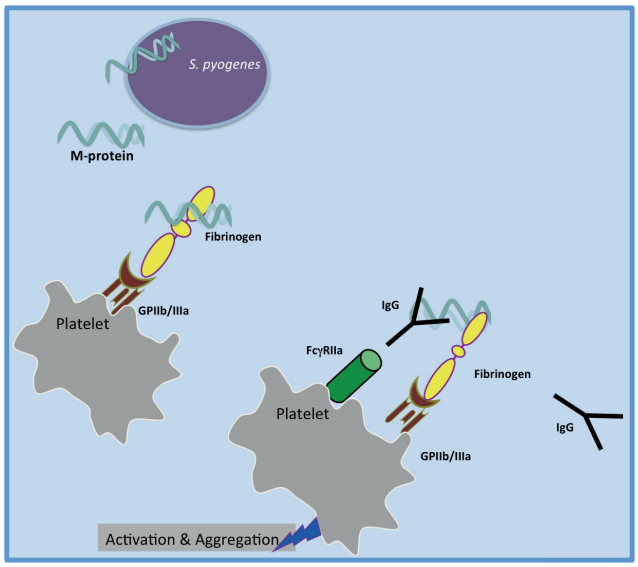

Figure 8: Schematic image of platelet activation and aggregation in response to *S. pyogenes* M1 protein.

Bacteria play a role in infective endocarditis where platelet thrombin adhere and accumulate on the heart valve and in septicaemia where a decrease in the platelet count is seen as an indicator of illness (161). Bacteria have developed resistance to platelet antimicrobial peptides that are aimed at killing the bacteria (162). Although demonstrated by few studies, platelets have also been shown to bind and internalise bacteria for example *S. aureus* (163-165). Platelets have been shown to engulf *S. aureus* and human immunodeficiency virus (HIV) in a subcellular component and the bacteria appeared to be associated with platelet granule secretory products (166). This work also demonstrated that platelets, once activated, could increase their ability to internalise bacteria, therefore it was considered to be important that platelet activation had previously occurred in order for the process to take place more effectively.

Platelet-Neutrophil Complexes

Platelets can form complexes and communicate with leukocytes by the formation of platelet-leukocyte complexes (PLC) during vessel injury and inflammation (167). Platelets can interact and form complexes at different levels with the respective cell types. They form complexes most easily with monocytes {Michelson: 2001ep}, followed by neutrophils. Furthermore, platelets bind monocytes stronger than neutrophils under vascular shear (168). PLC interactions are observed in diseases including, inflammatory diseases for example inflammatory lung disease such as cystic fibrosis (169), allergy (170), atherosclerosis (171), autoimmune diseases for example rheumatoid arthritis (172) and infection for example in sepsis progression and septic shock (173,174).

Platelets interact with neutrophils through different receptors, mainly selectins and integrins (Table 2). The most well studied is platelet P-selectin (CD62P) interacting with the PSGL-1 (P-selectin glycoprotein ligand-1, CD162) receptor on the neutrophil (175).The signalling through PSGL-1 results in the up regulation of the β2-integrin Mac-1 or CD11b/CD18 complex on neutrophils (176). CD11b/CD18 may bind directly to the platelet GPIb receptor or indirectly bind to the plasma protein fibrinogen which then associates with the platelet GPIIb/IIIa receptor (167). Blocking of the platelet GPIb receptor has been shown to prevent PNC formation at the endothelium (177).

At the endothelium neutrophil rolling can occur on platelets adhered to the endothelium via PNC formation (178,179). Activated platelets release chemokines, adhere to the endothelium and may subsequently recruit neutrophils to the endothelium and enhance their transmigration (180,181).

Other adhesion partners between platelets and leukocytes have been reported, although these are not as well studied. Intracellular adhesion molecule-2 (ICAM-2) is the main β2-integrin ligand present on activated platelets and can interact with neutrophil CD11b/CD18 or CD11a/Cd18, promoting neutrophil adhesion (182). In addition, neutrophil CD11a/Cd18 can interact with the platelet receptor junctional adhesion molecule 3 (JAM-3) (183). Activated platelets express CD40 ligand (CD40L) (184) which can interact with neutrophil CD40 (185). The table below (Table 2) outlines some of the most well studied interactions that occur between platelets and neutrophils.

Platelet	Neutrophil
P-selectin	PSGL-1
ICAM-2	CD11a/CD18
JAM-3	CD11a/CD18
CD40L	CD40
GPIIbIIIa	CD11b/CD18
GPIbα	CD11b/CD18

Table 2: The table demonstrates the most well studied mechanisms of interactions between platelets and neutrophils of which platelet p-selectin interacting with its coresponding receptor PSGL-1 on neutrophils is the most well known.

Platelets in complex with neutrophils have been shown to have enhanced functions while combined. Platelet binding can induce neutrophils to release neutrophil extracellular traps (NETs) which can entrap bacteria (186,187). PNC formation facilitates transcellular metabolism of arachidonic acid metabolites, which enhances the synthesis of proinflammatory and vasoconstrictive compounds amplifying the levels of neutrophil eicosanoids such as lipoxins and leukotrienes (188) (189).

PNCs have been reported to occur during sepsis and in some patient studies PNCs are speculated to contribute to the development of multiorgan failure (190) (173,186). It is however unclear whether PNC formation is directly mediated by the bacteria or is an indirect consequence of the overwhelming immune dysregulation observed in sepsis. Bacterial LPS has been shown to mediate PNC formation (186). Bacteria isolated from patients with bacteraemia generated PNCs *ex-vivo* in blood samples from the same patient from which the bacteria were isolated, indicating that PNC formation may occur in direct response to pathogeneic bacteria (191).

4. NEUTROPHILS-TRAVELLERS AND ENGULFERS

In the nineteenth century, the German researcher Paul Ehrlich was the first to distinguish the different leukocytes on the basis of their nuclear morphology and granule content by utilising special cell staining techniques. The neutrophil is the most abundant leukocyte in the blood stream and has an important immune role. They are also designated as polymorphonuclear leukocytes (PMN) since they have a multilobed nucleus and contain large numbers of intracellular granules and vesicles (192). The exciting, yet relatively short-lived life of a neutrophil begins in the bone marrow during granulopoiesis when a hematopoietic stem cell differentiates first into the common myeloid progenitor cells and then into polymorphonuclear leukocytes (193). Each day 10^{11} neutrophils are produced from the bone marrow from the hematopoietic stem cell (194). They have been considered to have relatively short lifespans with a half-life of approximately 8 h in humans (195) and approximately 1.5 hours in mice in the blood circulation, but more recent studies suggest they have longer lifespans (196). The neutrophil is an exceptionally efficient phagocyte displaying an immense ability to engulf and destroy microbes. If they do not encounter an infectious agent, neutrophils enter the reticuloendothelial organs or return to the bone marrow to undergo programmed cell death. Maintaining neutrophil homeostasis is very important and is regulated by the rate of differentiation and proliferation of the neutrophil precursors in the bone marrow.

Neutrophil Granules

Neutrophil granules can be subdivided into three main subsets. The utilisation of multiple techniques over the last number of years, including fractionation techniques, immune electron microscopy and flow cytometry have shown that neutrophils possess three main granule types; Azurophilic (primary), specific (secondary) and gelatinase (tertiary) subsets (197). Neutrophils also contain secretory vesicles whose origin may differ from the other granule types (198,199). The granules contain about 300 proteins (Figure 9) which include receptors which can become part of the cell membrane, proteolytic and bactericidal proteins and pro-inflammatory molecules (200) (201) which can all be rapidly transported to the cell surface when necessary.

During the transition from myeloblast to promyelocyte the granules begin to appear. Neutrophil granules are released in an hierarchical order and this is the opposite to that of which they were formed (202). The azurophilic granules are the largest (203) and are only fully mobilised after a strong stimulation that release molecules such as HBP and myeloperoxidase (MPO). The secondary (specific) granules are smaller than the azurophilic, contain many plasma molecules and play a role in oxidative burst (204). These are rich in antimicrobial substances such as neutrophil gelatinase associated lipocalin (N-GAL), lactoferrin, lysozyme among others and these granules contribute to the contents of the phagosome during bacterial clearance. The secretory vesicles and tertiary granules are more easily mobilised and play a role in neutrophil transmigration (205). Granules are not released until a signal transduction event is initiated whereby signals are sent to the cytoplasm to activate their movement to the membrane for cell degranulation and granule secretion. Neutrophil degranulation requires calcium, guanosine triphosphate (GTP) and ATP hydrolysis.

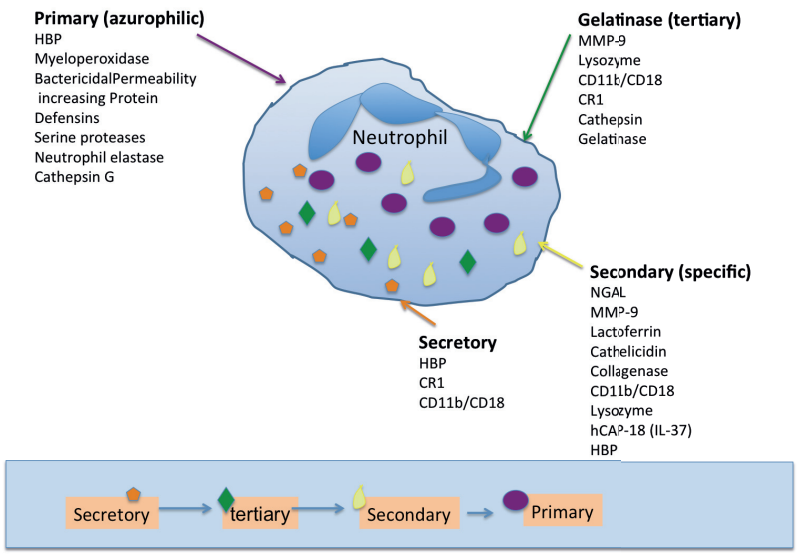

Figure 9. Neutrophil granule subsets.

Neutrophil Function

Neutrophils are one of the first responders at the site of inflammation, adhering and migrating rapidly to the necessary sites and are crucial for bacterial clearance. This is one of the neutrophils primary functions – extravasation to the site of inflammation and/or damage. This process is discussed in more detail later in this thesis. It is complex and involves a number of processes including neutrophil rolling, arrest, spreading, crawling and transmigration (206). The neutrophil granules facilitate transmigration across the endothelium, particularly the secretory and gelatinase (tertiary) granules. Secretory granules can incorporate molecules into their membrane such as selectins which then play a role in the initial steps of transmigration (207). Tertiary granules contain membrane and matrix degrading proteins such as collagenase that degrades collagen (208).

The importance of neutrophil function is demonstrated in patients with leukocyte adhesion deficiency (LAD), a rare yet life-threatening genetic condition. There is a deficiency of CD18, an adhesive glycoprotein on the surface of leukocytes that facilitates cellular interactions such as adhesion and subsequent leukocyte transmigration (209). Leukocytes are unable to transmigrate to the site of infection and thus are unable to clear pathogens. This results in these individuals being severely immunocompromised and unable to clear bacterial infection, therefore they experience recurrent necrotic soft tissue infections and impaired wound healing.

Neutrophils express a vast array of receptors on their surfaces that play important roles in pathogen recognition and phagocytosis of microbes. They express pathogen recognition receptors such as TLRs which are essential for PAMP recognition or DAMP recognition and generation of antimicrobial responses (210) but also opsonic receptors including the Fc and complement receptors.

Neutrophils migrate in a directional manner in a process called chemotaxis, which was first described by Leber in 1888. They move towards a chemical gradient in response to chemotactic factors or chemoattractants such as platelet activating factor (PAF), formyl-methionyl-leucylphenylalanine (fMLF), complement factor 5a (C5a) and chemokines such as interleukin 8 (IL-8). When the orientation of the chemoattractant changes the neutrophil cytoskeleton reorganises and it moves in the direction of that chemoattractant.

The neutrophil may use this method when migrating towards its target (see review (211)). fMLF is a proteinogenic amino acid found in bacteria and is a potent chemotactic factor when the peptides are released during infection. The formyl peptide receptor (FPR) receptor on the neutrophil recognise these peptides and initiates a cell signalling event. The neutrophil express two formyl peptide receptors, known as FPR1 and FPR2 and are both G-protein coupled receptors (212,213).

Pathogen Elimination

Phagocytosis is an active receptor mediated process that is actin driven and assists in the clearance of bacteria. The process was originally shown in 1977 to be IgG dependent (214), but later studies revealed a role for other receptors, including the complement receptors (215). There are a number of key steps involved in the process of phagocytosis, which involves receptor mediated particle recognition by the phagocyte, particle uptake into a vesicle, formation of a phagolysosome and finally clearance of the pathogen digested particles (216). Initially, the phagocyte must recognise many types of pathogen targets for which it has evolved many receptors on its membrane surface. PRRs on the neutrophil recognise PAMPs (5) or the phagocyte Fcγ receptors and complement receptor 3 (CR3, CD11b/CD18, αMβ2 integrin) (217) recognise pathogens that are opsonised by antibodies or complement components bound to their surface (218). When signalling networks are initiated and cytoskeletal actin rearrangements occur the plasma membrane surrounds the microbe and engulfs it, forming the phagosome. The microbe, once within the membrane bound phagosome is entrapped and fuses with the lysosome to form a phagolysosome (219). The phagolysosome is an acidic compartment in which the pathogen is broken down and processed for antigen presentation (220).

Neutrophils also have an oxidative (respiratory) burst mechanism. Neutrophils generate reactive oxygen species (ROS), which essentially are oxygen-derived molecules that are oxidising agents or converted to radicals. The process of ROS production requires nicotinamide adenine dinucleotide phosphate-oxidase (NADPH) (221). Chronic granulomatous disease (CGD) is an inherited disorder, which occurs due to a defect or deficiency in the subunits, which make up the NADPH oxidase enzyme system (222). The disease is characterised by recurrent bacterial infections due to the inability of phagocytes, including neutrophils, to perform one of their functions in releasing reactive oxygen species.

Another mechanism by which neutrophils can entrap and clear bacteria is through the release of NETs (223). NET formation occurs when neutrophils undergo a special type of controlled cell death called NETosis where the nuclear chromatin is dissolved and extracellular strands of "sticky" threads of DNA are released. These strands of DNA also contain antimicrobial peptides. Platelets may have a role in NET formation in response to LPS and *Escherichia coli* (186).

5. THE ENDOTHELIUM - MORE THAN JUST A BARRIER BREACHED!

The endothelium consists of a single monolayer of specialised cells that provide the inner lining to blood vessels and as such is a major interphase for circulating immune cells in their passage from blood to tissues. In this way the endothelium can be considered an important barrier between blood and underlying tissues. Endothelial cells arise from the precursor hemangioblast, which can either differentiate into hematopoietic stem cells (HSCs) or into angioblasts (224). The hematopoietic and angiogenic lineage is derived from the mesodermal stem cells during embryonic development. The endothelium has the ability to secrete cytokines, chemokines and express adhesion molecules and is therefore viewed as an important host immunological organ (225). Endothelial cells maintain normal homeostasis by maintaining an antithrombotic state, regulating secretion of vasodilator and vasoconstrictor molecules (prostacyclin and nitric oxide), control vascular tone and blood pressure (226) and control leukocyte migration and fluid permeability across the vessel wall (227).

Activation of the Endothelium

Endothelium cell activation occurs in response to "danger" signals from pathogens and/or inflammatory mediators. Activating agonists such as thrombin, TNFα and bradykinin induce endothelial signalling events and vascular leakage (228). Endothelial cells express functional PRRs, which include the TLRs- TLR 1,2,4,5 and 6 that recognise bacterial PAMPs (229) such as bacterial lipoproteins, peptidoglycan, carbohydrates and endotoxins for example LPS released from gram negative bacteria (230). PRRs also recognise DAMPs, which are generated in response to tissue damage. The *S. pyogenes* M protein has recently been shown to be recognised as a PAMP by keratinocytes and is involved in the upregulation of many signalling partners (77).

Leukocyte Cell Migration

The initial events in leukocyte transmigration involve leukocyte capture, rolling and adhesion. Mediators of acute inflammation stimulate molecules such as selectins to be translocated from endothelial cells and facilitate the initial rolling process. There are three selectins involved -E, -P and -L selectins. Endothelial p-selectin, found in WPBs of endothelial cells is the primary selectin involved in leukocyte capture and rolling, which interacts with selectin ligands on leukocytes, particularily PSGL-1 and this facilitates rapid leukocyte adhesion (231). Once the leukocyte is in contact with the endothelium and the rolling process has occurred, further endothelial adhesion molecules are upregulated and chemokines such as IL-8, GROα and monocyte chemoattractant protein-1 (MCP-1) are secreted by the endothelium (232,233). The contact between chemokines and leukocyte chemokine receptors leads to the activation of leukocyte integrins.

Leukocytes express β2 integrins, and when upregulated, they tightly adhere to the endothelium (234). This stage of firm leukocyte arrest involves leukocyte-function associated antigen-1 (LFA-1/ (CD11a/CD18) and Mac-1 (CD11b/CD18) that can bind to ICAM-1 and ICAM-2 on endothelial cells promoting firm leukocyte arrest (235). Once leukocytes are firmly attached the process of leukocyte transmigration takes place. Adhesion molecules involved with transmigration include platelet endothelial cell adhesion molecule-1 (PECAM-1), vascular endothelial (VE) -cadherin, vascular cell adhesion molecule-1 (VCAM-1), integrin associated protein (IAP,CD47) and very late antigen 4 (VLA-4, α4β1) (236).

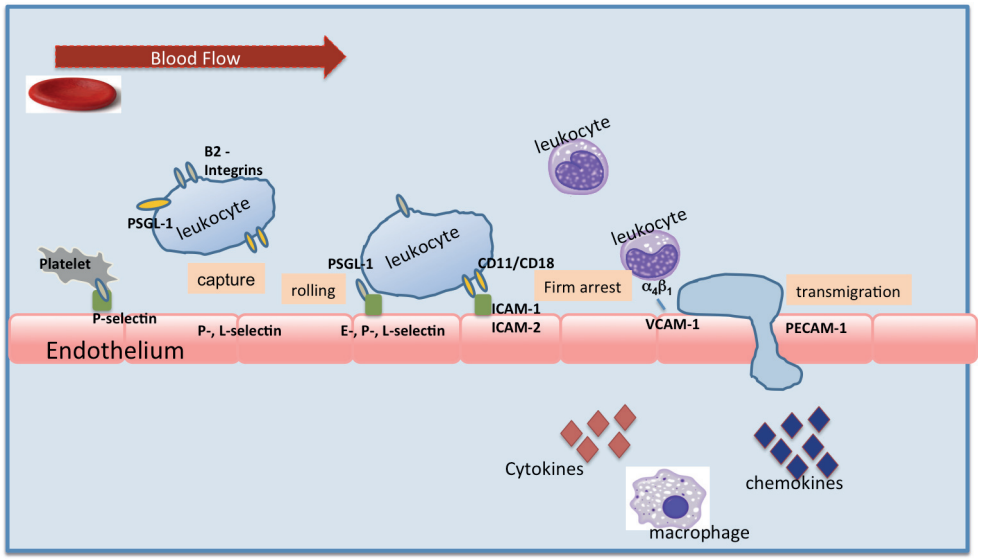

Figure 10: The diagram indicates the main events and interacting patners involved in leukocyte transmigration.

Endothelial cells play an important role in vascular healing. Upon vascular damage platelets become activated and adhere to subendothelial components, initially to vWF, which is bound to collagen at the subendothelium. Platelets interact with adhesion molecules such as P-selectin, PECAM-1 and integrins (237). Once adhered platelets aggregate together forming a platelet plug that seals vessel damage.

The endothelium normally maintains an antithrombotic state (238) with molecules such as activated protein C (APC), of which thrombomodulin is a cofactor for thrombin-induced activation of APC and the anticoagulant proteins, tissue factor pathway inhibitor (TFPI) and antithrombin (AT). These molecules primarily prevent blood from clotting by inhibiting the activation of clotting factors on a normal endothelial cell surface. The inhibitor, plasminogen activator inhibitor-1 (PAI-1) (239) plays a role in the fibrinolytic system. When endothelial cells become activated, the endothelium changes to a prothrombotic state and the clotting factor thrombin generated by the coagulation system can bind to receptors (PARs) on endothelial cells and platelets, which stimulates endothelial cytokine and chemokine production. TF is a potent activator of the coagulation cascade and is constitutively expressed by cells surrounding blood vessels (240) and is induced by cytokines from endothelial cells. TF is also present on monocytes and macrophages (241) and platelets and granulocytes play a role in its generation (242). In addition both the *S. pyogenes* M1 and M3 serotypes have been shown to stimulate procoagulant activity in endothelial cells via TF upregulation (243).

PRESENT INVESTIGATIONS

When *S. pyogenes* disseminates into the bloodstream, life-threatening disease such as sepsis can result. Upon interacting with our immune system this pathogen or its bacterial surface M protein has multiple effects and can activate several cells including platelets, neutrophils and endothelial cells which are investigated in this thesis. Platelets are a key focus throughout the studies of this thesis and we have investigated their function in both *in vitro* and *in vivo* model systems.

PAPER I

Background: Platelets can interact and form complexes with other immune cells. In this paper we investigate the formation of platelet- neutrophil complexes (PNCs). We focused on the relative contribution of each cell type involved in PNC formation and the functional effect these complexes have on neutrophils. During severe bacterial infection both the coagulation and inflammatory systems are activated (244) and we investigated these systems by comparing different agonists throughout this study. These included ADP, fMLF, the coagulation factor thrombin and the *S. pyogenes* virulence factor, M1 protein. It has previously been shown *in vitro* that in order for platelets to become activated in response to M1 protein, fibrinogen binding and the presence of anti-M1 IgG antibodies are required in those individuals (75). Therefore the effects of this protein were investigated in this study in different donors that could then be categorised as "responders" and "non-responders". PNC formation and neutrophil activation was investigated in a whole blood environment, thus resembling normal physiological conditions.

The main aims were to investigate the effects of distinct agonists on neutrophil function in PNCs and to distinguish any differences between the agonists.

Results & Conclusions: The agonist thrombin gave rise to platelet dependent PNC formation in all donors investigated and the neutrophils in response to this agonist demonstrated enhanced phagocytic and killing abilities, while chemotaxis was unaffected. In comparison, M1 protein only demonstrated PNC formation in certain donors that contained specific IgG against M1 protein and a direct correlation was made between donors with anti-M1 IgG antibodies and their level of PNC formation.

Neutrophil functional readouts differed in PNCs generated in response to M1 protein as compared to thrombin or ADP in combination with fMLF. Those PNCs generated in response to the M1 protein diminished the phagocytic and chemotactic functions of the neutrophils. In addition immunofluorescent and scanning electron microscopy revealed that PNCs in response to M1 protein were entrapped in a platelet and fibrinogen network.

PAPER II

Background: *S. pyogenes* has been shown to aggregate and subsequently disaggregate platelets over time in platelet rich plasma (245) and this bacteria has the ability to evade the immune detection systems and escape neutrophil phagocytosis (246) (63,68,71). The aim of the study was to investigate the role of platelet activation and aggregation in response to *S. pyogenes* for leukocyte function in blood. To this end we investigated the interactions between washed bacteria, platelets and leucocytes in whole blood, with a particular focus on bacterial survival.

Results & Conclusions: We demonstrate for the first time that platelet aggregation, platelet-neutrophil complex formation and platelet- monocyte complex formation occurs in direct response to *S. pyogenes* in whole blood. This results in platelet dependent neutrophil and monocyte activation, tissue factor up-regulation and clot formation. Furthermore, bacteria were directly associated with all of these homotypic and heterotypic cell aggregates, as visualised with immunofluorescent microscopy. Bacteria were entrapped within these cell aggregates over time, however bacterial killing was not observed and viable bacteria could be removed by sonication of the samples after one hour. We propose that bacterial entrapment in platelets or platelet leukocyte complexes might contribute to bacterial survival in human blood.

PAPER III and IV

Background: Platelets have been reported to play a role during invasive bacterial infections and the inflammatory response that follows and their numbers decrease dramatically during the progression of sepsis. In these two papers we assessed the role of platelets during the pathophysiology of sepsis in an animal model of *S. pyogenes*, infection. There have been many studies of platelet-bacteria interactions *in vitro* and *ex vivo* but animal models of platelet function during infection have been lacking. Thrombocytopenia occurs during severe and invasive bacterial infection and there is a correlation between the platelet count and the severity of sepsis (94,95,247). It is not clear whether platelets play a protective role in the prevention of sepsis or whether platelet activation contributes to the pathogenesis of the syndrome, therefore in paper III we investigated how platelets contribute to the acute response to bacterial infection using a mouse model of *S. pyogenes* bloodstream infection in platelet depleted mice as compared with healthy controls. Platelet activation was observed in paper III, therefore paper IV was initiated as a follow up study to investigate the kinetics of platelet activation during the progression of sepsis in the same animal model. A particular focus of this study was to determine which platelet activation assays could provide prognostic information on the progression of *S. pyogenes* sepsis and whether platelet activation was associated with organ damage.

Results & Conclusions: Collectively, the results of paper III and IV demonstrate an important role for platelets during the pathophysiology of *S. pyogenes* infection. In paper III platelets are reported to contribute to bacterial dissemination from the blood to the organs of animals, infected with *S. pyogenes*. The bacterial load in the blood, spleen and lungs was significantly decreased in platelet-depleted animals. These animals also exhibited reduced plasma IL6 levels and weight loss during the infection, suggesting that the pathogenesis of infection was decreased in these animals at this time point. We also observed that there was a significant increase in PNC formation during *S. pyogenes* infection as compared with uninfected controls, therefore PNC formation may be important during bacterial infection.

Paper IV was initiated to investigate the kinetics of platelet activation and in particular PNC formation throughout the progression of *S. pyogenes* sepsis and the results suggest that PNC formation may be a more robust biomarker to demonstrate platelet activation during disease progression. In our model neutrophil activation occurred early and remained high during the course of infection but platelet activation and PNC formation was more discriminatory between different time points.

The platelet count progressively decreased in blood during the infection while PNC formation was first increased and then decreased during the later stages of infection. The PNCs do not correlate to neutrophil activation, which implies that PNCs in this model are platelet dependent events, as we have previously demonstrated *in vitro*.

As *S. pyogenes* infection progressed to a late stage of sepsis, organ damage was observed at the same time point as PNCs were decreased in the blood of the animals and thrombocytopenia was most profound. Platelet aggregates were detected in the liver therefore suggesting that platelet activation and PNC formation precedes thrombocytopenia and platelets may contribute to the pathogenesis of organ damage during sepsis.

Very little work has been carried out on the therapeutic potential of targeting PNCs, as most studies are still at the investigation and mechanistic phases but further studies should aim to better understand their functional role and their future potential use as diagnostic or therapeutic tools.

PAPER V

Background: The endothelium constitutes the inner lining of blood vessels with a single layer of cells and is a semi-permeable barrier that plays an important role in regulating the movement of fluids, proteins and the transmigration of cells from blood to the tissue. Endothelial permeability and endothelial responses are altered in the presence of activating substances, such as TNFα and thrombin and when bacteria are present in the bloodstream. In this paper we investigated the effects of the Gram-positive bacterial protein, M1 protein on endothelial cells. Previously, M1 protein in complex with a plasma protein has been shown to induce the release of HBP from neutrophils (51) and HBP can increase endothelial cell permeability. In addition M1 protein activates platelets (75) and within platelet-neutrophil complexes we have shown that the neutrophils are dysfunctional (248). The aim of this paper was to determine whether M1 protein released from *S. pyogenes* binds to and activates endothelial cells, and the consequences of this for endothelial cell permeability. We investigated endothelial cell function either with M1 protein alone or in the presence of a plasma cofactor, fibrinogen.

Results & Conclusions: M1 protein increased endothelial vascular permeability in a Rho kinase and TLR-2 dependent manner, at levels equivalent to the positive control, thrombin. M1 protein has previously been shown to bind human monocytes by TLR-2 engagement and increase cytokine production from these cells (76). It has also been shown that M1 protein induces vascular nitric oxide production via TLR-2 (249). The results of these studies are in agreement with our TLR-2 findings and we demonstrate M1 protein can bind to both human and immortalised endothelial cell lines.

M1 protein may contribute to endothelial dysfunction and vascular leakage during infection. M1 protein has been shown to induce a proinflammatory cytokine response in epithelial cells (77). This may suggest that M1 protein induces an activation of the innate immune response, however in our studies there is a relative lack of cytokine production from the immortalised cell line and weak cytokine release from the primary human endothelial cells in response to M1 protein, indicating that the protein alone induces a weak response. However, in the presence of the plasma protein fibrinogen binding of M1 protein to endothelial cells and cytokine production were increased. It could be speculated that M1 protein requires another bridging molecule in addition to TLR-2 for an enhanced endothelial response. In addition, M1 protein has been shown to lose its specialised structure at 37°C (250) and perhaps the plasma protein herein is playing a role in stabilising M1 protein.

All together the findings of this thesis provide new evidence for the role of platelets, neutrophils, monocytes and endothelial cells during the pro-inflammatory response that is initiated during the pathogenesis of infection. The combination of pro-inflammatory stimuli, *S. pyogenes* and its virulence factor M1 protein, used throughout these studies contribute and affect these cells which are stimulated and play central roles in the immune response. M1 protein directly binds endothelial cells and effects vascular function, directly activates platelets, which in turn can interact with leukocytes whereby the neutrophils are dysfunctional and entrapped in aggregates which may accumulate in damaged organs and ultimately contribute to the outcome of sepsis.

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REFERENCES

1. Schippa S, Conte M. Dysbiotic Events in Gut Microbiota: Impact on Human Health. Nutrients. 2014 Dec;6(12):5786–805.

2. Hollowell JG AOGELBNMPC. Hematological and Iron-Related Analytes— Reference Data for Persons Aged 1 Year and Over: United States, 1988–94. 2005 Feb 15;1–168.

3. Ross DW, Ayscue LH, Watson J, Bentley SA. Stability of hematologic parameters in healthy subjects. Intraindividual versus interindividual variation. Am J Clin Pathol. 1988 Sep;90(3):262–7.

4. Loos M, Heinz HP. Generation of the classical pathway C3 convertase (EAC4b2a) by proteolytic enzymes. Acta Pathol Microbiol Immunol Scand Suppl. 1984;284:67–74.

5. Medzhitov R. Approaching the asymptote: 20 years later. Immunity. 2009 Jun;30(6):766–75.

6. Janeway CAJ, Medzhitov R. Innate immune recognition. Annu Rev Immunol. 2002;20:197–216.

7. Matzinger P. The danger model: a renewed sense of self. Science. 2002 Apr;296(5566):301–5.

8. Gallucci S, Matzinger P. Danger signals: SOS to the immune system. Curr Opin Immunol. 2001 Feb;13(1):114–9.

9. Beutler B. Innate immunity: an overview. Mol Immunol. 2004 Feb;40(12):845–59.

10. Le Y, Murphy PM, Wang JM. Formyl-peptide receptors revisited. Trends Immunol. 2002 Oct 31;23(11):541–8.

11. Medzhitov R. Origin and physiological roles of inflammation. Nature. 2008 Jul;454(7203):428–35.

12. Muller WA. Getting Leukocytes to the Site of Inflammation. Veterinary Pathology. 2013 Jan 23;50(1):7–22.

13. Dahlbäck B. Blood coagulation. Lancet. 2000 6 May;355(9215):1627–32.

14. Monroe DM. What Does It Take to Make the Perfect Clot? Arteriosclerosis, Thrombosis, and Vascular Biology. 2006 Jan 1;26(1):41–8.

15. Esmon CT. The interactions between inflammation and coagulation. Br J Haematol. 2005 Nov;131(4):417–30.

16. Maskrey BH, Megson IL, Whitfield PD, Rossi AG. Mechanisms of Resolution of Inflammation: A Focus on Cardiovascular Disease. Arteriosclerosis, Thrombosis, and Vascular Biology. 2011 Apr 20;31(5):1001–6.

17. Crotty S, Ahmed R. Immunological memory in humans. Semin Immunol. 2004 Jun;16(3):197–203.

18. Broere F, Apasov SG, Sitkovsky MV, van Eden W. T cell subsets and T cell-mediated immunity. Basel: Principles of Immunopharmacology; 2011. pp. 15–27.

19. Fridman WH. Fc receptors and immunoglobulin binding factors. FASEB J. 1991 Sep;5(12):2684–90.

20. Nimmerjahn F, Ravetch JV. Fcgamma receptors: old friends and new family members. Immunity. 2006 Jan;24(1):19–28.

21. Witko-Sarsat VR, Rieu P, Descamps-Latscha BA, Lesavre P, Halbwachs-Mecarelli L. Neutrophils: Molecules, Functions and Pathophysiological Aspects. 2000 May 2;80:617–53.

22. Repp R, Valerius T, Sendler A, Gramatzki M, Iro H, Kalden JR, et al. Neutrophils express the high affinity receptor for IgG (Fc gamma RI, CD64) after in vivo application of recombinant human granulocyte colony-stimulating factor. Blood. 1991 Aug;78(4):885–9.

23. Rosenbach FJ. Mikro-Organismen bei den Wund-Infections-Krankheiten des Menschen. 1887.

24. Stromberg A, Schwan A, Cars O. Throat carrier rates of beta-hemolytic streptococci among healthy adults and children. Scand J Infect Dis. 1988;20(4):411–7.

25. Begovac J, Bobinac E, Benic B, Desnica B, Maretic T, Basnec A, et al. Asymptomatic pharyngeal carriage of beta-haemolytic streptococci and streptococcal pharyngitis among patients at an urban hospital in Croatia. Eur J Epidemiol. 1993 Jul;9(4):405–10.

26. Carapetis JR, Steer AC, Mulholland EK, Weber M. The global burden of group A streptococcal diseases. Lancet Infect Dis. 2005 Nov;5(11):685–94.

27. McCarty M. VARIATION IN THE GROUP-SPECIFIC CARBOHYDRATE OF GROUP A STREPTOCOCCI: I. IMMUNOCHEMICAL STUDIES ON THE CARBOHYDRATES OF VARIANT STRAINS. Journal of Experimental Medicine. 1955 Jul 1;102(1):11–28.

28. LANCEFIELD RC. The antigenic complex of streptococcus haemolyticus. I. Demonstration of a type- specific substance in extracts of streptococcus haemolyticus. J Exp Med. 1928 Jan;47(1):91–103.

29. Ferretti JJ, McShan WM, Ajdic D, Savic DJ, Savic G, Lyon K, et al. Complete genome sequence of an M1 strain of Streptococcus pyogenes. Proc Natl Acad Sci U S A. 2001 Apr;98(8):4658–63.

30. Hanski E, Caparon M. Protein F, a fibronectin-binding protein, is an adhesin of the group A streptococcus Streptococcus pyogenes. Proc Natl Acad Sci U S A. 1992 Jul;89(13):6172–6.

31. Hanski E, Horwitz PA, Caparon MG. Expression of protein F, the fibronectin-binding protein of Streptococcus pyogenes JRS4, in heterologous streptococcal and enterococcal strains promotes their adherence to respiratory epithelial cells. Infect Immun. 1992 Dec;60(12):5119–25.

32. Dale JB, Washburn RG, Marques MB, Wessels MR. Hyaluronate capsule and surface M protein in resistance to opsonization of group A streptococci. Infect Immun. 1996 May;64(5):1495–501.

33. Svensson MD, Sjöbring U, Luo F, Bessen DE. Roles of the plasminogen activator streptokinase and the plasminogen-associated M protein in an experimental model for streptococcal impetigo. Microbiology. 2002 Dec;148(Pt 12):3933–45.

34. Nelson DC, Garbe J, Collin M. Cysteine proteinase SpeB from Streptococcus pyogenes - a potent modifier of immunologically important host and bacterial proteins. Biol Chem. 2011 Dec;392(12):1077–88.

35. Pawel-Rammingen von U. Streptococcal IdeS and its impact on immune response and inflammation. J Innate Immun. 2012;4(2):132–40.

36. Pawel-Rammingen von U, Johansson BP, Björck L. IdeS, a novel streptococcal cysteine proteinase with unique specificity for immunoglobulin G. EMBO J. 2002 Apr;21(7):1607–15.

37. Akesson P, Moritz L, Truedsson M, Christensson B, Pawel-Rammingen von U. IdeS, a highly specific immunoglobulin G (IgG)-cleaving enzyme from Streptococcus pyogenes, is inhibited by specific IgG antibodies generated during infection. Infect Immun. 2006 Jan;74(1):497–503.

38. Akesson P, Sjoholm AG, Björck L. Protein SIC, a novel extracellular protein of Streptococcus pyogenes interfering with complement function. J Biol Chem. 1996 Jan;271(2):1081–8.

39. Dano K, Andreasen PA, Grondahl-Hansen J, Kristensen P, Nielsen LS, Skriver L. Plasminogen activators, tissue degradation, and cancer. Adv Cancer Res. 1985;44:139–266.

40. Ponting CP, Marshall JM, Cederholm-Williams SA. Plasminogen: a structural review. Blood Coagulation & Fibrinolysis. 1992 Oct;3(5):605–14.

41. Bajaj AP, Castellino FJ. Activation of human plasminogen by equimolar levels of streptokinase. J Biol Chem. 1977 Jan;252(2):492–8.

42. Váradi A, Patthy L. Location of plasminogen-binding sites in human fibrin(ogen). Biochemistry. 1983 May 10;22(10):2440–6.

43. Strickland DK, Morris JP, Castellino FJ. Enhancement of the streptokinase-catalyzed activation of human plasminogen by human fibrinogen and its plasminolysis products. Biochemistry. 1982 Feb;21(4):721–8.

44. Sun H, Ringdahl U, Homeister JW, Fay WP, Engleberg NC, Yang AY, et al. Plasminogen is a critical host pathogenicity factor for group A streptococcal infection. Science. 2004 Aug;305(5688):1283–6.

45. Sriskandan S, Faulkner L, Hopkins P. Streptococcus pyogenes: Insight into the function of the streptococcal superantigens. International Journal of Biochemistry and Cell Biology. 2007;39(1):12–9.

46. Spaulding AR, Salgado-Pabon W, Kohler PL, Horswill AR, Leung DYM, Schlievert PM. Staphylococcal and streptococcal superantigen exotoxins. Clin Microbiol Rev. 2013 Jul;26(3):422–47.

47. MacIsaac CM, Curtis N, Cade J, Visvanathan K. Superantigens in sepsis. New Insights Into an Old Enemy Proceedings of the XVIth Lancefield International Symposium on Streptococci and Streptococcal Diseases, held in Palm Cove, Australia. 2006 Apr 1;1289 IS -:121–4.

48. BHARDWAJ N, YOUNG JW, NISANIAN AJ, BAGGERS J, STEINMAN RM. Small Amounts of Superantigen, When Presented on Dendritic Cells, Are Sufficient to Initiate T-Cell Responses. J Exp Med. 1993;178(2):633–42.

49. Pahlman LI, Olin AI, Darenberg J, Mörgelin M, Kotb M, Herwald H, et al. Soluble M1 protein of Streptococcus pyogenes triggers potent T cell activation. Cell Microbiol. 2008 Feb;10(2):404–14.

50. Berge A, Björck L. Streptococcal cysteine proteinase releases biologically active fragments of streptococcal surface proteins. J Biol Chem. 1995 Apr 27;270(17):9862–7.

51. Herwald H, Cramer H, Mörgelin M, Russell W, Sollenberg U, Norrby-Teglund A, et al. M Protein, a Classical Bacterial Virulence Determinant, Forms Complexes with Fibrinogen that Induce Vascular Leakage. Cell. 2004 Feb 5;116(3):13–3.

52. Akesson P, Schmidt KH, Cooney J, Björck L. M1 protein and protein H: IgGFc- and albumin-binding streptococcal surface proteins encoded by adjacent genes. Biochem J. 1994 Jun 14;300 (Pt 3):877–86.

53. Fischetti VA. Streptococcal M protein: molecular design and biological behavior. Clin Microbiol Rev. 1989 Jul;2:285–314.

54. Fischetti VA. Streptococcal M protein. Sci Am. 1991 Jun;264(6):58–65.

55. McNamara C, Zinkernagel AS, Macheboeuf P, Cunningham MW, Nizet V, Ghosh P. Coiled-coil irregularities and instabilities in group A Streptococcus M1 are required for virulence. Science. 2008 Mar 7;319(5868):1405–8.

56. Frick I-M, Schmidtchen A, Sjöbring U. Interactions between M proteins of Streptococcus pyogenes and glycosaminoglycans promote bacterial adhesion to host cells. Eur J Biochem. 2003 May;270(10):2303–11.

57. Cue D, Lam H, Cleary PP. Genetic dissection of the Streptococcus pyogenes M1 protein: regions involved in fibronectin binding and intracellular invasion. Microb Pathog. 2001 Nov;31(5):231–42.

58. Cue D, Dombek PE, Lam H, Cleary PP. Streptococcus pyogenes serotype M1 encodes multiple pathways for entry into human epithelial cells. Infect Immun. 1998 Oct;66(10):4593–601.

59. Rezcallah MS, Hodges K, Gill DB, Atkinson JP, Wang B, Cleary PP. Engagement of CD46 and alpha5beta1 integrin by group A streptococci is required for efficient invasion of epithelial cells. Cell Microbiol. 2005 May;7(5):645–53.

60. LANCEFIELD RC. Current knowledge of type-specific M antigens of group A streptococci. J Immunol. 1962 Aug 31;89:307–13.

61. Facklam RF, Martin DR, Lovgren M, Johnson DR, Efstratiou A, Thompson TA, et al. Extension of the Lancefield classification for group A streptococci by addition of 22 new M protein gene sequence types from clinical isolates: emm103 to emm124. Clin Infect Dis. 2001 Dec 31;34(1):28–38.

62. Stevens DL. Streptococcal toxic shock syndrome. Clinical Microbiology and Infection. European Society of Clinical Infectious Diseases; 2002 Apr 5;8(3):133–6.

63. Berggård K, Johnsson E, Morfeldt E. Binding of human C4BP to the hypervariable region of M protein: a molecular mechanism of phagocytosis resistance in Streptococcus pyogenes. Mol Microbiol. 2001 Oct;2:539–51.

64. Thern A, Stenberg L, Dahlbäck B, Lindahl G. Ig-binding surface proteins of Streptococcus pyogenes also bind human C4b-binding protein (C4BP), a regulatory component of the complement system. J Immunol. 1994 Dec 31;154(1):375–86.

65. Johnsson E, Thern A, Dahlbäck B, Hedén LO, WIKSTROM M, Lindahl G. A highly variable region in members of the streptococcal M protein family binds the human complement regulator C4BP. J Immunol. 1996 Oct 1;157(7):3021–9.

66. Kantor FS. FIBRINOGEN PRECIPITATION BY STREPTOCOCCAL M PROTEIN: I. IDENTITY OF THE REACTANTS, AND STOICHIOMETRY OF THE REACTION. Journal of Experimental Medicine. 1965 May 1;121(5):849–59.

67. WHITNACK E, BEACHEY EH. Inhibition of Complement-Mediated Opsonization and Phagocytosis of Streptococcus-Pyogenes by D-Fragments of Fibrinogen and Fibrin Bound to Cell-Surface M-Protein. J Exp Med. 1985;162(6):1983–97.

68. Carlsson F, Sandin C, Lindahl G. Human fibrinogen bound to Streptococcus pyogenes M protein inhibits complement deposition via the classical pathway. Mol Microbiol. Blackwell Science Ltd; 2005;56(1):28–39.

69. Ringdahl U, Svensson HG, Kotarsky H, Gustafsson M, Weineisen M, Sjobring U. A role for the fibrinogen-binding regions of streptococcal M proteins in phagocytosis resistance. Mol Microbiol [Internet]. 2000 Aug 31;37(6):1318–26. Available from: http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=10998165&retmode=ref&cmd=prlinks

70. Berge A, Kihlberg BM, Sjoholm AG, Björck L. Streptococcal protein H forms soluble complement-activating complexes with IgG, but inhibits complement activation by IgG-coated targets. J Biol Chem. 1997;272(33):20774–81.

71. Staali L, Mörgelin M, Björck L, Tapper H. Streptococcus pyogenes expressing M and M-like surface proteins are phagocytosed but survive inside human neutrophils. Cell Microbiol. 2003 Apr;5(4):253–65.

72. Kahn F, Mörgelin M, Shannon O, Norrby-Teglund A, Herwald H, Olin AI, et al. Antibodies against a surface protein of Streptococcus pyogenes promote a pathological inflammatory response. PLoS Pathog. 2007 Dec 31;4(9):e1000149–9.

73. Soehnlein O, Oehmcke S, Ma X, Rothfuchs AG, Frithiof R, van Rooijen N, et al. Neutrophil degranulation mediates severe lung damage triggered by streptococcal M1 protein. Eur Respir J. 2008 Jul 31;32(2):405–12.

74. Lauth X, Köckritz-Blickwede von M, McNamara CW, Myskowski S, Zinkernagel AS, Beall B, et al. M1 protein allows Group A streptococcal survival in phagocyte extracellular traps through cathelicidin inhibition. J Innate Immun. 2009;1(3):202–14.

75. Shannon O, Hertzén E, Norrby-Teglund A, Mörgelin M, Sjöbring U, Björck L. Severe streptococcal infection is associated with M protein-induced platelet activation and thrombus formation. Mol Microbiol. 2007 Sep;65(5):1147–57.

76. Pahlman LI, Mörgelin M, Eckert J, Johansson L, Russell W, Riesbeck K, et al. Streptococcal M protein: a multipotent and powerful inducer of inflammation. J Immunol. 2006 Jul 14;177(2):1221–8.

77. Persson ST, Wilk L, Mörgelin M, Herwald H. Vigilant Keratinocytes Trigger Pathogen-Associated Molecular Pattern Signaling in Response to Streptococcal M1 Protein. Infect Immun. 2015 Nov 30;83(12):4673–81.

78. Pahlman LI, Malmström E, Mörgelin M, Herwald H. M protein from Streptococcus pyogenes induces tissue factor expression and pro-coagulant activity in human monocytes. Microbiology. 2007 Jul 31;153(Pt 8):2458–64.

79. BenNasr A, Herwald H, Sjobring U, Renne T, MullerEsterl W, Björck L. Absorption of kininogen from human plasma by Streptococcus pyogenes is followed by the release of bradykinin. Biochem J. 1997;326:657–60.

80. Bone RC, Balk RA, Cerra FB, Dellinger RP, Fein AM, Knaus WA, et al. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. 1992. pp. 1644–55.

81. Levy MM, Fink MP, Marshall JC, Abraham E, Angus D, Cook D, et al. 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. 2003. pp. 1250–6.

82. Angus DC, van der Poll T. Severe Sepsis and Septic Shock. The Boston Medical and Surgical Journal. 2013 Aug 29;369(9):840–51.

83. Angus DC, Linde-Zwirble WT, Lidicker J, Clermont G, Carcillo J, Pinsky MR. Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care. Crit Care Med. 2001 Jul;29(7):1303–10.

84. Tupchong K, Koyfman A, Foran M. Sepsis, severe sepsis, and septic shock: A review of the literature. African Journal of Emergency Medicine. 5(3):127–35.

85. Gaieski DF, Edwards JM, Kallan MJ, Carr BG. Benchmarking the incidence and mortality of severe sepsis in the United States. Crit Care Med. 2013 May;41(5):1167–74.

86. Brun-Buisson C, Meshaka P, Pinton P, Vallet B. EPISEPSIS: a reappraisal of the epidemiology and outcome of severe sepsis in French intensive care units. Intensive Care Med. 2004 Apr;30(4):580–8.

87. Hotchkiss RS, Monneret G, Payen D. Sepsis-induced immunosuppression: from cellular dysfunctions to immunotherapy. Nat Rev Immunol. 2013 Nov 30;13(12):862–74.

88. Hamers L, Kox M, Pickkers P. Sepsis-induced immunoparalysis: mechanisms, markers, and treatment options. Minerva Anestesiol. 2015 Apr;81(4):426–39.

89. Kovach MA, Standiford TJ. The function of neutrophils in sepsis. Curr Opin Infect Dis. 2012 Jun;25(3):321–7.

90. Alves-Filho JC, de Freitas A, Spiller F, Souto FO, Cunha FQ. The role of neutrophils in severe sepsis. Shock. 2008 Oct;30 Suppl 1:3–9.

91. Kangelaris KN, Prakash A, Liu KD, Aouizerat B, Woodruff PG, Erle DJ, et al. Increased expression of neutrophil-related genes in patients with early sepsis-induced ARDS. Am J Physiol Lung Cell Mol Physiol. 2015 Jun;308(11):L1102–13.

92. Tapper H, Herwald H. Modulation of hemostatic mechanisms in bacterial infectious diseases. Blood. 2000 Oct;96(7):2329–37.

93. Hoffman M, Cooper ST. Thrombin enhances monocyte secretion of tumor necrosis factor and interleukin-1 beta by two distinct mechanisms. Blood Cells Mol Dis. 1995;21(2):156–67.

94. Vandijck DM, Blot SI, De Waele JJ, Hoste EA, Vandewoude KH, Decruyenaere JM. Thrombocytopenia and outcome in critically ill patients with bloodstream infection. Heart & Lung. 2010;39(1):21–6.

95. SHARMA B, SHARMA M, MAJUMDER M, STEIER W, SANGAL A, KALAWAR M. Thrombocytopenia in septic shock patients--a prospective observational study of incidence, risk factors and correlation with clinical outcome. Anaesthesia and Intensive Care. 2007 Dec;35(6):874–80.

96. Wong HR, Cvijanovich N, Wheeler DS, Bigham MT, Monaco M, Odoms K, et al. Interleukin-8 as a stratification tool for interventional trials involving pediatric septic shock. Am J Respir Crit Care Med. 2008 Aug 1;178(3):276–82.

97. Gautam N, Maria Olofsson A, Herwald H, Iversen LF, Lundgren-Åkerlund E, Hedqvist P, et al. Heparin-binding protein (HBP/CAP37): A missing link in neutrophil-evoked alteration of vascular permeability. Nat Med. Nature Publishing Group; 2001 Oct 1;7(10):1123–7.

98. Linder A, Akesson P, Inghammar M, Treutiger C-J, Linner A, Sunden-Cullberg J. Elevated plasma levels of heparin-binding protein in intensive care unit patients with severe sepsis and septic shock. Crit Care. 2012;16(3):–R90.

99. Linder A, Christensson B, Herwald H, Björck L, Akesson P. Heparin-Binding Protein: An Early Marker of Circulatory Failure in Sepsis. Clin Infect Dis. 2009 Oct;49(7):1044–50.

100. Panacek EA, Marshall JC, Albertson TE, Johnson DH, Johnson S, MacArthur RD, et al. Efficacy and safety of the monoclonal anti-tumor necrosis factor antibody F(ab')2 fragment afelimomab in patients with severe sepsis and elevated interleukin-6 levels. Crit Care Med. 2004 Nov;32(11):2173–82.

101. Riedemann NC, Guo R-F, Ward PA. Novel strategies for the treatment of sepsis. Nat Med. 2003 May;9(5):517–24.

102. Daly ME. Determinants of platelet count in humans. Haematologica. 2010 Dec 31;96(1):10–3.

103. WRIGHT JH. The Origin and Nature of the Blood Plates. The Boston Medical and Surgical Journal. 1906 Jun 7;154(23):643–5.

104. Wendling F, Maraskovsky E, Debili N, Florindo C, Teepe M, Titeux M, et al. cMpl ligand is a humoral regulator of megakaryocytopoiesis. Nature. 1994 Jun 16;369(6481):571–4.

105. K K. The mpl ligand: molecular and cellular biology of the critical regulator of megakaryocyte development. Stem Cells. 1994 Jan 1;12 Suppl 1:91–6; discussion 96–7.

106. Kuter DJ. Biology and Chemistry of Thrombopoietic Agents. Semin Hematol. 2010 Jan 1;47(3):243–8.

107. Lok S, Kaushansky K, Holly RD, Kuijper JL, Lofton-Day CE, Oort PJ, et al. Cloning and expression of murine thrombopoietin cDNA and stimulation of platelet production in vivo. Nature. 1994 Jun 15;369(6481):565–8.

108. Kaushansky K. Thrombopoietin. Trends in Endocrinology & Metabolism. 1997 Mar;8(2):45–50.

109. Richardson JL, Shivdasani RA, Boers C, Hartwig JH, Italiano JE. Mechanisms of organelle transport and capture along proplatelets during platelet production. Blood. 2005 Dec 15;106(13):4066–75.

110. Leven RM, Yee MK. Megakaryocyte morphogenesis stimulated in vitro by whole and partially fractionated thrombocytopenic plasma: a model system for the study of platelet formation. Blood. 1987 Mar 31;69(4):1046–52.

111. Leven RM. Megakaryocyte motility and platelet formation. Scanning Microscopy. 1987 Nov 30;1(4):1701–9.

112. Tablin F, Castro M, Leven RM. Blood platelet formation in vitro. The role of the cytoskeleton in megakaryocyte fragmentation. J Cell Sci. 1990 Aug 31;97 (Pt 1):59–70.

113. Thon JN, Montalvo A, Patel-Hett S, Devine MT, Richardson JL, Ehrlicher A, et al. Cytoskeletal mechanics of proplatelet maturation and platelet release. The Journal of Cell Biology. 2010 Nov 15;191(4):861–74.

114. Italiano JE, Lecine P, Shivdasani RA, Hartwig JH. Blood Platelets Are Assembled Principally at the Ends of Proplatelet Processes Produced by Differentiated Megakaryocytes. The Journal of Cell Biology. 1999 Dec;147:1299–312.

115. COHEN JA, LEEKSMA CH. Determination of the life span of human blood platelets using labelled diisopropylfluorophosphonate. J Clin Invest. 1956 Aug 31;35(9):964–9.

116. Manning KL, Novinger S, Sullivan PS, McDonald TP. Successful determination of platelet lifespan in C3H mice by in vivo biotinylation. Lab Anim Sci. 1996 Oct;46(5):545–8.

117. Ault KA, Knowles C. In vivo biotinylation demonstrates that reticulated platelets are the youngest platelets in circulation. Exp Hematol. 1995 Jul 31;23(9):996–1001.

118. White JG, Escolar G. The blood platelet open canalicular system: a two-way street. Eur J Cell Biol. 1991 Dec;56(2):233–42.

119. Escolar G, White JG. The platelet open canalicular system: a final common pathway. Blood Cells. 1991;17(3):467–85–discussion486–95.

120. Zharikov S, Shiva S. Platelet mitochondrial function: from regulation of thrombosis to biomarker of disease. Biochem Soc Trans. 2013 Feb;41(1):118–23.

121. Ravi S, Chacko B, Sawada H, Kramer PA, Johnson MS, Benavides GA, et al. Metabolic plasticity in resting and thrombin activated platelets. PLoS ONE. 2015;10(4):e0123597–7.

122. Whiteheart SW. Platelet granules: surprise packages. Blood. 2011 Aug 4;118(5):1190–1.

123. Flaumenhaft1 R. α-granules: a story in the making. Blood. 2012 Dec 13;120:4908–9.

124. Coppinger JA, Cagney G, Toomey S, Kislinger T, Belton O, McRedmond JP, et al. Characterization of the proteins released from activated platelets leads to localization of novel platelet proteins in human atherosclerotic lesions. Blood. 2004 Mar 14;103(6):2096–104.

125. Assoian RK, Komoriya A, Meyers CA, Miller DM, Sporn MB. Transforming growth factor-beta in human platelets. Identification of a major storage site, purification, and characterization. J Biol Chem. 1983 Jun;258:7155–60.

126. Hsu-Lin SS, Berman CLC, Furie BCB, August DD, Furie BB. A platelet membrane protein expressed during platelet activation and secretion. Studies using a monoclonal antibody specific for thrombin-activated platelets. J Biol Chem. 1984 Jul 24;259(14):9121–6.

127. MCEver RP, Beckstead JH, Moore KL, Marshall-Carlson L, Bainton DF. GMP-140, a platelet alpha-granule membrane protein, is also synthesized by vascular endothelial cells and is localized in Weibel-Palade bodies. J Clin Invest. 1989 Jul;84(1):92–9.

128. Krijgsveld J, Zaat S, Meeldijk J, van Veelen PA, Fang G, Poolman B, et al. Thrombocidins, microbicidal proteins from human blood platelets, are C-terminal deletion products of CXC chemokines. J Biol Chem. 2000;275(27):20374–81.

129. Woronowicz K, Dilks JR, Rozenvayn N, Dowal L, Blair PS, Peters CG, et al. The Platelet Actin Cytoskeleton Associates with SNAREs and Participates in α-Granule Secretion. Biochemistry. 2010 Jun;49(21):4533–42.

130. Flaumenhaft R. Molecular basis of platelet granule secretion. Arteriosclerosis, Thrombosis, and Vascular Biology. 2003 Jul 1;23(7):1152–60.

131. Cauwenberghs S, Feijge MAH, Harper AGS, Sage SO, Curvers J, Heemskerk JWM. Shedding of procoagulant microparticles from unstimulated platelets by integrin-mediated destabilization of actin cytoskeleton. FEBS Lett. 2006 Oct;580(22):5313–20.

132. Andonegui G, Kerfoot SM, McNagny K, Ebbert KVJ, Patel KD, Kubes P. Platelets express functional Toll-like receptor-4. Blood. 2005 Sep 30;106(7):2417–23.

133. Aslam R, Speck ER, Kim M, Crow AR, Bang KWA, Nestel FP, et al. Platelet Toll-like receptor expression modulates lipopolysaccharide-induced thrombocytopenia and tumor necrosis factor-alpha production in vivo. Blood. 2006 Jan 15;107(2):637–41.

134. Cognasse F, Hamzeh H, Chavarin P, Acquart S, Genin C, Garraud O. Evidence of Toll-like receptor molecules on human platelets. Immunol Cell Biol. 2005 Apr;83(2):196–8.

135. VANCURA S, STEINER M. Identification of Fc and F(ab')2IgG receptors on human platelets. Biochemistry. 1987 Jan 27;84:3575–9.

136. Ghazizadeh SS, Bolen JBJ, Fleit HBH. Physical and functional association of Src-related protein tyrosine kinases with Fc gamma RII in monocytic THP-1 cells. J Biol Chem. 1994 Mar 24;269(12):8878–84.

137. Boylan B, Gao C, Rathore V, Gill JC, Newman DK, Newman PJ. Identification of FcgammaRIIa as the ITAM-bearing receptor mediating alphaIIbbeta3 outside-in integrin signaling in human platelets. Blood. 2008 Sep 30;112(7):2780–6.

138. Shattil SJ, Newman PJ. Integrins: dynamic scaffolds for adhesion and signaling in platelets. Blood. 2004 Sep 14;104(6):1606–15.

139. Offermanns S. Activation of platelet function through G protein-coupled receptors. Circ Res. 2006 Dec 7;99(12):1293–304.

140. Packham MA, Mustard JF. Platelet adhesion. Prog Hemost Thromb. 1984;7:211–88.

141. Savage B, Sixma JJ, Ruggeri ZM. Functional self-association of von Willebrand factor during platelet adhesion under flow. Proc Natl Acad Sci U S A. 2002 Jan 8;99(1):425–30.

142. Konstantopoulos K, Chow TW, Turner NA, Hellums JD, Moake JL. Shear stress-induced binding of von Willebrand factor to platelets. Biorheology. 1997 Jan;34(1):57–71.

143. Morales LD, Martin C, Cruz MA. The interaction of von Willebrand factor-A1 domain with collagen: mutation G1324S (type 2M von Willebrand disease) impairs the conformational change in A1 domain induced by collagen. J Thromb Haemost. 2006 Feb;4(2):417–25.

144. De Luca M, Facey DA, Favaloro EJ, Hertzberg MS, Whisstock JC, McNally T, et al. Structure and function of the von Willebrand factor A1 domain: analysis with monoclonal antibodies reveals distinct binding sites involved in recognition of the platelet membrane glycoprotein Ib-IX-V complex and ristocetin-dependent activation. Blood. 2000 Jan;95(1):164–72.

145. Moroi M, Jung SM. A mechanism to safeguard platelet adhesion under high shear flow: von Willebrand factor–glycoprotein Ib and integrin α2β1–collagen interactions make complementary, collagen-type-specific contributions to adhesion. J Thromb Haemost. 2007 Mar;5:1340–2.

146. Nieswandt B, Pleines I, Bender M. Platelet adhesion and activation mechanisms in arterial thrombosis and ischaemic stroke. Journal of Thrombosis and Haemostasis. 2011 Jun 30;9 Suppl 1:92–104.

147. Nieswandt B, Watson SP. Platelet-collagen interaction: is GPVI the central receptor? Blood. 2003 Jul 14;102(2):449–61.

148. Hawiger J. Mechanisms involved in platelet vessel wall interaction. Thromb Haemost. 1995 Jul;74(1):369–72.

149. Gale AJ. Current Understanding of Hemostasis. Toxicologic Pathology. 2011 Mar 4;39(1):273–80.

150. Donovan JW, Mihalyi E. Conformation of fibrinogen: calorimetric evidence for a three-nodular structure. Proc Natl Acad Sci U S A. 1974 Oct;71(10):4125–8.

151. Mosesson MW. Fibrinogen and fibrin structure and functions. J Thromb Haemost. 2005 Aug;3(8):1894–904.

152. Powers ME, Becker REN, Sailer A, Turner JR, Bubeck Wardenburg J. Synergistic Action of Staphylococcus aureus alpha-Toxin on Platelets and Myeloid Lineage Cells Contributes to Lethal Sepsis. Cell Host Microbe. 2015 Jun;17(6):775–87.

153. Wilson M, Blum R, Dandona P, Mousa S. Effects in humans of intravenously administered endotoxin on soluble cell-adhesion molecule and inflammatory markers: a model of human diseases. Clin Exp Pharmacol Physiol. 2001 May;28(5-6):376–80.

154. Heptinstall S. Platelet activation by an extracellular adherence protein from Staphylococcus aureus acting via modulation of sulfhydryl groups on platelets. Arteriosclerosis, Thrombosis, and Vascular Biology. 2012 Aug;32(8):1751–2.

155. HAWIGER J, STECKLEY S, HAMMOND D, CHENG C, SHEILATIMMONS AGAD. Staphylococci-induced Human Platelet Injury Mediated by Protein A and Immunoglobulin G Fc Fragment Receptor. J Clin Invest. 1979 Oct;64:931–7.

156. Kurpiewski GE, Forrester LJ, Campbell BJ, Barrett JT. Platelet aggregation by Streptococcus pyogenes. Infect Immun. 1983 Feb 1;39(2):704–8.

157. Kerrigan SW, Clarke N, Loughman A, Meade G, Foster TJ, Cox D. Molecular Basis for Staphylococcus aureus-Mediated Platelet Aggregate Formation Under Arterial Shear In Vitro. Arteriosclerosis, Thrombosis, and Vascular Biology. 2007 Nov 15;28(2):335–40.

158. O'Brien L, Kerrigan SW, Kaw G, Hogan M, Penadés J, Litt D, et al. Multiple mechanisms for the activation of human platelet aggregation by Staphylococcus aureus: roles for the clumping factors ClfA and ClfB, the serine-aspartate repeat protein SdrE and protein A. Mol Microbiol. 2002 Apr 30;44(4):1033–44.

159. Loughman A, Fitzgerald JR, Brennan MP, Higgins J, Downer R, Cox D, et al. Roles for fibrinogen, immunoglobulin and complement in platelet activation promoted by Staphylococcus aureus clumping factor A. Mol Microbiol. 2005 Jun 30;57(3):804–18.

160. Sjobring U. Induction of platelet thrombi by bacteria and antibodies. Blood. 2002 Aug 1;100(13):4470–7.

161. Moreau D, Timsit J-F, Vesin A, Garrouste-Orgeas M, de Lassence A, Zahar J-R, et al. Platelet Count Decline: An Early Prognostic Marker in Critically Ill Patients With Prolonged ICU Stays. Chest. American College of Chest Physicians; 2007;131(6):1735–41.

162. Yeaman MR, Bayer AS, Koo SP, Foss W, Sullam PM. Platelet microbicidal proteins and neutrophil defensin disrupt the Staphylococcus aureus cytoplasmic membrane by distinct mechanisms of action. J Clin Invest. 1998 Jan 1;101(1):178–87.

163. Clawson CC, White JG. Platelet interaction with bacteria. V. Ultrastructure of congenital afibrinogenemic platelets. Am J Pathol. 1980 Jan 1;98(1):197–211.

164. Clawson CC. Platelet Interaction with Bacteria: III. Ultrastructure. Am J Pathol. American Society for Investigative Pathology; 1973 Mar 1;70(3):449–63.

165. Clawson CC, Rao GH, White JG. Platelet interaction with bacteria. IV. Stimulation of the release reaction. Am J Pathol. 1975 Oct 31;81(2):411–20.

166. Youssefian T, Drouin A, Massé JM, Guichard J. Host defense role of platelets: engulfment of HIV and staphlococcus aureus occurs in a specific subcellular compartment and is enhanced by platelet activation. Blood. 2002 Jun;99(11):4021–9.

167. Li N, Hu H, Lindqvist M, Wikström-Jonsson E, Goodall AH, Hjemdahl P. Platelet-leukocyte cross talk in whole blood. Arteriosclerosis, Thrombosis, and Vascular Biology. 2000 Dec;20(12):2702–8.

168. Ahn KC, Jun AJ, Pawar P, Jadhav S, Napier S, McCarty OJT, et al. Preferential binding of platelets to monocytes over neutrophils under flow. Biochem Biophys Res Commun. 2005 Apr;329(1):345–55.

169. O'Sullivan BP, Linden MD, Frelinger AL, Barnard MR, Spencer-Manzon M, Morris JE, et al. Platelet activation in cystic fibrosis. Blood. 2005 Jun 15;105(12):4635–41.

170. Pitchford SCS, Yano HH, Lever RR, Riffo-Vasquez YY, Ciferri SS, Rose MJM, et al. Platelets are essential for leukocyte recruitment in allergic inflammation. Journal of Allergy and Clinical Immunology. 2003 Jun 30;112(1):109–18.

171. Huo Y, Schober A, Forlow SB, Smith DF, Hyman MC, Jung S, et al. Circulating activated platelets exacerbate atherosclerosis in mice deficient in apolipoprotein E. Nat Med. 2002 Dec 31;9(1):61–7.

172. Joseph JE, Harrison P, Mackie IJ, Isenberg DA, Machin SJ. Increased circulating platelet-leucocyte complexes and platelet activation in patients with antiphospholipid syndrome, systemic lupus erythematosus and rheumatoid arthritis. Br J Haematol. 2001 Jun;115(2):451–9.

173. Gawaz MM, Fateh-Moghadam SS, Pilz GG, Gurland HJH, Werdan KK. Platelet activation and interaction with leucocytes in patients with sepsis or multiple organ failure. Eur J Clin Invest. 1995 Oct 31;25(11):843–51.

174. Kirschenbaum LA, Adler D, Astiz ME, Barua RS, Saha D, Rackow EC. Mechanisms of platelet-neutrophil interactions and effects on cell filtration in septic shock. Shock. 2002 Jun;17(6):508–12.

175. Moore KL, Patel KD, Bruehl RE, Li F, Johnson DA, Lichenstein HS, et al. P-selectin glycoprotein ligand-1 mediates rolling of human neutrophils on P-selectin. The Journal of Cell Biology. 1995 Feb;128(4):661–71.

176. Piccardoni P. Platelet/polymorphonuclear leukocyte adhesion: a new role for SRC kinases in Mac-1 adhesive function triggered by P-selectin. Blood. 2001 Jul 1;98(1):108–16.

177. Simon DI, Chen Z, Xu H, Li CQ, Dong JF, McIntire LV, et al. Platelet glycoprotein Ibα is a counterreceptor for the leukocyte integrin Mac-1 (CD11b/CD18). J Exp Med. 2000 Jul;192(2):193–204.

178. Xu TT, Zhang LL, Geng ZHZ, Wang H-BH, Wang J-TJ, Chen MM, et al. P-selectin cross-links PSGL-1 and enhances neutrophil adhesion to fibrinogen and ICAM-1 in a Src kinase-dependent, but GPCR-independent mechanism. Cell Adh Migr. 2007 Jun 30;1(3):115–23.

179. Diacovo TGT, Roth SJS, Buccola JMJ, Bainton DFD, Springer TAT. Neutrophil rolling, arrest, and transmigration across activated, surface-adherent platelets via sequential action of P-selectin and the beta 2-integrin CD11b/CD18. Blood. 1996 Jun 30;88(1):146–57.

180. Lam FWF, Burns ARA, Smith CWC, Rumbaut RER. Platelets enhance neutrophil transendothelial migration via P-selectin glycoprotein ligand-1. Am J Physiol Heart Circ Physiol. 2011 Jan 31;300(2):H468–75.

181. Thomas Weissmüller ELCPRMSPLBGTFSPC. PMNs facilitate translocation of platelets across human and mouse epithelium and together alter fluid homeostasis via epithelial cell–expressed ecto-NTPDases. J Clin Invest. American Society for Clinical Investigation; 2008 Nov 3;118(11):3682.

182. Diacovo TG, deFougerolles AR, Bainton DF, Springer TA. A functional integrin ligand on the surface of platelets: intercellular adhesion molecule-2. J Clin Invest. 1994 Sep;94(3):1243–51.

183. Santoso S, Sachs UJH, Kroll H, Linder M, Ruf A, Preissner KT, et al. The Junctional Adhesion Molecule 3 (JAM-3) on Human Platelets is a Counterreceptor for the Leukocyte Integrin Mac-1. Journal of Experimental Medicine. 2002 Sep 2;196(5):679–91.

184. Buchner K, Henn V, Grafe M, de Boer OJ, Becker AE, Kroczek RA. CD40 ligand is selectively expressed on CD4(+) T cells and platelets: implications for CD40-CD40L signalling in atherosclerosis. J Pathol. 2003 Oct;201(2):288–95.

185. Cerletti C, Tamburrelli C, Izzi B, Gianfagna F, de Gaetano G. Platelet-leukocyte interactions in thrombosis. Thromb Res. Elsevier Ltd; 2012 Mar 1;129(3):263–6.

186. Clark SR, Ma AC, Tavener SA, McDonald B, Goodarzi Z, Kelly MM, et al. Platelet TLR4 activates neutrophil extracellular traps to ensnare bacteria in septic blood. Nat Med. 2007 Mar 25;13(4):463–9.

187. Caudrillier A, Kessenbrock K, Gilliss BM, Nguyen JX, Marques MB, Monestier M, et al. Platelets induce neutrophil extracellular traps in transfusion-related acute lung injury. J Clin Invest. 2012 Jul;122(7):2661–71.

188. Fiore S, Serhan CN. Formation of lipoxins and leukotrienes during receptor-mediated interactions of human platelets and recombinant human granulocyte/macrophage colony-stimulating factor-primed neutrophils. J Exp Med. 1990 Nov 1;172(5):1451–7.

189. Palmantier RR, Borgeat PP. Thrombin-activated platelets promote leukotriene B4 synthesis in polymorphonuclear leucocytes stimulated by physiological agonists. Br J Pharmacol. 1991 Jul 31;103(4):1909–16.

190. Russwurm S, Vickers J, Meier-Hellmann A, Spangenberg P, Bredle D, Reinhart K, et al. Platelet and leukocyte activation correlate with the severity of septic organ dysfunction. Shock. 2002 Apr;17(4):263–8.

191. Johansson D, Shannon O, Rasmussen M. Platelet and Neutrophil Responses to Gram Positive Pathogens in Patients with Bacteremic Infection. PLoS ONE. 2011 Nov 29;6(11):e26928.

192. VEDA P. WHY ARE NEUTROPHILS POLYMORPHONUCLEAR? European Journal of inflammation. 2011 Apr 1;9(2):85–93.

193. Borregaard N. Neutrophils, from Marrow to Microbes. Immunity. Elsevier Inc; 2010 Nov 24;33(5):657–70.

194. Furze RC, Rankin SM. Neutrophil mobilization and clearance in the bone marrow. Immunology. 2008 Nov;125(3):281–8.

195. Galli SJ, Borregaard N, Wynn TA. Phenotypic and functional plasticity of cells of innate immunity: macrophages, mast cells and neutrophils. Nat Immunol. 2011 Oct 19;12(11):1035–44.

196. Pillay J, Braber den I, Vrisekoop N, Kwast LM, de Boer RJ, Borghans JAM, et al. In vivo labeling with 2H2O reveals a human neutrophil lifespan of 5.4 days. Blood. 2010 Jul;116(4):625–7.

197. Faurschou M, Borregaard N. Neutrophil granules and secretory vesicles in inflammation. Microbes Infect. 2003 Nov;5(14):1317–27.

198. Borregaard N, Miller LJ, Springer TA. Chemoattractant-regulated mobilization of a novel intracellular compartment in human neutrophils. Science. 1987 Sep;237(4819):1204–6.

199. Borregaard N, Kjeldsen L, Rygaard K, Bastholm L, Nielsen MH, Sengeløv H, et al. Stimulus-dependent secretion of plasma proteins from human neutrophils. J Clin Invest. 1992 Jul;90(1):86–96.

200. Häger M, Cowland JB, Borregaard N. Neutrophil granules in health and disease. J Intern Med. 2010 Jul;268(1):25-34

.

201. Sun R, Iribarren P, Zhang N, Zhou Y, Gong W, Cho EH, et al. Identification of Neutrophil Granule Protein Cathepsin G as a Novel Chemotactic Agonist for the G Protein-Coupled Formyl Peptide Receptor. The Journal of Immunology. 2004 Jun 21;173(1):428–36.

202. Sengeløv H, Kjeldsen L, Borregaard N. Control of exocytosis in early neutrophil activation. J Immunol. 1993 Feb;150(4):1535–43.

203. Pippin DJ, Pippin KD. Size of azurophil lysosomes of human PMNs determined by stereological morphometry. Ultrastruct Pathol. 2003 Jul;27(4):261–3.

204. Borregaard N, Heiple JM, Simons ER, Clark RA. Subcellular localization of the b-cytochrome component of the human neutrophil microbicidal oxidase: translocation during activation. J Cell Biol. 1983 Jul;97(1):52–61.

205. Sengeløv H, Follin P, Kjeldsen L, Lollike K, Dahlgren C, Borregaard N. Mobilization of granules and secretory vesicles during in vivo exudation of human neutrophils. J Immunol. 1995 Apr;154(8):4157–65.

206. Ley K, Laudanna C, Cybulsky MI, Nourshargh S. Getting to the site of inflammation: the leukocyte adhesion cascade updated. Nat Rev Immunol. 2007 Sep;7(9):678–89.

207. Borregaard N, Kjeldsen L, Sengeløv H, Diamond MS, Springer TA, Anderson HC, et al. Changes in subcellular localization and surface expression of L-selectin, alkaline phosphatase, and Mac-1 in human neutrophils during stimulation with inflammatory mediators. J Leukoc Biol. 1994 Jul;56(1):80–7.

208. Kjeldsen L, Bjerrum OW, Askaa J, Borregaard N. Subcellular localization and release of human neutrophil gelatinase, confirming the existence of separate gelatinase-containing granules. Biochem J. 1992 Oct;287 (Pt 2):603–10.

209. Rosenzweig SD, Holland SM. Recent Insights into the Pathobiology of Innate Immune Deficiencies. Curr Allergy Asthma Rep. 2011 Aug 4;11(5):369–77.

210. Sabroe I, Dower SK, Whyte M. The role of toll-like receptors in the

regulation of neutrophil migration, activation, and apoptosis. Clin Infect Dis. 2005;41:S421–6.

211. Phillipson M, Kubes P. The neutrophil in vascular inflammation. Nat Med. 2011 Nov 7;17(11):1381–90.

212. Fu H. Ligand recognition and activation of formyl peptide receptors in neutrophils. J Leukoc Biol. 2005 Dec 19;79(2):247–56.

213. Dufton N, Perretti M. Therapeutic anti-inflammatory potential of formyl-peptide receptor agonists. Pharmacol Ther. 2010 Aug;127(2):175–88.

214. Kaplan G. Differences in the mode of phagocytosis with Fc and C3 receptors in macrophages. Scand J Immunol. 1977;6(8):797–807.

215. Brown EJ. Complement receptors, adhesion, and phagocytosis. Infect Agents Dis. 1992 Apr;1(2):63–70.

216. Groves E, Dart AE, Covarelli V, Caron E. Molecular mechanisms of phagocytic uptake in mammalian cells. Cell Mol Life Sci. 2008 Mar 7;65(13):1957–76.

217. GRIFFIN FM, GRIFFIN JJA, LEIDER JE, SILVERSTEIN ASC. Studies of the macrophage complement receptor. Alteration of receptor function upon mac- rophage activation. J. Exp. Med. 141, 1278–1290. 2003 Apr 4;:1–20.

218. Ehlenberger AG, Nussenzweig V. The role of membrane receptors for C3b and C3d in phagocytosis. J Exp Med. 1977 Jan 31;145(2):357–71.

219. Nordenfelt P, Tapper H. Phagosome dynamics during phagocytosis by neutrophils. J Leukoc Biol. 2011 Aug 1;90(2):271–84.

220. Stuart LM, Ezekowitz RAB. Phagocytosis: elegant complexity. Immunity. 2005 May;22(5):539–50.

221. Bedard K, Krause K-H. The NOX family of ROS-generating NADPH oxidases: physiology and pathophysiology. Physiol Rev. 2007 Jan;87(1):245–313.

222. Segal AW. The NADPH oxidase and chronic granulomatous disease. Mol Med Today. 1996 Mar;2(3):129–35.

223. Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS, et al. Neutrophil extracellular traps kill bacteria. Science. 2004 Mar;303(5663):1532–5.

224. Adamo L, García-Cardeña G. The vascular origin of hematopoietic cells. Developmental Biology. 2012 Feb;362(1):1–10.

225. Redl H. The Endothelium as an Immune Organ: Active Player and Passive Target? Immune Response in the Critically Ill. Berlin, Heidelberg: Springer Berlin Heidelberg; 2000. pp. 292–311.

226. Vallance P, Collier J, Moncada S. Effects of endothelium-derived nitric oxide on peripheral arteriolar tone in man. Lancet. 1989 Oct;2(8670):997–1000.

227. Sandoo A, van Zanten JJCSV, Metsios GS, Carroll D, Kitas GD. The endothelium and its role in regulating vascular tone. Open Cardiovasc Med J. 2009 Dec 31;4:302–12.

228. Wojciak-Stothard B, Ridley AJ. Rho GTPases and the regulation of endothelial permeability. Vascul Pharmacol. 2002 Nov;39(4-5):187–99.

229. Pegu A, Qin S, Fallert Junecko BA, Nisato RE, Pepper MS, Reinhart TA. Human Lymphatic Endothelial Cells Express Multiple Functional TLRs. The Journal of Immunology. 2008 Feb 21;180(5):3399–405.

230. Henneke P, Golenbock DT. Innate immune recognition of lipopolysaccharide by endothelial cells. Crit Care Med. 2002 May;30(5 Suppl):S207–13.

231. Geng JG, Bevilacqua MP, Moore KL, McIntyre TM, Prescott SM, Kim JM, et al. Rapid neutrophil adhesion to activated endothelium mediated by GMP-140. Nature. 1990 Feb;343(6260):757–60.

232. Goebeler M, Yoshimura T, Toksoy A, Ritter U, Brocker EB, Gillitzer R. The chemokine repertoire of human dermal microvascular endothelial cells and its regulation by inflammatory cytokines. J Invest Dermatol. 1997 Apr;108(4):445–51.

233. Weber KS, Hundelshausen von P, Clark-Lewis I, Weber PC, Weber C. Differential immobilization and hierarchical involvement of chemokines in monocyte arrest and transmigration on inflamed

endothelium in shear flow. Eur J Immunol. 1999 Jan 31;29(2):700–12.

234. Alon R, Feigelson S. From rolling to arrest on blood vessels: leukocyte tap dancing on endothelial integrin ligands and chemokines at sub-second contacts. Semin Immunol. 2002 Apr;14(2):93–104.

235. Phillipson M, Heit B, Colarusso P, Liu L, Ballantyne CM, Kubes P. Intraluminal crawling of neutrophils to emigration sites: a molecularly distinct process from adhesion in the recruitment cascade. J Exp Med. 2006 Nov;203(12):2569–75.

236. Muller WA. Mechanisms of Transendothelial Migration of Leukocytes. Circ Res. 2009 Jul 30;105(3):223–30.

237. Body SC. Platelet activation and interactions with the microvasculature. J Cardiovasc Pharmacol. 1996;27 Suppl 1:S13–25.

238. Bombeli T, Mueller M, Haeberli A. Anticoagulant properties of the vascular endothelium. Thromb Haemost. 1997 Mar;77(3):408–23.

239. Smyth JV, FRCS, Rooney OB, Dodd PD, FRCS, Walker MG, et al. Production of plasminogen activator inhibitor-1 by human saphenous vein endothelial cells seeded onto endarterectomy surfaces in vitro. journal of vascular surgery. 1997 Jan 30;25:722–5.

240. Mackman N. Role of Tissue Factor in Hemostasis, Thrombosis, and Vascular Development. Arteriosclerosis, Thrombosis, and Vascular Biology. 2004 Mar 4;24(6):1015–22.

241. Osterud B, Flaegstad T. Increased tissue thromboplastin activity in monocytes of patients with meningococcal infection: related to an unfavourable prognosis. Thromb Haemost. 1983 February 28;49(1):5–7.

242. Osterud B. Tissue factor expression by monocytes: regulation and pathophysiological roles. Blood Coagulation & Fibrinolysis. 1998 Mar;9 Suppl 1:S9–14.

243. Bryant AE, Hayes-Schroer SM, Stevens DL. M type 1 and 3 group A streptococci stimulate tissue factor-mediated procoagulant activity in human monocytes and endothelial cells. Infect Immun. 2003 Mar 31;71(4):1903–10.

244. Levi M, Keller TT, van Gorp E. Infection and inflammation and the coagulation system. Cardiovascular …. 2003.

245. Svensson L, Baumgarten M, Mörgelin M, Shannon O. Platelet activation by Streptococcus pyogenes leads to entrapment in platelet aggregates, from which bacteria subsequently escape. Infect Immun. 2014 Oct;82(10):4307–14.

246. Horstmann RD, Sievertsen HJ. Antiphagocytic activity of streptococcal M protein: selective binding of complement control protein factor H. 1988.

247. Gawaz M, Dickfeld T, Bogner C, Moghadam-Fateh S, F JN. Platelet function in septic multiple organ dysfunction syndrome. Intensive Care Med. 2012 Jan 25;23(4):379–85.

248. Hurley SM, Kahn F, Nordenfelt P, Mörgelin M, Sørensen OE, Shannon O. Platelet-Dependent Neutrophil Function Is Dysregulated by M Protein from Streptococcus pyogenes. Infect Immun. 2015 Sep;83(9):3515–25.

249. Sigurdardottir T, Björck V, Herwald H, Mörgelin M, Rutardottir S, Törnebrant J, et al. WHAT'S NEW IN SHOCK, JULY 2010? . Shock. 2010 Jun 30;34(1):98–104.

250. Nilson B, Frick IM, Akesson P, Forsen S, Björck L, Akerström B, et al. Structure and Stability of Protein-H and the M1 Protein From Streptococcus-Pyogenes - Implications for Other Surface-Proteins of Gram-Positive Bacteria. Biochemistry. 1995;34(41):13688–98.