Platelet-dependent pulmonary recruitment of neutrophils in abdominal sepsis

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2012

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Academic Thesis

With permission from the Medical Faculty at Lund University for the presentation of this PhD thesis in a public forum in Clinical Research Centre (CRC), Entrance 72, Skåne University Hospital, Malmö, on Friday 23rd of March, 2012 at 13.00.

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LUND
UNIVERSITY

Department of Clinical Science, Malmö
Section for Surgery
Skåne University Hospital
Lund University, Sweden 2012

Organization	Document name
LUND UNIVERSITY Department of Clinical Science, Malmö Section for Surgery Skåne University Hospital Lund University, Sweden	DOCTORAL DISSERTATION
	Date of issue February 10, 2012
	Sponsoring organization
Author(s) Milladur Rahman	

Title and subtitle

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Abstract

Sepsis and subsequent multiple organ failure remain the major cause of mortality in intensive care units. Leukocyte-mediated tissue damage is a key feature in septic lung injury. Accumulating data suggest that platelets play a role in inflammation and tissue injury. However, the role of platelets in sepsis-induced leukocyte recruitment and lung edema formation in abdominal sepsis is not demonstrated yet. We hypothesized that platelets may play a significant role in pulmonary neutrophil recruitment and tissue damage in abdominal sepsis. For this purpose, we used the mice cecal ligation and puncture (CLP) model of abdominal sepsis. CLP causes significant pulmonary damage characterized by neutrophil infiltration, increased levels of CXC chemokines and increased edema formation in the lung. CLP also provoked Mac-1 expression on circulating neutrophils. Interestingly, depletion of platelets reduced CLP-induced lung damage, neutrophil recruitment in the bronchoalveolar space and edema formation as well as up-regulation of Mac-1 on neutrophils. However, blocking of platelet-neutrophil aggregates formation did not attenuate CLP-induced lung damage and neutrophil activation suggesting that platelets regulate sepsis-induced lung damage via up-regulation of Mac-1 in a contact independent manner. We also found that plasma levels of soluble CD40L was significantly increased in septic mice. Use of CD40L-deficient mice confirmed that platelet-derived CD40L is a pivotal mediator of neutrophil activation and recruitment in abdominal sepsis and this platelet mediated neutrophil activation was indirect and mediated via formation of MIP-2 and CXCR2 signaling. In addition, we observed a significant increase of soluble CD40L levels in septic patients. Interestingly, we found that inhibition of matrix MMPs reduced Mac-1 up-regulation on neutrophils and CXC chemokine formation in the septic lung injury. We also found that MMP-9 levels are significantly increased in septic mice but not MMP-2. In vitro studies revealed that activated platelets up-regulate surface expression of MMP-9 and that inhibition of MMP-9 decreased platelet shedding of CD40L. Use of MMP-9-deficient mice suggested that MMP-9 regulates platelet CD40L shedding in abdominal sepsis. Moreover, pulmonary infiltration of neutrophils as well as edema formation and lung injury were markedly decreased in septic animals lacking MMP-9. Plasma levels of MMP-9 were significantly increased in patients with septic shock compared to healthy controls. Taken together, platelets regulate neutrophil activation in abdominal sepsis via MMP-9-dependent shedding of platelet-derived CD40L. Thus, MMP-9 and CD40L may constitute novel and effective therapeutic targets in abdominal sepsis.

Key words: abdominal sepsis, platelet, neutrophil, lung, inflammation

Classification system and/or index termes (if any):

Supplementary bibliographical information:		Language English
ISSN and key title: 1652-8220		ISBN 978-91-86871-88-8
Recipient's notes	Number of pages 110	Price
	Security classification	

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Lund University, Faculty of Medicine Doctoral Dissertation Series 2012:26
ISBN 978-91-86871-88-8
ISSN 1652-8220

Printed in Sweden by Media-Tryck, Lund University, Lund 2012

To my Family

There are in fact two things, science and opinion; the former begets knowledge, the later ignorance. ***- Hippocrates***

Table of Contents

Abbreviations

APC	allophycocyanin
CARS	compensatory anti-inflammatory response syndrome
CD	cluster of differentiation
CLP	cecal ligation and puncture
CXCL2/MIP-2	macrophage inflammatory protein-2
DAMPs	damage-associated molecular patterns
ECM	extracellular matrix
ELISA	enzyme linked immunosorbent assay
FACS	fluorescence activated cell sorting
FITC	fluorescein isothiocyanate
i.p.	intraperitoneal
i.v.	intravenous
ICAM-1	intercellular adhesion molecule-1
ICU	intensive care unit
IL	interleukin
KC	cytokine-induced neutrophil chemoattractant
kD	kilo Dalton
LFA-1	lymphocyte function antigen-1
LPS	lipopolysaccharide
LRRs	leucine-rich repeats
LTA	lipoteichoic acid
Mac-1	membrane activate complex-1
MCP-1	monocyte chemotactic protein-1
MFI	mean fluorescence intensity
MMPs	matrix metalloproteinases
MOF	multiple organ failure
MPO	myeloperoxidase
NF-kB	nuclear factor kappa B
NOD	nucleotide-binding oligomerization domain
PAMPs	pathogen-associated molecular patterns
PAMPs	pathogen-associated molecular patterns
PE	phycoerythrin
PF-4	platelet factor-4
PG	peptidoglycan
PMNL	polymorphonuclear leukocyte
PRRs	pattern recognition molecular receptors
PSGL-1	P-selectin glycoprotein ligand-1
ROS	reactive oxygen species
s.c.	subcutaneous
SEM	standard error of the mean
SIRS	systemic inflammatory response syndrome
TIMPs	tissue inhibitors of metalloproteinases
TLR	toll-like receptor
TNF-α	tumor necrosis factor-α
VCAM-1	vascular cell adhesion molecule-1

List of original papers

The thesis is based on the following original papers and will be referred in the text by their sequential numbers:

I. Asaduzzaman M, Lavasani S, **Rahman M**, Zhang S, Braun OO, Jeppsson B, Thorlacius H. Platelets support pulmonary recruitment of neutrophils in abdominal sepsis. *Crit Care Med* 37: 1389-1396, 2009. [#]

II. **Rahman M**, Zhang S, Chew M, Ersson A, Jeppsson B, Thorlacius H. Platelet-derived CD40L (CD154) mediates neutrophil upregulation of Mac-1 and recruitment in septic lung injury. *Ann Surg* 250: 783-790, 2009. [#]

III. Chew M*, **Rahman M***, Ihrman L, Erson A, Zhang S, Thorlacius H. Soluble CD40L (CD154) is increased in patients with shock. *Inflamm Res* 59: 979-982, 2010. [$]

IV. **Rahman M**, Roller J, Zhang S, Syk I, Menger M, Jeppsson B, and Thorlacius H. Metalloproteinases regulate CD40L shedding from platelets and pulmonary recruitment of neutrophils in abdominal sepsis. *Inflamm Res* 2011. (accepted)

V. **Rahman M**, Zhang S, Zhang S, Chew M, Syk I, Jeppsson B, and Thorlacius H. Platelet shedding of CD40L is regulated by matrix metalloproteinase-9 in abdominal sepsis. Submitted to *Crit Care Med*. 2012

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Introduction

Sepsis describes a complex clinical syndrome that results from body's systemic response to infection [1]. Sepsis develops when the initial, appropriate host response to an infection fails and becomes amplified, and then dysregulated [1-3]. It is considered as one of the leading cause of death across the world and highest in non-coronary intensive care units. The documented incidence was approximately 3.0 cases per 1,000 populations in North America [4, 5]. The mortality rate is generally between 30-40% in the elderly and 50% or greater in patients with more severe syndrome, septic shock [1, 5]. The annual cost for sepsis is €7.6 billion in Europe and €17.4 billion in the US [4, 6]. The major cause of mortality in patients with sepsis is multiple organ failure. Patients usually develop a single organ failure, typically acute lung injury followed by the failure of other organs, for instance, the liver and kidney, resulting in multiple organ dysfunctions [1]. Management of patients with sepsis is largely limited to supportive therapies, which is partly due to an incomplete understanding of the underlying patho-physiology [7, 8].

Abdominal sepsis is characterized by intestinal perforation in which toxins and microbes contaminate the abdominal cavity [9, 10]. Fecal bacteria stimulate local production of various pro-inflammatory substances, which are subsequently released into the circulation. Moreover, bacteria can directly invade the gut-blood barrier and trigger an inflammatory host response in the vascular compartment [11]. In general, neutrophils constitute body's first line of defense against invading microorganisms. They are able to eliminate harmful pathogens by their ability to rapidly exit blood vessels and migrate to extravascular sites of infected organs [12, 13]. However, excessive activation and accumulation of neutrophils cause pulmonary tissue damage in sepsis [14-17]. The complex signalling cascades triggering neutrophil activation and recruitment in the lung by a mixed bacterial flora and their released products are largely unknown [18, 19]. Numerous studies have shown that inhibition of neutrophil recruitment may protect against sepsis-induced lung injury [16, 17, 20].

Platelets are considered to be essential for haemostasis, thrombosis and wound healing, but a growing body of evidence indicates that platelets play a role in inflammation and tissue injury [21-24]. Of interest, some recent studies have reported that platelets may exert a supportive role in the recruitment of leukocytes in the microvasculature [22, 25, 26]. The ability of platelets to store, produce and release several pro-inflammatory and anti-inflammatory factors, make it an important modulator of other immune cells function [24, 27]. The mechanism behind platelet-dependent pulmonary recruitment of leukocytes in abdominal sepsis is remained to be elucidated.

Background

Sepsis

Sepsis is characterized by body's host response to an infection. This host response is known as systemic inflammatory response syndrome (SIRS) and is characterized by an elevated or lowered body temperature, i.e. under 36 °C (97 °F) or over 38 °C (100 °F), an elevated heart rate (above 90 beats per minute), high respiratory rate (above 20 breaths per minute or a partial pressure of carbon dioxide in the blood of less than 4.3 kilopascals), abnormal white blood cell counts (above 12 x 10^9 per liter) [28, 29]. Sepsis is differentiated from SIRS by the presence of a known or suspected pathogen. Severe sepsis arises when sepsis is associated with organ

dysfunction, hypotension, organ hypoperfusion, and septic shock develops when sepsis is associated with hypotension despite adequate fluid resuscitation [29]. The clinical appearance of a patient with SIRS due to infection and other causes, for example, burns, polytrauma, chemical pneumonitis and pancreatitis, is similar. Sepsis is a medical emergency due to an interruption of oxygen and nutrients to the tissues of vital organs such as brain, intestine, liver, kidney and lung.

Pathogenesis of Sepsis

Microbial components

The initiation of complex signaling cascades of immune system by bacteria or bacterial components is very complex. Bacterial motifs which are recognized by innate immune system are called pathogen-associated molecular patterns (PAMPs) [30]. A microbiological diagnosis revealed that about 60% of the cases are caused by gram-negative bacteria and remainders are caused by gram-positive bacteria [31]. Lipopolysaccharide (LPS; also known as endotoxin) of gram-negative bacteria play the dominant role in immunepathogenesis of sepsis. The LPS molecule is embedded on the outer membrane of bacterial lipid bilayer and the lipid A portion of this molecule is the most toxic part and involved in the activation of host cells [1, 32]. It has the ability to directly activate host immune cells i.e. macrophages, endothelial cells, and complement, leading to the release of several pro-inflammatory mediators including TNF-α, IL-1, IL-6, high-mobility group box-1 (HMGB-1), macrophage migratory inhibitory factor (MIF), platelet-activating factor (PAF), nitric oxide (NO), complements and eicosanoids [33-36]. LPS causes cardiac dysfunction and decreased systemic vascular resistance, leading to shock and death [18, 37]. CD14/TLR4/MD2 receptor complex of host cells is involved in the recognition of most gram-negative bacteria and their products [38].

Microbial products from gram-positive bacteria including lipoteichoic acid (LTA), peptidoglycan (PG), flagellin, microbial DNA can binds to cell surface receptors and stimulate for cytokine production [39, 40]. CD14/TLR2 receptor complex is involved in binding with these components. Some of the gram-positive bacteria can produce potent exotoxins. Toxic shock syndrome toxin-1 (TSST-1) of *staphylococcus aureus* and pyrogenic exotoxins from *streptococcus pyogenes* have been reported to induce IL-1 and TNF- α production from human monocytes [41, 42]. Staphylococcal enterotoxins, enterotoxin A is considered to be more potent than TSST-1in its ability to cause fever, cachexia, multiple organs dysfunction and death [43]. In addition to other classical immune cells, platelets are also susceptible to gram-positive bacteria and their toxins, after exposure platelets reported to secret granular constitutes such as PF-4, sCD40L, RANTES [44, 45].

It has been reported that different toxins from gram-negative or gram-positive organisms activate the host immune system in a distinctly different manner. For example, LPS reported to activate macrophages and stimulate TNF-α production [46, 47], whereas superantigens from gram-positive bacteria do not provoke clear-cut TNF-α production but activate primarily T-lymphocytes casing FasL-dependent apoptosis [48]. However, activation of immune system during polymicrobial sepsis by mixed bacterial flora is much more complex and largely unknown [18, 19].

Host recognition and pro-inflammatory response

The discovery of LPS receptor is a significant step toward the understanding of host response to an infection. Toll like receptors (TLRs) are essential members of

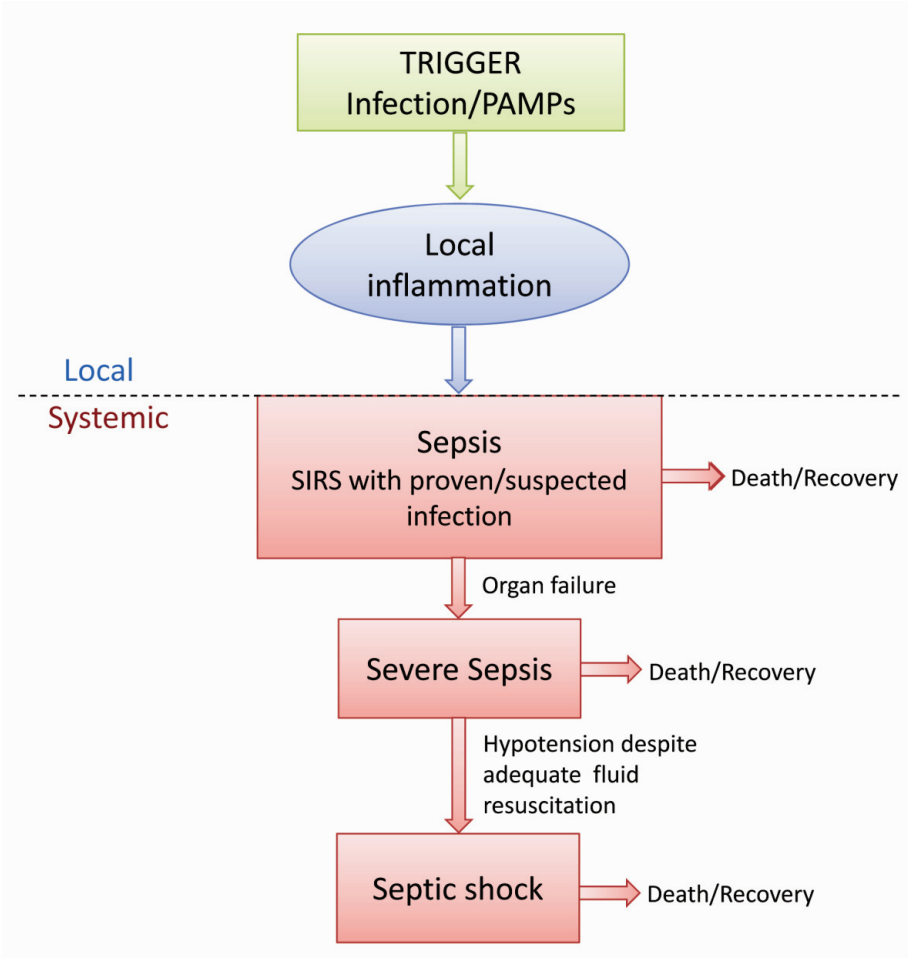

Figure 1. A simple schematic illustration of pathophysiology of sepsis.

a family of pattern recognition receptors (PRRs) that alert the innate immune response system to the presence of a microbial invader. It was first discovered in Drosophila in 1985 by Christiane Nüsslein-Volhard [49]. Innate immune function lacks the precision like adaptive immune system; however, it compensates that lack by its ability of phagocytosis and clearance of pathogens by its cellular components such as neutrophils, monocytes, macrophages, and natural killer cells [50, 51]. The transmembrane part of TLRs involved in the detection of LPS and many other microbial mediators, such as PG, lipopeptides, and LTA [52-54]. Injury of host components in sepsis causes cell lysis, thus releases many pro-inflammatory damage-associated molecular pattern molecules (DAMPs). DAMPs in turn stimulate PRRs such as TLRs or NOD-like receptors (NLRs) [55].

There are some other pattern-recognition molecules such as alternative complement components [56], mannose-binding lectin [57], and CD14 [58]. Originally CD14 is identified as the essential co-receptor that mediates LPS dependent activation of monocytes but later it is shown that it can be activated by PG [59]. CD14 is also found in the circulation as a soluble CD14 (sCD14) and many cells which are constitutively CD14

negative such as dendritic cells, fibroblasts, and vascular endothelium cells, can respond to LPS by interacting with sCD14 [60]. Ten major human TLRs and 2 NLRs have been identified by genome researcher so far. TLR4 is the primary LPS receptor whereas TLR1, TLR2, and TLR6 recognize an array of other microbial mediators that serve as PAMPs. TLR5 recognizes bacterial flagellin from either gram-negative or gram-positive bacteria. TLR9 recognizes unmethylated CpG motifs found in bacterial DNA. The natural ligand for TLR10 is not identified yet. Recently monocytic intracellular proteins NOD1 and NOD2 have been identified to bind and confer responsiveness to LPS and PG [61, 62].

Ligands binding with the receptors leading to the activation of transcription factors, such as nuclear factor κB (NK-κB), activator protein 1, interferon regulatory factor 3 [63, 64]. Activation of transcription factors induce *de novo* expression of multiple genes, leading to the production and release of inflammatory molecules; including, cytokines, chemokines, adhesion molecules, and clotting factors [64]. NOD-1 and NOD2 are cytosolic proteins also known as CARD4 and CARD15 respectively. NOD1 or NOD2 interact with RICK (also known as Rip2 or CARDIK) protein to form a signaling platform-the inflammasome that signals via caspase-1 resulting in the induction of IL-1β and IL-8 [62].

Anti-inflammatory response

Animal as well as clinical studies have shown that the initial hyper-inflammatory response in sepsis is quickly followed by a sustained counter-inflammatory state [65, 66]. Counter-inflammatory cytokines including IL-10 and antagonists such as soluble TNF receptors and IL-1 receptor antagonist, decoy receptor such as IL-1 receptor type II and inactivation of complement cascade and anti-inflammatory cytokines resulted in immunosuppression or immunoparalysis in sepsis. During this hypo-inflammatory phase immune system is unable to mount appropriate host-defense responses against pathogens known as compensatory anti-inflammatory response syndrome (CARS) [67, 68]. CARS increased susceptibility to infections which are the most common cause of mortality of septic patients [68]. It is characterized by loss of phagocytic function, decreased major histo-compatibility complex type II, loss of delayed type hypersensitivity response where T-cells undergo apoptosis as well as fail to proliferate and produce interferon-γ (IFN-γ) [66, 69, 70]. Thus, resulting in loss of important cross talk between innate and adaptive immune systems [65, 71]. Moreover, several studies have reported that the number of regulatory T-cells increase during CARS which further reduce the ability of the immune system to mount anti-bacterial responses [72, 73]. Some investigators believe that it is the counter-inflammatory response that cause an insufficient host defense against infection and resulted in progressive organ failure and death in sepsis [51, 74].

Organ failure

The pathogenesis of organ dysfunction in sepsis is poorly understood. Tissue perfusion and hypoxia considered as main factors of organ failure [1, 75, 76]. Although immune system is the motor of multiple organ failure (MOF), gut is proposed to be one of the major pistons that turns this motor [77]. Clinical observations explored that bacteremia, sepsis, and MOF could exist even in the absence of an identifiable focus of infection [77]. Several human and animal studies favor an association between gut barrier failure and bacteria or bacterial toxins translocation, and then development of multiple organ failure [78-80]. In this context, it is important to note that lung is the most sensitive and critical end-organ in abdominal sepsis and lung injury constitute significant cause of mortality in spite of

aggressive surgical interventions, antibiotic and immunomodulating therapies [18]. Failure of gut barrier allows passage of bacteria and endotoxin from the gut lumen to the portal or systemic circulation which in turn leads to local activation of the immune inflammatory system and the local production of cytokines and other immune inflammatory mediators [77]. Disorders of coagulation system due to activation of coagulation pathways by bacterial components are common in sepsis [81]. Activation of coagulation pathways resulted in series of proteolytic cascades, and the net result is enhanced fibrin deposition, thus cause microvascular occlusion and accumulation of tissue exudates resulting in inadequate oxygenation and disorders in homeostasis of microvascular [1]. Excessive neutrophil infiltration also causes tissue damage by releasing lysosomal enzymes and reactive oxygen species [48, 82]. Pro-inflammatory cytokines, in particular IL-1 and IL6 are the main inducers of coagulation cascades. Increased synthesis of nitric oxide by cytokines contributes to the hypotension and resistance to vasopressor drugs that result in vasodilatory shock [83].

Leukocyte recruitment

Chemokines

Chemokines are low molecular weight (8-10 kD) chemotactic cytokines that involved in leukocytes recruitment by activating and regulating integrins via G-protein coupled receptors called chemokine receptor [84, 85]. Although chemokines are essential for host defense against bacteria, overproduction of these mediators has been shown to play an important role in the pathogenesis of sepsis. Two major subfamilies, CXC and CC chemokines have been investigated mostly in sepsis. However, CXC chemokines are most studied neutrophil chemoattractant in sepsis [86-89]. In humans, systemic administration of endotoxin in healthy volunteers leads to increase of IL-8 levels [90]. Furthermore, human PMNs are shown to response to CXC chemokines, IL-8RA and IL-8RB [91, 92]. In mouse CXC chemokines, KC (cytokine-induced neutrophil chemo-attractant, mouse IL-8 homologs) and MIP-2 (macrophage inflammatory protein -2) have been reported to attract leukocytes [85, 93, 94] and share a common receptor, CXCR2 [86]. Furthermore, expression of CXCR2, but not CXCR1, has been shown to be reduced on neutrophils of septic shock patient [95] and CXCR2 also found to be elevated in the lung and plasma in sepsis [86]. Mice deficient of CXCR2 or treated with CXCR2-specific antagonist have been shown to be protective against sepsis [96, 97]. Blockade of CXCR2 by an inhibitor reduced polymorphonuclear influx, lung protein leakage and the lung tissue level of KC and MIP-2 in septic animal [86].

Adhesion molecules

Leukocytes recruitment from the circulation to the inflamed tissue is a complex and multi-step process. Most of the literatures describe this process as initial tethering, rolling and adhesion on the endothelium, and finally transmigration [14, 98]. All of these steps are mediated by close interaction between leukocytes and microvascular endothelial cells. Rolling of leukocytes on the surface of endothelium is mediated by the selectin family of adhesion molecules including P- selectin (CD62P), E-selectin (CD62E) and L-selectin (CD62L) and their corresponding ligands and generally considered as the first step of this multistep process [99, 100]. Leukocytes are considered to roll when their velocity is approximately 50 times lower than the base line [101]. Many pro-inflammatory agents like TNF-α, histamine, leukotrienes have been reported to regulate rolling by controlling the upregulation of P-selectin [104, 105].
However, rolling is not a prerequisite for subsequent adhesion and transmigration in

narrow lumens, for example, in liver sinusoids, lung capillaries [102, 103].

The rolling phase of leukocytes can be shifted towards an irreversible firm adhesion stage, if appropriate chemotactic stimulus is present. Leukocyte arresting on vascular beds is predominantly mediated by integrins which binds with their constitutive or inducible ligands [106]. Integrins are expressed on the surface of leukocyte at a low affinity state but in the presence of appropriate stimulus they are activated to mediate firm adhesion [106, 107]. The adhesion molecules are β2-integrins composed of β-subunit (CD18) and different type of α-subunits (CD11a-d). An abundant amount of integrins are expressed on the surface of leukocyte comprising LFA-1, CD11a), membrane activate complex-1 (Mac-1, CD11b), p150,95 (CD11c) and less abundant αdβ2 (CD11d). When activated these integrins bind with their transmembrane glycoproteins ligands which are members of the immunoglobulin superfamily namely ICAMs (ICAM-1 to ICAM-5), vascular cell adhesion molecule-1 (VCAM-1) and junctional adhesion molecules (JAMs) [108].

Some investigators have shown that ICAM-1 is important in supporting neutrophil recruitment in the lung during sepsis [15, 109]. It has been shown that LFA-1 and Mac-1 play an important role in abdominal sepsis by supporting pulmonary infiltration of neutrophils [17]. Furthermore, ICAM-1 is known to interact with both LFA-1 and Mac-1 for firm adhesion in a stimulus and organ dependent manner [16, 110, 111]. One recent study has reported that LFA-1 may involve in first stable contact and Mac-1 may involve in more established adhesion with endothelium cells in inflamed organs and inhibition of either one would be sufficient to reduced neutrophil recruitment [112].

Platelets in inflammation

Platelets or thrombocytes are small (2–3 μm diameters) and irregularly shaped cell fragments. They are produced as fragments of megakaryocyte through an endomitotic process rather than by straightforward cellular duplication [113]. Platelets are lack of nucleus but they do process mitochondria, residual of endoplasmic reticulums, network of actin and myosin filaments. Under normal condition, platelets are preferentially circulated along with the vessel wall without any interactions [114, 115]. In response to stimuli (such as thrombin, trypsin, collagen, adenosine diphosphate, epinephrine, arachidonic acid metabolites, PAF and vasopressin), platelets are transformed irreversibly from its discoid shape to extend numerous pseudopodia and become highly adhesive to each other or to other cells [116].

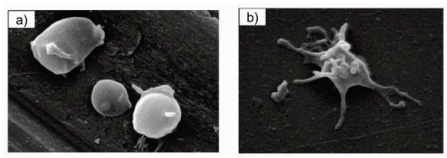

Figure 2. Scanning electron microscope images of resting (a) and activated (b) platelets (Courtesy: David Erlinge).

Platelet activation is accompanied by secretion of numerous substances from their specialized granules. They contribute to the inflammation process either by direct interaction with other immune cells or by secreting inflammatory mediators [24, 117, 118]. In addition to mitochondrial lysosomes and peroxisomes, platelets contain α-granules and dense granules. The α-granules are the largest and most common, containing a number of factors, including P-selectin, factor V, factor VIII, von Willebrand factor, thrombospondin, fibronectin, fibrinogen, β-thromboglobulin, PF-4, and platelet-derived growth factor. The α-granules also reported to contain MMP- 2 and 9 [117, 119, 120]. The dense granules contain

several vasoconstrictive agent and pro-inflammatory molecules such as adenosine and guanine nucleotides, calcium, histamine, and serotonin. The cytoplasm can contain a number of other substances, including serotonin, epinephrine, norepinephrine, nitric oxide, and cytokines, such as transforming growth factor-β, vascular endothelial growth factor, CD40L and IL-1β. Thus, release of platelet contents may have contributions on attracting neutrophils and leukocytes, in mediating ongoing inflammatory responses such as in sepsis.

Accumulating data suggest that platelets play important roles in several diverse processes. In addition to their classical role in homeostasis and thrombosis, platelets can recruit leukocytes and progenitor cells to sites of vascular injury [27, 121]. For example, depletion of platelets has been shown to decrease leukocyte accumulation in models of localized inflammation in the lung and liver [122, 123]. Leukocytes activated by interactions with platelets also reported to release granular contents such as MPO [117, 124]. Activated platelets described to regulate endothelial expression of ICAM-1 and adhesion of neutrophils [118, 125]. It is also shown that platelets deposit pro-inflammatory compounds, such as CCL5 and PF-4 on inflamed endothelium and thereby promote local recruitment of leukocytes [126]. In mice, pretreatment with anti- platelet drug such as clopidogrel prior to administration of LPS prevents thrombocytopenia; reduce lung fibrin accumulation [127].

CD40L in inflammation

CD40 ligand (CD40L, CD154, TRAP) is a trimeric 33 kD transmembrane protein [128, 129]. It was first identified on $CD4^+$ T-lymphocytes [2], and shown to be involved in T cell dependent humoral immunity by interaction with CD40 expressed on B lymphocytes [130]. CD40L is also present on platelets, monocytes, macrophages, and endothelial cells [131-133]. Platelets are estimated to contain more than 95% of the CD40L in the circulatory system [134]. A small amount of CD40L is expressed on the surface of unstimulated platelets, but within minutes of activation CD40L is expressed on the surfaces of activated platelets *in vitro* [135]. CD40L is subsequently cleaved from the platelet surface as an 18 kD soluble fragment (soluble CD40L, sCD40L, or sCD154) that remains trimeric and contains a TNF-homologous domain [136, 137]. The trimeric form of sCD40L has structural domains which are considered important for its biological multi-functionality. For example, TNF homology domain is important for binding with CD40. The lysine-arginine-glutamic acid (KGD) motif binds with glycoprotein GPIIb/IIIa and the trimeric structure of CD40L involves in induction of signaling reactions when binds with receptors [134].

Table 1. Schematic features of CD40L (SwissProt).

Description	Length (amino acid)	Positions (amino acid)	Graphical view
CD40 ligand	261	1-261	
Cytoplasmic domain	22	1-22	
Transmembrane	24	23-46	
Extracellular domain	215	47-261	
Soluble CD40 ligand	149	113-261	

CD40L expressed on the surfaces of activated platelets is capable of initiating various inflammatory response, including expression of tissue factor, up-regulation of various adhesion molecules (ICAM-1, VCAM-1, E-selectin), and release of chemokines (MCP-1, IL-6, IL-8) [125, 138]. Like TNF-α, CD40L on platelets is reported to induce endothelial cells to secrete chemokines and express adhesion molecules, thereby involve in recruitment and extravasation of leukocytes at the site of injury [138]. Increased levels of sCD40L are detected in patients with cardiovascular diseases, inflammatory bowel disease, as well as autoimmune

diseases [139-141]. It is also proposed that sCD40L may involve in self-perpetuating feedback loop which is closely associated with platelet regulatory functions such as secretion of α-granules and dense granules, activation of integrin β3, and morphologic changes in resting platelets [137, 142]. The physiologically important cleavage event that releases sCD40L from the platelet surface is believed to be catalyzed by an unidentified MMP activity [136, 143].

Matrix Metalloproteinases in inflammation

MMPs comprise a large family of more than 25 structurally and functionally related Ca^{2+} containing and Zn^{2+} dependent endopeptidases [144]. MMPs are capable of cleave protein components of extracellular matrix (ECM) and non-ECM molecules such as growth factors and their receptors, chemokines, cytokines, adhesion molecules and surface receptors [145]. They belong to a larger family of proteases known as metzincin superfamily and involve in fundamental process such as cell proliferation, differentiation, adhesion, migration, angiogenesis, apoptosis, and inflammation. Based on ECM specificity they have been divided into following major classes– collagenases, gelatinases, stromelysins, and matrilysisns. They are multi-domain proteins and their activity is tightly control by compartmentalization and inhibited by their natural inhibitors (TIMPs) or by acute-phase reactant α_2-macroglobulin [144]. Loss of control may lead to an imbalance of MMPs activities with implication of disease processes [146].

MMPs were initially discovered to play an exclusive role in cancer; however, overexpression of MMPs in many pathological conditions suggested their involvement in the pathophysiology of inflammatory diseases [146-148]. For example, MMP-9 levels are reported to increase in healthy human volunteers after injection of bacterial LPS [149], both pro-MMP-9 and pro-MMP-2 and active MMP-9 were detected in the plasma of patients with gram-negative sepsis [150], increases levels of MMP-9 were detected in experimental pancreatitis [151], and increase of MMP-7 and MMP-10 were observed in the pulmonary infection *with pseudomonas aeruginosa* [152]. In this context it is important to note that MMP-9, gelatinase B, is reported to be a complex family member in terms of domain structure and regulation of its activity [153]. Most of the MMPs are released as inactive proforms and activated in the extracellular environment or at the cell surfaces by proteolytic cleavage [146, 154]. Fibroblasts, endothelial cells, and epithelial cells secret mainly MMP-1, MMP-2 and MMP-9, whereas PMNs and alveolar macrophages secret MMP-8 and MMP-9 [154].

Platelets have been shown to express MMP-1, MMP-2, MMP-3, MMP-9 and their endogenous inhibitors TIMP-1, TIMP-2, TIMP-4 [155-159]. Formation of platelet-leukocyte complex have been shown to be associated with expression and activation of MMPs [160]. However, the exact source of MMPs is not clear here, could involve both cells. In addition, platelets from patients with Crohn's disease have been shown to have increase MMP-9 activity [161]. Activated platelets are also shown to release MMP-2 in the circulation of patients with acute coronary syndromes [162]. In contrast, platelet activation is shown to link with MMP-9 activation in the coronary circulation in acute myocardial infarction [163].

Accumulating studies have shown that treatment with broad-spectrum inhibitors of MMPs not only attenuate levels of cytokines and MMPs but also reduce mortality in the experimental sepsis models [164-166]. A number of synthetic MMP inhibitors, such as phenanthroline, BB94, MMP inhibitor-1, GM6001, GW280264X and TAPI-2, have been

shown to inhibit platelet MMPs. Effect of certain MMP inhibitors on platelets have been shown to be effective in inhibition of platelet adhesion, aggregation and GPIb shedding *in vitro*. The efficacy of MMP inhibitors is limited to MMP-dependent platelet modulation and can be enhanced by co-administration of classical inhibitors of platelet function such as aspirin or ADP blockers [155, 167]. Lack of selective inhibitor is the main drawback to study a specific MMP in inflammatory processes.

Treatment of sepsis

Human sepsis is a complex and evolving disease. Despite overwhelming research efforts and clinical trials, the management of critically ill patients with sepsis is largely supportive apart from antibiotic therapies. The definition of patient population and the timing of delivery of potential therapy are critical in emergency room setting [168, 169]. The initial management includes supportive care to correct physiological abnormalities, such as hypoxia and hypotension and distinguish sepsis from systemic inflammatory response syndrome (SIRS) for surgical procedure [8, 170, 171]. Many anti-inflammatory and anti-coagulant drugs showing promising results in the laboratory setting, fail to show significant survival benefit in recent randomized human trials [172, 173]. Concerns have been expressed on the rapidity of progress from the simple animal studies to clinical trials without checking the efficacy of potential therapeutic targets in a range of models [174] and much of the preclinical data was often based on lethal bacterial toxin based studies which did not replicate the septic patient status adequately [175]. Despite this, several therapies for treatment of specific populations of septic patients such as recombinant activated protein C [176], low dose corticosteroids [177], intensive insulin therapy [178] have been shown to reduce mortality. However, each of these approaches only improves septic survival by 10% [179]. One reason might be that sepsis manifests itself as multiple processes, making therapeutic intervention difficult. There has yet to be a therapy offered that significantly modifies the outcome of this disease. Therefore, an improved understanding of immune-pathology of sepsis is important to facilitate the development of effective therapy against sepsis.

Aims

1. To determine the role of platelets in sepsis-induced leukocyte recruitment and lung edema formation in polymicrobial sepsis.
2. To define the role of platelet-derived CD40L on activation of neutrophils and pulmonary accumulation in abdominal sepsis.
3. To determine plasma levels of sCD40L in patients with septic and non-septic shock.
4. To examine the role of MMPs in controlling CD40L release from platelets and subsequent activation and infiltration of neutrophils in the lung in abdominal sepsis.
5. To determine the role of specific MMP in the regulation of platelet shedding of CD40L as well as neutrophil recruitment and lung tissue injury in abdominal sepsis.

Materials and Methods

Animals

Experiments were performed using male C57BL/6 wild-type, CD40L-deficient (B6.129S2-Cd40lgtm1Tmx/J, Jackson Laboratory, Bar Harbor, ME) male C57BL/6 mice, MMP-9 deficient (B6.FVB(Cg)-Mmp9tm1Tvu/J) male C57BL/6 mice and recombination-activating gene (RAG) gene-deficient (B6.129S7-Rag1tm1Mom/J, Jackson Laboratory) male C57BL/6 mice weighing 20-25 g. Animals were kept on a controlled room with 12 hours light-dark cycle. All experimental procedures were performed in accordance with the legislation on the protection of animals and were approved by the Regional Ethical Committee for Animal Experimentation at Lund University, Sweden.

Experimental sepsis model

Several experimental animal models have been used to study sepsis and sepsis associated systemic inflammatory response. However, cecal ligation and puncture (CLP) model in rodents is considered as the gold standard of experimental sepsis research [19, 180, 181]. This model of sepsis is known to closely mimic the pathophysiology of septic human patients. However, one major concern of CLP model is its consistency. The underlying inflammatory response and outcome of the CLP depends on several factors i.e. the length of cecum ligated, size of needle used and number of punctures and fluid resuscitation. Thus, a standardized performance of the CLP procedure is ensured to develop a high-grade of sepsis by ligating 75% of cecum and puncturing twice with 21-gauge needle in the anti-mesenteric side of the cecum. (Positions of ligature and puncture are shown in Figure 3).

In brief, mice were anesthetized and the lower quadrant of the abdomen was disinfected with alcohol. The abdomen was opened by midline incision to exteriorize the cecum which was filled with feces by milking stool backwards from the ascending colon and a ligature was placed below the ileocecal valve. Care was taken not to breach or damage the mesenterial blood vessels. The cecum was soaked with PBS (pH 7.4) and then punctured twice with a 21-gauge needle. The cecum was then returned into the peritoneal cavity and the abdominal wall was closed with a suture. Sham mice underwent the same surgical procedures, *i.e.*, laparotomy and resuscitation, but the cecum was not ligated nor punctured. The mice were then returned to their cages and provided with food and water *ad libitum*. Animals were anesthetized 4-24 hours after CLP induction. The left lung was ligated and excised for edema measurement. The right lung was used for collecting bronchoalveolar lavage fluid (BALF) in which the number of neutrophils were quantified. Next, the lung was perfused with PBS through the right ventricle of the heart and one part was fixed in formaldehyde for histology and the remaining lung tissue was weighed, snap-frozen in liquid nitrogen and stored at -80°C for later ELISA and MPO assays as described below.

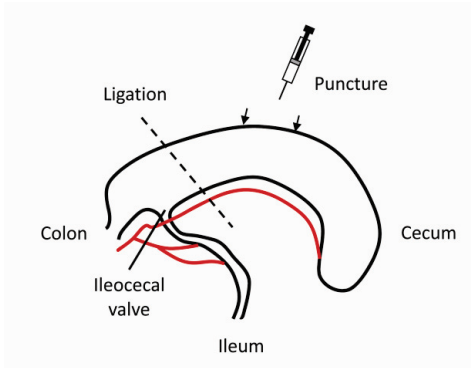

Figure 3. Schematic illustration of characterized positions of cecal ligation and puncture to induce high-grade sepsis.

Patients

The study design was a single-center prospective observational cohort study of critically ill patients admitted to the mixed-bed ICU of Malmö University Hospital, Sweden between December 2005 and May 2008. The local ethics committee approved the study and informed consent was obtained from all patients or their next-of-kin. In the study III, 9 healthy controls and 53 consecutive shock patients with or without sepsis were included. In the study V, 9 healthy controls and 29 consecutive patients with septic shock were included. For inclusion in the study, patients should be aged over 18, fulfill the SIRS criteria [29] and exhibit circulatory failure, defined as failure to maintain mean arterial pressure $\geq$ 70 mmHg despite adequate fluid resuscitation accordingly to the surviving sepsis campaign algorithm [171]. Exclusion criteria were pregnancy, abnormalities of coagulation, fibrinolytic therapy, compromised immunity or a "Do Not Resuscitate" order. Patients were defined as septic or not based on standard published criteria [176]. All patients were enrolled into the study within 6 hours of the diagnosis of shock. Sepsis was defined as a known infection or a suspected infection exhibiting one of following: leukocytes in a normally sterile body fluid, perforated viscus, and radiographic evidence of pneumonia in association with the production of purulent sputum or a syndrome associated with a high risk of infection. Acute Physiology and Chronic Health Evaluation (APACHE) II scores [182] were calculated at admission. All patients were treated according to international guidelines for the management of sepsis and septic shock [171].

Antibodies and biochemical substances

Animals were anaesthetized by intraperitoneal (*i.p.*) administration of 7.5 mg Ketamine hydrochloride (Hoffman-La Roche, Basel, Switzerland) and 2.5 mg xylazine (Janssen Pharmaceutica, Beerse, Belgium) per 100 g body weight. To study the role of platelets in abdominal sepsis, 1.0 mg/kg of a monoclonal antibody directed against murine CD42b (GP1bα, rat IgG, Emfret Analytics GmbH & Co. KG, Wurzburg, Germany) was administered *i.p.* 2 h prior to CLP. To evaluate the functional importance of PSGL-1, Mac-1 and CD40L, monoclonal antibodies directed against murine CD162 (PSGL-1, clone 2PH1, rat IgG_1), CD11b (Mac-1, clone M1/70, rat IgG_{2b}) and CD40L (clone MR1, 10 mg/kg, eBioscience Inc., San Diego, CA, USA), were administered respectively and a non-functional isotype-matched control antibody (clone R3-34, rat IgG_1) at a concentration of 1.6 mg/kg was also administered in CLP mice. All antibodies were purchased from BD Biosciences (Pharmingen, San Jose, CA, USA) except mentioned. Antibodies and PBS (100 µl) were administered *i.v.* immediately before CLP induction. In order to delineate the role of MMPs, a potent and broad-spectrum hydroxamic acid inhibitor of MMPs, GM6001 (Galardin, N-[(2R)-2-(hydroxamidocarbonylmethyl)-4-methyl-pentanoyl]-L-tryptophan methyl-amide; Calbiochem, Darmstadt, Germany) was given (40 mg/kg) *i.p.* 1 hour before the CLP induction in study IV. In the final study, a selective inhibitor of MMP-9 (MMP-9 inhibitor I, 10 µM) (Calbiochem) was used for *in vitro* studies (Figure 4. Chemical structures of MMP inhibitors).

Systemic leukocyte and platelet counts

Blood was collected from tail vein and was mixed with Turks solution (0.2 mg gentian violet in 1 ml glacial acetic acid, 6.25% v/v) in a 1:20 dilution. Leukocytes were identified as monomorphonuclear (MNLs) and polymorphonuclear (PMNLs) cells in a Burker chamber.

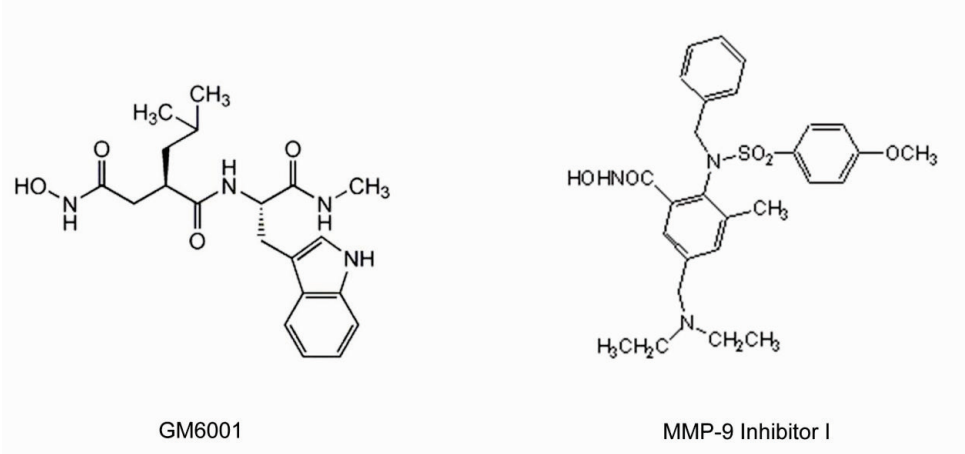

Figure 4. Chemical structures of MMP inhibitors (calbiochem).

For platelets counting, blood was mixed with Stromatol solution (Mascia Brunelli spa, Viale Monza, Milan, Italy) in a dilution of 1:500 after collecting from tail vein and then counted in a Burker chamber.

Lung edema

The left lung was excised, washed in PBS, and gently dried using a blotting paper before weighing. The tissue was then dried at 60°C for 72 hours and re-weighed. The change in the ratio of wet weight to dry weight was used as indicator of lung edema formation.

MPO activity

The enzyme MPO is abundant in PMNLs and has been used as a reliable marker for the detection of neutrophil accumulation in inflamed tissue [183, 184]. In brief, frozen lung tissue was thawed and homogenized in 1 ml of 0.5% hexadecyltrimethyl-ammonium bromide. Next, the sample was freeze-thawed, after which the MPO activity of the supernatant was measured. The enzyme activity was determined spectrophotometrically as the MPO-catalysed change in absorbance in the redox reaction of H_2O_2 (450 nm, with a reference filter 540 nm, 25°C). Values were expressed as MPO units per g tissue.

Enzyme linked immunosorbent assay

For measuring lung chemokines, the lung sample was thawed and homogenized in PBS. MIP-2 and KC were analyzed by using double antibody Quantikine ELISA kits (R & D Systems) using recombinant murine MIP-2 and KC as standards. The minimal detectable protein concentrations are less than 0.5 pg/ml. For soluble CD40L analysis, plasma was collected on ice using citrate as anticoagulant and centrifuged for 20 minutes at 2000 x g immediately after collection. An additional centrifugation at 10000 x g for 10 minutes at 4°C was employed for complete removal of platelets and stored at -20 °C for further use. Plasma samples were then diluted with a sterile buffer (10% fetal calf serum in PBS, pH-7.4) and analyzed by using commercially available ELISA kits (R & D Systems). Plasma levels of sCD40L and MMP-9 in septic patients were analyzed by use of commercially available ELISA kits (Bender MedSystems, Vienna, Austria), using recombinant human CD40L and recombinant human MMP-9 as standards, respectively.

Flow cytometry

Flow cytometry was performed for analysis of the number of binding platelets and Mac-1 expression on circulating neutrophils (I, II and IV) and for analysis of CD40L (II, IV, V) and MMP-9 (V) on platelets. Blood was collected into heparinized syringes at 4 h post CLP induction. Immediately after collection, blood samples were incubated with an anti-CD16/CD32 antibody blocking Fcγ III/II receptors in order to reduce non-specific labelling for 10 min at room temperature (RT) and then incubated with FITC-conjugated 7/4 (clone 7/4, rat IgG_{2a}, abcam, Cambridge, CB4 0FW, UK), APC-conjugated anti-Gr-1 (clone RB6-8C5, Rat IgG_{2b}) and PE-conjugated anti-CD41 antibodies (clone MWReg30, Integrin α_{IIb} chain, rat IgG_1) to detect the percentage of neutrophil-platelet aggregates by considering neutrophils as cells positive for Gr-1 and 7/4 and platelets as $CD41^+$ cells. Another set of samples were stained with PE-conjugated anti-Gr-1 (clone RB6-8C5, rat IgG_{2b}), FITC-conjugated anti-CD41 (clone MWReg30, Integrin α_{IIb} chain, rat IgG_1) and APC-conjugated anti-Mac-1 (clone M1/70, Integrin α_M chain, rat IgG_{2b}) antibodies to detect surface expression of Mac-1 on neutrophils. For CD40L expression, blood samples or platelets were incubated with FITC-conjugated anti-CD41 (clone MWReg30, integrin αIIb chain, rat IgG1) and PE-conjugated anti-CD40L (clone MR1, hamster IgG, eBioscience, San Diego, CA, USA) antibodies. Cells were fixed with 1% formaldehyde solution; erythrocytes were lysed using red blood cell lysing buffer (Sigma Chemical Co., St. Louis, MO, USA) and neutrophils and/or platelets were recovered following centrifugation. For MMP-9 expression analysis, platelets were incubated with the anti-CD16/CD32 antibody for 10 min on ice followed by staining with rabbit anti-mouse MMP-9. This was followed by staining with FITC-conjugated anti-rabbit IgG. Flow-cytometric analysis was performed according to standard settings on a FACScalibur flow cytometer (Becton Dickinson, Mountain View, CA, USA) and a viable gate was used to exclude dead and fragmented cells.

Intravital fluorescence microscopy

The lung microcirculation was examined by using intravital fluoresce microscopy in study IV. A midline laparotomy was performed and extended to the side along the lower border of the right rib cage from the subxyphoidal to the midaxillary level. Under transient lowering of the stroke volume to 100 µl, the right diaphragm was incised to create a right sided pneumothorax. Then, the diaphragm was stepwise coagulated and incised along the ventral chest wall to the midaxillary level. A parasternal thoracotomy was performed up to the level of the 4^{th} intercostal space after coagulating the internal mammary and the intercostal vessels. By this, the main part of the right thorax wall could be averted to the side. During the preparation, great care was taken not to directly manipulate the lung tissue and the lung surface was intermittently rinsed with saline (37 °C). A micromanipulator was used to fix a coverslip horizontally on the surface of right lung. Horizontal movements of the lung tissue could be minimized by modulating a positive end-

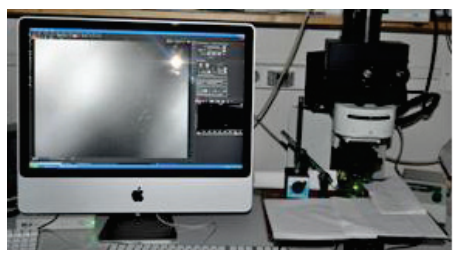

Figure 5. Intravital fluorescence microscopy used to study lung microcirculation.

expiratory pressure between 5 and 7 cm H_2O and adjusting stroke volume (minimum 150 µl) and stroke frequency (minimum 100 strokes/min). Immediately after surgical preparation, the mice were

put on the microscopic stage. Intravital fluorescence microscopy was performed after retrobulbar injection of 0.1 ml 0.1% rhodamine 6G (Sigma-Aldrich, Taufkirchen, Germany) for direct staining of white blood cells and 0.1 mL 5% FITC-dextran (MW 150 000, contrast enhancement; Sigma Chemical Co.). The subpleural pulmonary microvasculature was visualized by means of a modified Olympus microscope (BX50WI, Olympus Optical Co. GmbH, Hamburg, Germany) equipped with a 100 W mercury lamp and filter sets for blue (450-490 nm excitation and > 520 nm emission wave length) and green (530-560 nm excitation; > 580 nm emission wave length) light epi-illumination. Microscopic images were televised by means of a charge-coupled device video camera and recorded digitally. By means of a 20x objective (NA 0.4) a magnification of x990 was achieved. With this setup, all parts of the subpleural pulmonary microvasculature, i.e. arterioles, venules and capillaries could be identified. For the measurement of 3-5 venules and capillaries, Regions of Interest (ROIs) were selected randomly in each animal. Leucocyte rolling was determined by counting the number of such cells passing a reference point in the venule per 20 s. Firm adhesion was measured by counting the number of cells adhering to venular endothelium and remaining stationary for 20 s.

Histology

Lung samples were fixed in 4% formaldehyde phosphate buffer overnight and then dehydrated and paraffin-embedded. Six μm sections were stained with hematoxylin and eosin. In study V, lung injury was quantified in a blinded manner by adoption of a modified pre-existing scoring system as described previously (22), including size of alveolar spaces, thickness of alveolar septas, alveolar fibrin deposition and PMN infiltration graded on a 0 (absent) to 4 (extensive) scale. In each tissue sample, 5 random areas were scored and mean value was calculated. Histology score was the sum of all four parameters.

Statistics

Data are presented as mean values $\pm$ standard errors of the means (SEM) in study I, II, III, IV and median (25^{th}–75^{th} percentiles) in the study V. Statistical evaluations were performed using Kruskal-Wallis one way analysis of variance on ranks followed by multiple comparisons versus control group (Dunnett's method). Mann-Whitney Rank Sum Test was used for comparing two groups. In study III, Comparisons between groups on categorical variables were carried out with chi-square test or *z*-test. The association between continuous variables was carried out using Spearman's rank correlation coefficient. Comparisons of continuous variables between groups were carried out using Kruskal-Wallis one-way analysis of variance on ranks. $P < 0.05$ was considered significant and n represents the number of subjects in each group. Statistical analysis was performed using SigmaStat® software for windows version 3.5 (Systat Software, Chicago, Illinois, USA).

Table 2. Histology scoring system used in study V

Alveolar spaces: Alveolar spaces were scored using medium power field 40X

Score	Definition
0	normal alveolar microarchitecture
1	occasional reduction of alveolar space
2	progressive reduction of alveolar space
3	diffuse reduction of alveolar space
4	extensive destruction of tissue architecture

The thickness of the alveolar septa: The thickness of the alveolar septa were scored in oil emersion high power field (HPF)

Score	Definition
0	thin alveolar septa
1	occasional thickening of alveolar septa
2	progressive thickening of alveolar septa
3	diffuse thickening of alveolar septa
4	massive thickening of alveolar septa

Fibrin deposition: The fibrin deposition within the alveolar space were scored in oil emersion high power field (HPF)

Score	Definition
0	absent of fibrin deposition within the alveolar space
1	occasional fibrin deposition within the alveolar space
2	progressive fibrin deposition within the alveolar space
3	diffuse fibrin deposition within the alveolar space
4	massive fibrin deposition within the alveolar space

PMN infiltration: Infiltrated PMN were counted in interstitial and intraalveolar spaces in high power field 100X (HPF)

Score	Definition
0	0-10 PMN cells
1	11-20 PMN cells
2	21-30 PMN cells
3	31-50 PMN cells
4	More than 50 PMN cells

Table 3. Patient demographics in study III

	Total	Septic shock	Non-septic shock	*P* value
Males/females	37/16	25/11	12/5	1.00*
Age (years)	65 (55-74)	65 (57-73)	62 (50-76)	0.83#
APACHE II	24 (19-29)	24 (19-28)	25 (19-32)	0.80#
Number (%)	53	36 (67%)	17 (33)%	0.03†
ICU mortality	28.3%	25.0%	35.5%	0.64†
6-month mortality	39.6%	24.5%	47.1%	0.18†

Continuous variables are expressed as median (25^{th}–75^{th} percentiles). Categorical variables are expressed as frequencies and percentages.

* Chi-square test
# Mann-Whitney Rank Sum Test
† *z*-test

Table 4. Aetiology of shock patient used in study III (* sepsis samples used in the Study V)

Pat.	Sepsis	Aetiology
1*	Y	E. coli urosepsis.
2	N	Post operative heart failure and ileus
3*	Y	Septic shock, focus unknown. P. mirabilis cultured in blood.
4	N	Pancreatitis secondary to alcohol overconsumption.
5*	Y	Fulminant septic shock. E. coli och C. perfringens in blood.
6*	Y	Streptococcal sepsis post retained products of conception.
7*	Y	Septic shock, urinary focus with multiple organisms including E. faecalis.
8*	Y	Sepsis. Soft tissue focus.
9*	Y	Ulcerous colitis with multiple abdominal abcesses.
10	N	Pancreatitis secondary to alcohol overconsumption.
11*	Y	Pneumococcal sepsis, lung focus.
12*	Y	Pneumococcal sepsis and meningitis.
13*	Y	Enterococus urosepsis and aspiration pneumonia.
14	N	Post hemicolectomy, severe cardiac failure post-op.
15	N	Renal and hepatic failure, post-intoxication
16	N	Pancreatitis secondary to alcohol overconsumption.
17	N	Bleeding oesophageal varices.
18	N	Post sigmoid resection.
19	N	Pancreatitis, superior mesenteric vein thrombossis
20	N	Post arterial stenting with perioperative AMI.
21*	Y	Encephalopathy, Creutzfelt Jakob disease, septic shock. Staph spp in blood.
22*	Y	Staphylococcus aureus sepsis, soft tissue and lung focus.
23	N	Pancreatitis.
24*	Y	Sepsis, abdominal focus. No organism cultured.
25*	Y	Pneumococcal sepsis.
26*	Y	Fungal sepsis. Candida albicans in blood and bronchial brush cultures.
27*	Y	Urosepsis, E.coli.
28	Y	Legionella and pseudomonas pneumonia.
29*	Y	Pneumococcal pneumonia and sepsis with DIC.
30	Y	Soft tissue infection with sepsis and ARDS.
31*	Y	Sepsis with abdominal or lung focus, P. Aeruginosa and E. Faecium.
32	N	Intoxication, post-cardiac arrest
33	Y	Urosepsis. Staphylococcus aureus cultured in blood and urine.
34	Y	Post-operative bilateral pneumonia and wound infection.
35	Y	Haemophilus inlfuenzae pneumonia and sepsis.
36	Y	Traumatic finger amputation, postoperative sepsis.
37*	Y	Bowel ischaemia. S. milleri in blood cultures.
38	N	Encephalopathy of unknown origin, cardiac failure.
39	N	AAA, complicated by post-op bowel ischaemia
40	N	Unknown diagnosis, suspected sepsis, all investigations negative.
41*	Y	Perforated diverticulitis with multiple abcesses.
42*	Y	E.coli urosepsis.
43*	Y	Perianal abcess with Group G streptococcal sepsis.
44*	Y	Gallstone pancreatitis. Abdominal abcesses. Enterococcus faecium.
45*	Y	Pneumococcal pneumonia with sepsis.
46*	Y	Pneumonia with sepsis.
47*	Y	H. influenzae pneumonia with sepsis.
48*	Y	Necrotixing fasciitis, Group A streptococcus.
49	N	Multiorgan failure, diagnosis unclear. Possible AML. Culture negative.
50*	Y	Pneumonia with sepsis, Serratia spp. In blood cultures.
51	N	Thoraco-abdominal AA. Post-stenting.
52	Y	Cholecystitis, cholangitis and pancreatitis.
53*	Y	Klebsiella urosepsis.

Results and discussion

Role of platelets in abdominal sepsis

Accumulating data suggest that platelets play a role in inflammation. However, the role of platelets for pulmonary recruitment of sepsis is not demonstrated yet. Herein, we show for the first time that platelets constitute an important component in the pathophysiology of lung injury associated with abdominal sepsis. We use platelet-depleting antibody in order to reveal the role of platelets in CLP animal. We found that the pulmonary levels of MPO and the number of neutrophils in the bronchoalveolar space provoked by CLP was reduced by more than 50% in platelet-depleted mice, suggesting that platelets regulate a significant proportion of neutrophil accumulation in the lung in polymicrobial sepsis. CLP increased CXC chemokine production in septic lung injury. However, depletion of platelets had no effect on CLP-induced formation of MIP-2 and KC, suggesting that the effect of anti-GP1bα antibody on pulmonary infiltration of neutrophils is not related to local changes in CXC chemokine production in the lung. In addition, we found that platelet depletion not only decreased neutrophil recruitment but also attenuated sepsis-induced edema formation and tissue destruction in the lung, indicating that targeting platelet functions may protect against damage to the lung tissue in abdominal sepsis.

We also investigated the activation of circulating neutrophils in abdominal sepsis and found that perforation of the intestine increased Mac-1 expression on neutrophils and this up-regulation of Mac-1 was abolished by depletion of mice platelets. These observations suggest that platelets are important for mediating Mac-1 up-regulation on neutrophils in abdominal sepsis. Indeed, this platelet-mediated increase in Mac-1 was of functional importance in abdominal sepsis. We found that immunoneutralization of Mac-1 markedly decreased CLP-induced pulmonary infiltration of neutrophils, suggesting that systemic up-regulation of Mac-1 by platelets primes circulating neutrophils for subsequent tissue infiltration in the lung. This finding is in line with a previous study showing that Mac-1, together with LFA-1, contributes to pulmonary accumulation of neutrophils in abdominal sepsis [17]. Nonetheless, these findings showing that induction of Mac-1 expression on neutrophils is an important mechanism behind platelet-mediated recruitment of neutrophils in septic lung injury does not exclude the possibility that other mechanisms are operating in parallel.

Circulating leukocyte-platelet aggregates have been shown to be increased in a wide range of inflammatory diseases, such as ischemia reperfusion injury [185], pneumonia [186], hemodialysis [187] and acute myocardial disease [188] and the potential role of such aggregates in pathological inflammation has recently attracted a lot of attention. In fact, we observed that the percentage of neutrophil-platelet aggregates increased by more than two-fold in mice with abdominal sepsis. Knowing that PSGL-1 on neutrophils and P-selectin on platelets is important for aggregates formation [189, 190], we administered an anti-PSGL-1 antibody which indeed abolished sepsis-induced formation of neutrophil-platelet complexes. We also checked the role of Mac-1 on aggregates formation since Mac-1 is reported to support neutrophil-platelet interactions via GP1bα and/or GPIIIa/IIb [191, 192] but herein we found no change in the percentage of neutrophils binding platelets in the circulation, suggesting that Mac-1 may not be critical for neutrophil-platelet aggregation in abdominal sepsis. Nonetheless, immunoneutralization of PSGL-1 abolished aggregates formation but had no concomitant effect on CLP-induced Mac-1 expression on neutrophils, indicating that aggregate formation *per se*

is not necessary for the platelet-mediated up-regulation of Mac-1 on circulating neutrophils in polymicrobial sepsis. Moreover, we found that there was no difference in terms of Mac-1 expression on neutrophils exhibiting low compared to high levels of platelet binding, suggesting that up-regulation of Mac-1 on neutrophils mediated by platelets is independent of physical contacts between neutrophils and platelets in abdominal sepsis. In this context, it is important to mention that platelets contain numerous substances, including PF-4 [193], platelet activating factor [192], tromboxane A_2 [194] and ATP [195], all of which have the ability to activate neutrophils and increase expression of Mac-1.

Taken together, we can conclude that platelets play an important role in polymicrobial sepsis. This study shows that platelets regulate sepsis-induced infiltration of neutrophils in the lung via up-regulation of Mac-1 on circulating neutrophils in a contact-independent manner. Moreover, platelet depletion attenuates lung edema and tissue destruction in septic animals, suggesting that selected targeting of platelet inflammatory functions may be useful approach to protect against pulmonary injury in abdominal sepsis.

Role of platelet-derived CD40L on neutrophil activation and septic lung injury

Knowing that platelets mediated up-regulation of Mac-1 on neutrophils and neutrophil recruitment in abdominal sepsis is independent of physical contacts between neutrophils and platelets [176, 196], we focused on platelet derived products in activation and recruitment of neutrophils in abdominal sepsis. Interestingly, we observed that CLP animal has increased levels of CD40L in the plasma. This observation is line with previous experimental data showing increased expression of CD40L on peritoneal T-cells in CLP mice [197] and clinical data reporting elevated levels of CD40L in the blood of patients with meningococcal sepsis [198]. Concomitantly, we found a reduced expression of CD40L on the surface of platelets, indicating that the increased plasma levels of CD40L originate from platelets in CLP. This anticipation is later confirmed by our finding that depletion of platelets markedly attenuated (90% reduction) plasma levels of CD40L after induction of CLP.

We next asked whether CD40L has any important role in the pathophysiology of abdominal sepsis. Indeed, we found that inhibition of CD40L markedly decreased MPO activity and the number of neutrophils in the bronchoalveolar space provoked by CLP, suggesting that CD40L is an important molecule in regulating neutrophil trafficking into the inflamed lung. Additionally, we observed that blocking CD40L function not only reduced neutrophils accumulation but also attenuated sepsis-induced edema formation and tissue destruction in the lung, which suggest that targeting CD40L may protect against pulmonary damage in abdominal sepsis. We found that inhibition of CD40L had no effect on CLP-induced increase of circulating platelet-neutrophil aggregates which suggest that the impact of such aggregates appears to be very limited in terms of recruitment of neutrophils to the lung in sepsis [196].

Herein, we observed that CLP-induced Mac-1 expression on neutrophils was significantly reduced both in animals treated with an anti-CD40L antibody or mice lacking CD40L. Considering the finding that the majority of CD40L is originated from platelets in this model, these findings indicate that platelet-derived CD40L is potent regulator of Mac-1 expression on neutrophils in sepsis. This notion also helps to explain the role of CD40L in promoting CLP-induced accumulation of neutrophils in the lung. One of the receptors of CD40L is CD40,

which is mainly expressed on B-cells, dendritic cells and mast cells [199], but has also been detected on neutrophils [200], which lead us to investigate whether CD40L had the capacity to directly up-regulate Mac-1 on neutrophils. However, we found that co-incubation of peripheral murine neutrophils or bone marrow neutrophils with recombinant CD40L had no effect on Mac-1 expression. In this context, it interesting to note that CD40L has the capacity to stimulate the synthesis of CXC chemokines, such as IL-8, in macrophages and endothelial cells *in vitro* [138] and CXC chemokines are potent activators of neutrophils [93, 201, 202]. In the present study, we found that inhibition of CD40L function significantly reduced the CLP-induced elevation of plasma levels of MIP-2. Moreover, our results showed that administration of a MIP-2 receptor (CXCR2) antagonist markedly decreased CLP-induced Mac-1 expression on neutrophils as well as reduced pulmonary infiltration of neutrophils.

Taken together, these findings demonstrate that platelet-derived CD40L promotes MIP-2 secretion which, in turn, regulates neutrophil expression of Mac-1 and subsequent infiltration in the septic lung via CXCR2 signaling. However, the cellular source of MIP-2 in this chain of events remains to be determined. Knowing that both macrophages [202] and endothelial cells [203] are potent producers of MIP-2 as well as responsive to CD40L stimulation [134], it may be speculated that these cells may be involved. In this context, it should be noted that platelets also contain other compounds, such as platelet activating factor [192], tromboxane A_2 [194], and ATP [195], which have the capacity to activate neutrophils. Although our present results clearly show that platelet-derived CD40L indeed is a critical activator of neutrophils in sepsis they do not necessarily exclude the possibility that also other mechanisms are operating in parallel.

In conclusion we can say that platelet-derived CD40L is a pivotal mediator of neutrophil activation and recruitment in septic lung injury. Moreover, these findings suggest that CD40L mediates sepsis-induced neutrophil expression of Mac-1 in an indirect manner via formation of MIP-2 and CXCR2 signaling. We conclude that therapeutic strategies directed against CD40L may be useful to protect against pulmonary injury in abdominal sepsis.

Soluble CD40L in patients with shock

Once we established that platelet-derived sCD40L mediates neutrophil activation and plays a key role in septic-induced lung injury in mice [204], we asked whether sCD40L is increased in patients with shock with and without sepsis. Indeed, we found that sCD40L levels were significantly enhanced in all shock patients, regardless of sepsis or non-sepsis. Although sCD40L appears mainly to be released from activated platelets in sepsis [204] we did not observe a relationship between platelets counts on one-hand and sCD40L levels in any of the groups of shock patients. Moreover, we could not find any correlation between platelet counts on one hand and disease severity in shock patients.

However, we observed that the levels of sCD40L did not correlate with APACHE score or disease severity. This lack of correlation may either be due to a true absence of relationship or that sCD40L levels are underestimated in more severe disease. For example, dilution through high use of fluids, leakage to the interstitial space and urine or increased uptake at sites of thrombosis and inflammation may reduce the detectable plasma levels of sCD40L in patients with more severe disease. Although such factors may cause difficulties in finding a correlation between sCD40L and disease severity the relevance of sCD40L as a

potential target in patients with SIRS and sepsis requires further studies. Moreover, a single substance, such as sCD40L, would most likely respond more promptly to changes in inflammatory activity than the APACHE, which is a composite of multiple parameters. Nonetheless, this observation is in line with previous studies in meningococcal disease [205], acute pancreatitis [206] and colitis [207] reporting a lack of correlation between plasma levels of sCD40L and clinical scoring systems in critically ill patients. On the other hand, one study reported that sCD40L levels were higher in non-surviving sepsis patients compared to those surviving [208]. While our patients had similar APACHE scores, it is not clear from their study whether they had any difference in APACHE scores between surviving and non-surviving sepsis patients.

In conclusion, our data show that sCD40L levels are augmented in patients with SIRS and shock, regardless of whether this was due to sepsis or not. Although the functional role of sCD40L remains to be explored, these findings may open up a new focus to ameliorate pathological inflammation in shock patients.

Role of MMPs in abdominal sepsis

The mechanisms regulating CD40L release from platelets in abdominal sepsis are not known. MMPs belong to a large family of endopeptidases with capacity to cleave the majority of matrix proteins as well as many non-matrix targets, such as chemokines, cytokines, adhesion molecules and surface receptors. Based on these considerations the aim of the study IV was to define the role of MMPs in regulating cleavage of CD40L from platelets in abdominal sepsis. To elucidate MMPs role in platelet shedding of CD40L in abdominal sepsis, we used a broad-spectrum MMP inhibitor (GM600), which inhibits a set of metalloproteinases comprising MMP-1, MMP-2, MMP-3, MMP-8 and MMP-9.

We found that MMPs control CD40L shedding from platelets and subsequent up-regulation of Mac-1 on neutrophils as well as formation of CXC chemokines in the lung. Additionally, we observed that MMP inhibition not only reduced neutrophil recruitment but also decreased sepsis-induced edema formation and tissue destruction in the lung, suggesting that targeting MMPs may protect against pulmonary damage in abdominal sepsis. This notion is in line with most studies on MMP inhibition in models of endotoxemia and severe infections [165, 209, 210]. Moreover, we studied the detailed impact of MMPs on leukocyte-endothelium interactions; for this we used intravital fluorescence microscopy of the lung microcirculation. We were able to demonstrate that MMP inhibition decreased sepsis-induced leukocyte adhesion in venules but not capillary trapping of leukocytes. Considering that venular adhesion of leukocytes are mediated by specific adhesion molecules and that trapping of leukocytes in capillaries is dependent on size-restriction in the capillary lumen due to increased stiffness of [186, 211-213] our data suggest that MMPs mainly regulate the adhesion molecule-dependent accumulation of leukocytes in the lung. This notion is also supported by the observation herein that inhibition of MMPs decreased neutrophil expression of Mac-1, which is known to mediate pulmonary recruitment of neutrophils in abdominal sepsis [17]. Tissue navigation of neutrophils is regulated by secreted CXC chemokines [214]. In the present study, we observed that MMP inhibition attenuated sepsis-provoked-induced formation of MIP-2 and KC in the lung, which may also contribute to the protective effect of MMP inhibition in septic lung damage. The mechanism by which MMPs control CXC chemokine formation in the lung is not known at present.

Thus, study IV demonstrates that MMPs regulate platelet shedding of CD40L, Mac-1 up-regulation on neutrophils and CXC chemokine formation in the lung, which together helps to explain the MMP-dependent sepsis-induced neutrophil recruitment and tissue damage in the lung. Thus, based on our results, we suggest that MMPs may be a useful target to inhibit lung damage in abdominal sepsis.

Role of MMP-9 in CD40L shedding from platelets

Accumulating data suggest that certain MMPs, in particular MMP-2 and MMP-9 (gelatinase sub family), are elevated in the plasma of septic patients [149, 150, 215, 216]. Overwhelming data in the literarture implicate MMPs in numerous features of inflammatory functions, including cytokine production and leukocyte migration [217-219]. In the present study, we observed that plasma levels MMP-9 but not MMP-2 were increased in septic animals. This observation is in accordance with other studies reporting that MMP-9 levels are elevated in the circulation of infectious disease models [220, 221]. We next asked whether MMP-9 might be involved in platelet shedding of CD40L in sepsis. We found that the CLP-induced reduction in platelet surface expression of CD40L and concomitant increase of soluble CD40L levels in the plasma were significantly attenuated in mice lacking MMP-9, suggesting that MMP-9 indeed is a potent regulator of platelet shedding of CD40L in abdominal sepsis. It should be note that these findings do not exclude the possibility that other MMPs or proteases might also involve in the regulation of platelet shedding of CD40L in sepsis. For example, at least two *in vitro* studies showed that MMP-2 involved in CD40L shedding from platelet [135, 222].

In addition, we found that MMP-9 was up-regulated on the surface of activated platelets and that platelet shedding of CD40L is abolished in activated platelets from MMP-9 gene-deficient mice *in vitro*. Moreover, we observed that recombinant MMP-9 completely attenuated CD40L expression and increased shedding of CD40L in platelets lacking MMP-9. In addition, inhibition of MMP-9 pharmacologically abolished MMP-9 cleavage in activated platelets suggests that MMP-9 is an important regulator of platelet shedding of CD40L *in vitro*. In fact, this study is the first to demonstrate a role of MMP-9 in regulating platelet inflammatory functions in sepsis. In this context, it is interesting to note that soluble CD40L has been reported to induce formation and increased expression of MMP-9 [223-225]. Considered together with our observation that MMP-9 appears to regulate platelet release of soluble CD40L, it may be proposed that there might be self-amplifying loops involving reciprocal activation of CD40L and MMP-9 in sepsis, which requires further studies to confirm.

Knowing that neutrophil recruitment is a rate-limiting step in septic lung injury [17, 226], herein, we observed that pulmonary infiltration of neutrophils was greatly decreased in animals lacking MMP-9, indicating that MMP-9 is a significant regulator of sepsis-induced neutrophil infiltration in the lung. Additionally, we observed that not only neutrophil recruitment was reduced but also sepsis-induced edema formation and tissue destruction in the lung were markedly attenuated in MMP-9 gene-deficient mice. The published literature on the role of MMP-9 in severe infections is complex and contradictory. For example, one study reported that *i.p.* administration of *Escerichia coli* caused an increased accumulation of neutrophils and tissue damage in the lung [227] whereas another study reported that pulmonary challenge with *Fransciella tularensis* was associated with decreased neutrophil infiltration and lung damage [228] in MMP-9 gene-deficient mice. Moreover, Lee at al. (2005) demonstrated that MMP-9 exerts a

protective effect in corneal infection with *Pseudomonas* [229] whereas McClellan et al. (2006) reported diametrically opposite results in a similar model [230]. Although the reason for these discrepancies cannot be clarified herein, it is well known that mice challenged with different bacteria or bacterial toxins as well as different routes of administration display divergent phenotypes [46, 47, 231]. Nonetheless, our present findings demonstrate that MMP-9 is a fundamental regulator of pulmonary infiltration of neutrophils and tissue damage in CLP-induced polymicrobial sepsis which is a model more reminiscent of the events in human sepsis compared to other models based on challenge with a single bacteria or toxin [82, 232]. This notion is also supported by a previous study reporting that treatment with a broad-spectrum inhibitor of MMPs reduces sepsis-induced neutrophil infiltration and tissue damage in the lung [210].

Interestingly, we found that MMP-9 levels were significantly enhanced in patients with septic shock which not only supported by other clinical studies but also support a role of MMP-9 in patients with sepsis. However, we did not see any correlation between levels of MMP-9 with APACHE II score. This lack of correlation may have been due to several causes rather a true absence of relationship. For example, differences in the time-lag for sampling, dilution of plasma due to administration of fluids, interstitial leakage or increased uptake at sites of inflammation, might decrease detectable plasma levels of MMP-9 and, thus, lead to an underestimation of the MMP-9 levels in septic shock patients. Although such factors may cause difficulties in finding a correlation between MMP-9 and disease severity, the relevance of MMP-9 as a potential target in patients with sepsis requires further studies. In this context, it may be speculated that a single compound, such as MMP-9, most likely responds more promptly to changes in inflammatory activity than the APACHE II score, which is a composite of multiple clinical parameters. Moreover, we did not find any difference in MMP-9 levels between non-surviving and surviving sepsis patients.

In conclusion, we demonstrate for the first time that MMP-9 is up-regulated on activated platelets and regulates platelet shedding of CD40L in abdominal sepsis. Moreover, our novel results show that MMP-9 is important in controlling pulmonary accumulation of neutrophils and lung edema formation in polymicrobial sepsis. Thus, based on our data, we suggest that targeting MMP-9 may be a useful strategy in order to ameliorate pathological inflammation and lung damage in abdominal sepsis.

Summary

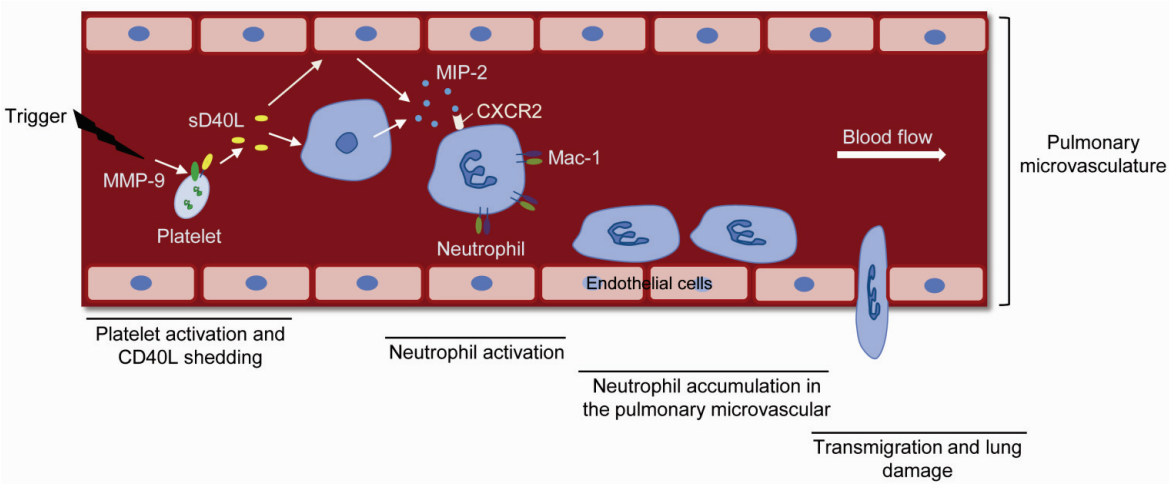

Figure 6. Schematic diagram summarizing the proposed mechanism of platelet-dependent pulmonary recruitment of neutrophils in abdominal sepsis.

Conclusions

1. Platelets play a key role in regulating infiltration of neutrophils and edema formation in the lung via upregulation of Mac-1 in a contact-independent manner in abdominal sepsis.
2. Platelet-derived CD40L is a pivotal mediator of neutrophil activation and recruitment in abdominal sepsis and CD40L mediated neutrophil activation is indirect via formation of MIP-2 and CXCR2 signaling.
3. Soluble CD40L levels are increased in patient with SIRS and shock, regardless of sepsis or non-sepsis.
4. Inhibition of MMPs reduces Mac-1 up-regulation on neutrophils and CXC chemokine formation in the septic lung injury, which might be related to controlling CD40L shedding from platelets.
5. MMP-9 mediates platelet-dependent pulmonary accumulation of neutrophils and tissue damage in polymicrobial sepsis by regulating CD40L shedding from platelets.

Taken together, these findings will hopefully reveal novel therapeutic targets and approaches that can improve survival of septic patient.

Sammanfattning på svenska

Sepsis, blodförgiftning, är ett potentiellt allvarligt tillstånd där bakterier eller deras toxiner aktiverar immunsystemet I blodbanan. Svår sepsis är associerad med organdysfunktion och hög mortalitet (30-60%). Cirka 200 per 100 000 invånare i Sverige drabbas årligen av svår sepsis. Akut lungskada är en central komponent hos patienter med sepsis och experimentella studier har visat att aktivering och ackumulering av vita blodkroppar är ett hastighetsberoende steg i sepsis-associerad lungskada. Trombocyter är kända för sin viktiga roll vid blödning och sårläkning men nyare data indikerar också att trombocyter är också viktiga vid inflammatoriska reaktioner. Den här avhandlingen fokuserar på den potentiella betydelsen av trombocyter vid sepsis. I det första arbetet observerades att om man tog bort trombocyterna från möss minskade aktiveringen och rekrytering av vita blodkroppar, neutropfila granulocyter, till lunga med minskad vävnadsskada som följd. Inhibering av PSGL-1 fullständigt blockerade aggregat bildningen mellan trombocyter och neutrofiler vid sepsis. Det vill säga att den här trombocyt-beroende aktivering av neutrofiler visade sig vara oberoende av fysisk kontakt mellan trombocyterna och neutrofilerna. Istället kunde det konstateras att någon eller några faktorer som utsöndras i löslig form från trombocyter i sin tur aktiverade cirkulerande neutrofiler vid sepsis. I arbete nummer två observerade vi att löslig form av CD40L ökade kraftigt i blodet vid sepsis och att den här ökningen försvann helt om man tog bort trombocyterna före induktion av sepsis. I det här arbetet identifierades CD40L vara den molekyl som utsöndras från trombocyter och som aktiverar neutrofiler i blodbanan. Blockering av CD40L minskade inte bara aktivering av neutrofiler utan reducerade också sepsis inducerad lungskada. CD40L-medierad aktivering av neutrofiler visade sig vara indirekt via bildningen av MIP-2 som är en potent stimulator av neutrofiler. I det tredje arbetet visade det sig att lösligt CD40L också ökade i blodet på patienter med sepsis jämfört med friska kontroller. Löslig CD40L ökade inte bara vid septisk chock utan också vid chock orsakad av andra faktorer än bakterier. De här resultaten indikerar att fynden i de två första djurexperimentella arbetena kan vara relevanta också hos patienter med sepsis. I arbete fyra undersöktes mekanismer som kan förklara hur CD40L frisätts från trombocyter vid sepsis. Blockering av en grupp av enzym, metalloproteinaser (MMP), visade sig hindra frisättning av CD40L från trombocyter och därmed aktiveringen av neutrofiler samt minskade lungskadan vid sepsis. Efter att ha konstaterat att något MMP kan vara involverat, mättes bildningen av relevanta kandidater, MMP-2 och MMP-9, i blodet. Det visade sig att MMP-9 men inte MMP-2 ökade i blodet vid sepsis. Med hjälp av möss som saknar MMP-9 kunde det fastställas att MMP-9 reglerade CD40L frisättningen från trombocyter vid sepsis. I direkta försök på isolerade trombocyter kunde det konstateras att MMP-9 ökar på ytan av aktiverade trombocyter och spelar en direkt avgörande roll för frisättning av CD40L. Det visade sig också att patienter hade förhöjda nivåer av MMP-9 i blodet

jämfört med friska kontroller vilket skulle kunna betyda att MMP-9 också spelar en funktionell roll vid sepsis. Sammanfattningsvis kan det konstateras att trombocyter spelar en viktig roll vid sepsis genom att aktivera cirkulerande neutrofiler via frisättning av CD40L. Dessutom visar den här avhandlingen att MMP-9 upregleras på aktiverade trombocyter och frisätter CD40L ligand. Mot bakgrund av att dessa molekyler också ökar vid hos patienter med sepsis skulle CD40L och MMP-9 kunna utgöra nya och mer specifika måltavlor för behandling av patienter med svår sepsis.

Acknowledgments

This thesis is the result of my years of research in Department of Surgery, Malmö, Lund University, Sweden. I would like to express my acknowledgements to everyone who have contributed to this thesis either directly or mentally.

First and foremost, I wish to show my utmost gratitude to professor **Henrik Thorlacius**, my principal PhD supervisor. I appreciate and commemorate his constant sincerity and encouragement that I have received during my whole study period. His profound and prolific scientific thinking on research has made him as a constant oasis of ideas and passions in science. He sets an example of a world-class researcher for his rigor and passion on research.

I am deeply grateful and thankful to my co-supervisor professor **Bengt Jeppsson,** whose encouragement, guidance and support from the initial to the final level enabled me to develop the thesis. I will always remember his sincerity and generosity. I also sincerely remember his wife **Christina** for all of her kind hospitality in Sweden and unforgettable time in Bangladesh.

I am delighted to interact with my co-supervisor **Ingvar Syk.** I really appreciate his advices and constructive criticism in some of my papers.

I am indebted to one of my Bangladeshi seniors and ex-member of our group, **Muhammad Asaduzzaman,** he has introduced me with Henrik and given me all kind of necessary advice and training at the beginning of my PhD study.

I would like to show my gratitude to **Michelle Chew,** one of my collaborators for her great enthusiasm and contributions in clinical studies.

The members of our group have contributed immensely to my personal and professional time. The group has been a source of friendship as well as good advice and collaboration. Especially, I would like to express my gratefulness to **Su Zhang** for her nice collaboration and great contributions**.** Special thanks go to **Yusheng Wang, Qing Liu, Songen Zhang, Andrada Röme, Darbaz Awla, Aree Abdulla, Karzan Palani, Zirak Hasan, Jonas Roller, Amr Al-haidari, Yongzhi Wang, Mohammed Merza, Ling Tao Luo** and **Susanne Eiswold.**

I warmly thankful to **Anita Alm**, who has provided me an outstanding and non-ending help whenever I needed. She was more as a friend than as an administrator. I will never forget her visit in Bangladesh.

My deepest gratitude to **Anne-Marie Rohrstock.** Her presence in the laboratory has made this work much easier and smoother.

Several individuals who in one way or another contributed and extended their valuable assistance in my PhD, namely, Professor **Michael Menger, Sara Regner, Anders Erssson, Lilian Ehrman, Jonas Menjer, Peter Ellmark, Oscar Braun, Mattias Lepsenyi, Ingrid Palmquist, Diya Adawi**, **Christina Stene**.

I am also grateful to all of my Bangladeshi friends and seniors especially people in Lund/Malmö, for their unlimited help in my personal life. I had very good time with them. I will never forget those memories.

I wish to express my love and gratitude to my beloved wife **Tanzina Azad** for her understanding on the nature of research work especially during the time of late night experiments and thesis writing. I am thankful to her for take caring our little daughter **Manha** without interrupting my work.

I am ever grateful to my family members especially my brothers (**Taslim** and **Wahab**) and sisters (**Jasmin** and **Sarmin**) and some of my uncles whose

contributions in my education are never forgettable. Last but not least, I would like to thank my parents who raised me with love and supported and inspired my education endlessly.

References

1. Cohen J. The immunopathogenesis of sepsis. Nature 2002; 420:885-91.

2. Warren HS. Strategies for the treatment of sepsis. N Engl J Med 1997; 336:952-3.

3. Zeni F, Freeman B, Natanson C. Anti-inflammatory therapies to treat sepsis and septic shock: a reassessment. Crit Care Med 1997; 25:1095-100.

4. 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; 29:1303-10.

5. Martin GS, Mannino DM, Eaton S, Moss M. The epidemiology of sepsis in the United States from 1979 through 2000. N Engl J Med 2003; 348:1546-54.

6. Bone RC, Sibbald WJ, Sprung CL. The ACCP-SCCM consensus conference on sepsis and organ failure. Chest 1992; 101:1481-3.

7. Hollenberg SM, Ahrens TS, Annane D, Astiz ME, Chalfin DB, Dasta JF, et al. Practice parameters for hemodynamic support of sepsis in adult patients: 2004 update. Crit Care Med 2004; 32:1928-48.

8. Sessler CN, Perry JC, Varney KL. Management of severe sepsis and septic shock. Curr Opin Crit Care 2004; 10:354-63.

9. Gorbach SL, Bartlett JG. Anaerobic infections. 1. N Engl J Med 1974; 290:1177-84.

10. Simon GL, Gorbach SL. Intestinal flora in health and disease. Gastroenterology 1984; 86:174-93.

11. Polk HC, Jr., Shields CL. Remote organ failure: a valid sign of occult intra-abdominal infection. Surgery 1977; 81:310-3.

12. Wagner JG, Roth RA. Neutrophil migration during endotoxemia. J Leukoc Biol 1999; 66:10-24.

13. von Andrian UH, Chambers JD, McEvoy LM, Bargatze RF, Arfors KE, Butcher EC. Two-step model of leukocyte-endothelial cell interaction in inflammation: distinct roles for LECAM-1 and the leukocyte beta 2 integrins in vivo. Proc Natl Acad Sci U S A 1991; 88:7538-42.

14. Reutershan J, Basit A, Galkina EV, Ley K. Sequential recruitment of neutrophils into lung and bronchoalveolar lavage fluid in LPS-induced acute lung injury. Am J Physiol Lung Cell Mol Physiol 2005; 289:L807-15.

15. Issekutz AC, Issekutz TB. The contribution of LFA-1 (CD11a/CD18) and MAC-1 (CD11b/CD18) to the in vivo migration of polymorphonuclear leucocytes to inflammatory reactions in the rat. Immunology 1992; 76:655-61.

16. Basit A, Reutershan J, Morris MA, Solga M, Rose CE, Jr., Ley K. ICAM-1 and LFA-1 play critical roles in LPS-induced neutrophil recruitment into the alveolar space. Am J Physiol Lung Cell Mol Physiol 2006; 291:L200-7.

17. Asaduzzaman M, Zhang S, Lavasani S, Wang Y, Thorlacius H. LFA-1 and MAC-1 mediate pulmonary recruitment of neutrophils and tissue damage in abdominal sepsis. Shock 2008; 30:254-9.

18. Parrillo JE. Mechanisms Of disease - pathogenetic mechanisms of septic shock. N Engl J Med 1993; 328:1471-1477.

19. Remick DG. Pathophysiology of sepsis. Am J Pathol 2007; 170:1435-44.

20. Nagase T, Uozumi N, Ishii S, Kume K, Izumi T, Ouchi Y, et al. Acute lung injury by sepsis and acid aspiration: a

key role for cytosolic phospholipase A2. Nat Immunol 2000; 1:42-6.

21. von Hundelshausen P, Weber C. Platelets as immune cells: bridging inflammation and cardiovascular disease. Circ Res 2007; 100:27-40.

22. Abdulla A, Awla D, Hartman H, Rahman M, Jeppsson B, Regner S, et al. Role of platelets in experimental acute pancreatitis. Br J Surg 2011; 98:93-103.

23. Lam FW, Burns AR, Smith CW, Rumbaut RE. Platelets enhance neutrophil transendothelial migration via P-selectin glycoprotein ligand-1. Am J Physiol Heart Circ Physiol 2011; 300:H468-75.

24. Semple JW, Freedman J. Platelets and innate immunity. Cell Mol Life Sci 2010; 67:499-511.

25. Singbartl K, Forlow SB, Ley K. Platelet, but not endothelial, P-selectin is critical for neutrophil-mediated acute postischemic renal failure. FASEB J 2001; 15:2337-44.

26. Salter JW, Krieglstein CF, Issekutz AC, Granger DN. Platelets modulate ischemia/reperfusion-induced leukocyte recruitment in the mesenteric circulation. Am J Physiol Gastrointest Liver Physiol 2001; 281:G1432-9.

27. Smyth SS, McEver RP, Weyrich AS, Morrell CN, Hoffman MR, Arepally GM, et al. Platelet functions beyond hemostasis. J Thromb Haemost 2009; 7:1759-66.

28. Levy MM, Fink MP, Marshall JC, Abraham E, Angus D, Cook D, et al. 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. Crit Care Med 2003; 31:1250-6.

29. 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. Chest 1992; 101:1644-55.

30. Janeway CA, Jr., Medzhitov R. Introduction: the role of innate immunity in the adaptive immune response. Semin Immunol 1998; 10:349-50.

31. Alberti C, Brun-Buisson C, Burchardi H, Martin C, Goodman S, Artigas A, et al. Epidemiology of sepsis and infection in ICU patients from an international multicentre cohort study. Intensive Care Med 2002; 28:108-21.

32. Seydel U, Oikawa M, Fukase K, Kusumoto S, Brandenburg K. Intrinsic conformation of lipid A is responsible for agonistic and antagonistic activity. Eur J Biochem 2000; 267:3032-9.

33. Shapiro L, Gelfand JA. Cytokines and sepsis: pathophysiology and therapy. New Horiz 1993; 1:13-22.

34. Wang H, Vishnubhakat JM, Bloom O, Zhang M, Ombrellino M, Sama A, et al. Proinflammatory cytokines (tumor necrosis factor and interleukin 1) stimulate release of high mobility group protein-1 by pituicytes. Surgery 1999; 126:389-92.

35. Tanaka Y, Nagai Y, Kuroishi T, Endo Y, Sugawara S. Stimulation of Ly-6G on neutrophils in LPS-primed mice induces platelet-activating factor (PAF)-mediated anaphylaxis-like shock. J Leukoc Biol 2011.

36. Wang B, Gong X, Wan JY, Zhang L, Zhang Z, Li HZ, et al. Resolvin D1 protects mice from LPS-induced acute lung injury. Pulm Pharmacol Ther 2011; 24:434-41.

37. Smedegard G, Cui LX, Hugli TE. Endotoxin-induced shock in the rat. A role for C5a. Am J Pathol 1989; 135:489-97.

38. Triantafilou M, Triantafilou K. The dynamics of LPS recognition: complex orchestration of multiple receptors. J Endotoxin Res 2005; 11:5-11.

39. Majcherczyk PA, Langen H, Heumann D, Fountoulakis M, Glauser MP, Moreillon P. Digestion of Streptococcus pneumoniae cell walls with its major peptidoglycan hydrolase releases branched stem peptides carrying proinflammatory activity. J Biol Chem 1999; 274:12537-43.

40. Morath S, Geyer A, Hartung T. Structure-function relationship of cytokine induction by lipoteichoic acid from Staphylococcus aureus. J Exp Med 2001; 193:393-7.

41. Fast DJ, Schlievert PM, Nelson RD. Toxic shock syndrome-associated staphylococcal and streptococcal pyrogenic toxins are potent inducers of tumor necrosis factor production. Infect Immun 1989; 57:291-4.

42. Todd JK, Franco-Buff A, Lawellin DW, Vasil ML. Phenotypic distinctiveness of Staphylococcus aureus strains associated with toxic shock syndrome. Infect Immun 1984; 45:339-44.

43. Parsonnet J. Mediators in the pathogenesis of toxic shock syndrome: overview. Rev Infect Dis 1989; 11 Suppl 1:S263-9.

44. McNicol A, Agpalza A, Jackson EC, Hamzeh-Cognasse H, Garraud O, Cognasse F. Streptococcus sanguinis-induced cytokine release from platelets. J Thromb Haemost 2011; 9:2038-49.

45. Bhakdi S, Muhly M, Mannhardt U, Hugo F, Klapettek K, Mueller-Eckhardt C, et al. Staphylococcal alpha toxin promotes blood coagulation via attack on human platelets. J Exp Med 1988; 168:527-42.

46. Ulevitch RJ, Mathison JC, Schumann RR, Tobias PS. A new model of macrophage stimulation by bacterial lipopolysaccharide. J Trauma 1990; 30:S189-92.

47. Wright SD, Ramos RA, Tobias PS, Ulevitch RJ, Mathison JC. CD14, a receptor for complexes of lipopolysaccharide (LPS) and LPS binding protein. Science 1990; 249:1431-3.

48. Renno T, Hahne M, Tschopp J, MacDonald HR. Peripheral T cells undergoing superantigen-induced apoptosis in vivo express B220 and upregulate Fas and Fas ligand. J Exp Med 1996; 183:431-7.

49. Lemaitre B, Nicolas E, Michaut L, Reichhart JM, Hoffmann JA. The dorsoventral regulatory gene cassette spatzle/Toll/cactus controls the potent antifungal response in Drosophila adults. Cell 1996; 86:973-83.

50. Hotchkiss RS, Tinsley KW, Swanson PE, Grayson MH, Osborne DF, Wagner TH, et al. Depletion of dendritic cells, but not macrophages, in patients with sepsis. J Immunol 2002; 168:2493-500.

51. Hotchkiss RS, Karl IE. The pathophysiology and treatment of sepsis. N Engl J Med 2003; 348:138-50.

52. Beutler B, Rietschel ET. Innate immune sensing and its roots: the story of endotoxin. Nat Rev Immunol 2003; 3:169-76.

53. Takeda K. Evolution and integration of innate immune recognition systems: the Toll-like receptors. J Endotoxin Res 2005; 11:51-5.

54. Ulevitch RJ. Therapeutics targeting the innate immune system. Nat Rev Immunol 2004; 4:512-20.

55. Chen GY, Nunez G. Sterile inflammation: sensing and reacting to damage. Nat Rev Immunol 2010; 10:826-37.

56. Guo RF, Ward PA. Role of C5a in inflammatory responses. Annu Rev Immunol 2005; 23:821-52.

57. Hibberd ML, Sumiya M, Summerfield JA, Booy R, Levin M. Association of variants of the gene for mannose-binding lectin with susceptibility to meningococcal disease. Meningococcal Research Group. Lancet 1999; 353:1049-53.

58. Hoffmann JA, Kafatos FC, Janeway CA, Ezekowitz RA. Phylogenetic perspectives in innate immunity. Science 1999; 284:1313-8.

59. Pugin J, Heumann ID, Tomasz A, Kravchenko VV, Akamatsu Y, Nishijima M, et al. CD14 is a pattern recognition receptor. Immunity 1994; 1:509-16.

60. Landmann R, Zimmerli W, Sansano S, Link S, Hahn A, Glauser MP, et al. Increased circulating soluble CD14 is associated with high mortality in gram-negative septic shock. J Infect Dis 1995; 171:639-44.

61. Inohara N, Nunez G. The NOD: a signaling module that regulates apoptosis and host defense against pathogens. Oncogene 2001; 20:6473-81.

62. Mitchell JA, Paul-Clark MJ, Clarke GW, McMaster SK, Cartwright N. Critical role of toll-like receptors and nucleotide oligomerisation domain in the regulation of health and disease. J Endocrinol 2007; 193:323-30.

63. Barton GM, Medzhitov R. Toll-like receptor signaling pathways. Science 2003; 300:1524-5.

64. Ozato K, Tsujimura H, Tamura T. Toll-like receptor signaling and regulation of cytokine gene expression in the immune system. Biotechniques 2002; Suppl:66-8, 70, 72 passim.

65. Oberholzer A, Oberholzer C, Moldawer LL. Sepsis syndromes: understanding the role of innate and acquired immunity. Shock 2001; 16:83-96.

66. Docke WD, Randow F, Syrbe U, Krausch D, Asadullah K, Reinke P, et al. Monocyte deactivation in septic patients: restoration by IFN-gamma treatment. Nat Med 1997; 3:678-81.

67. Bone RC, Grodzin CJ, Balk RA. Sepsis: a new hypothesis for pathogenesis of the disease process. Chest 1997; 112:235-43.

68. Moore LJ, McKinley BA, Turner KL, Todd SR, Sucher JF, Valdivia A, et al. The epidemiology of sepsis in general surgery patients. J Trauma 2011; 70:672-80.

69. Ayala A, Herdon CD, Lehman DL, Ayala CA, Chaudry IH. Differential induction of apoptosis in lymphoid tissues during sepsis: variation in onset, frequency, and the nature of the mediators. Blood 1996; 87:4261-75.

70. Bommhardt U, Chang KC, Swanson PE, Wagner TH, Tinsley KW, Karl IE, et al. Akt decreases lymphocyte apoptosis and improves survival in sepsis. J Immunol 2004; 172:7583-91.

71. Hotchkiss RS, Nicholson DW. Apoptosis and caspases regulate death and inflammation in sepsis. Nat Rev Immunol 2006; 6:813-22.

72. Monneret G, Debard AL, Venet F, Bohe J, Hequet O, Bienvenu J, et al. Marked elevation of human circulating CD4+CD25+ regulatory T cells in sepsis-induced immunoparalysis. Critical care medicine 2003; 31:2068-71.

73. Hiraki S, Ono S, Tsujimoto H, Kinoshita M, Takahata R, Miyazaki H, et al. Neutralization of interleukin-10 or transforming growth factor-beta decreases the percentages of CD4(+)CD25(+)Foxp3(+) regulatory T cells in septic mice, thereby leading to an improved survival. Surgery 2012; 151:313-22.

74. Wesche DE, Lomas-Neira JL, Perl M, Chung CS, Ayala A. Leukocyte apoptosis and its significance in sepsis and shock. J Leukoc Biol 2005; 78:325-37.

75. Abraham E, Singer M. Mechanisms of sepsis-induced organ dysfunction. Crit Care Med 2007; 35:2408-16.

76. Aird WC. The role of the endothelium in severe sepsis and multiple organ dysfunction syndrome. Blood 2003; 101:3765-77.

77. Swank GM, Deitch EA. Role of the gut in multiple organ failure: bacterial translocation and permeability changes. World J Surg 1996; 20:411-7.

78. Deitch EA. Multiple organ failure. Pathophysiology and potential future therapy. Ann Surg 1992; 216:117-34.

79. Leaphart CL, Tepas JJ, 3rd. The gut is a motor of organ system dysfunction. Surgery 2007; 141:563-9.

80. Border JR, Hassett J, LaDuca J, Seibel R, Steinberg S, Mills B, et al. The gut origin septic states in blunt multiple trauma (ISS = 40) in the ICU. Ann Surg 1987; 206:427-48.

81. Levi M, Ten Cate H. Disseminated intravascular coagulation. N Engl J Med 1999; 341:586-92.

82. Wichterman KA, Baue AE, Chaudry IH. Sepsis and septic shock--a review of laboratory models and a proposal. J Surg Res 1980; 29:189-201.

83. Landry DW, Oliver JA. The pathogenesis of vasodilatory shock. N Engl J Med 2001; 345:588-95.

84. Proudfoot AE. Chemokine receptors: multifaceted therapeutic targets. Nat Rev Immunol 2002; 2:106-15.

85. Rollins BJ. Chemokines. Blood 1997; 90:909-28.

86. Lomas-Neira JL, Chung CS, Grutkoski PS, Miller EJ, Ayala A. CXCR2 inhibition suppresses hemorrhage-induced priming for acute lung injury in mice. J Leukoc Biol 2004; 76:58-64.

87. Craciun FL, Schuller ER, Remick DG. Early enhanced local neutrophil recruitment in peritonitis-induced sepsis improves bacterial clearance and survival. J Immunol 2010; 185:6930-8.

88. Hayashida K, Parks WC, Park PW. Syndecan-1 shedding facilitates the resolution of neutrophilic inflammation by removing sequestered CXC chemokines. Blood 2009; 114:3033-43.

89. Kaneider NC, Agarwal A, Leger AJ, Kuliopulos A. Reversing systemic inflammatory response syndrome with chemokine receptor pepducins. Nat Med 2005; 11:661-5.

90. Luster AD. Chemokines--chemotactic cytokines that mediate inflammation. N Engl J Med 1998; 338:436-45.

91. Grob PM, David E, Warren TC, DeLeon RP, Farina PR, Homon CA. Characterization of a receptor for human monocyte-derived neutrophil chemotactic factor/interleukin-8. J Biol Chem 1990; 265:8311-6.

92. Samanta AK, Oppenheim JJ, Matsushima K. Identification and characterization of specific receptors for monocyte-derived neutrophil chemotactic factor (MDNCF) on human neutrophils. J Exp Med 1989; 169:1185-9.

93. Bacon KB, Oppenheim JJ. Chemokines in disease models and pathogenesis. Cytokine Growth Factor Rev 1998; 9:167-73.

94. Oquendo P, Alberta J, Wen DZ, Graycar JL, Derynck R, Stiles CD. The platelet-derived growth factor-inducible KC gene encodes a secretory protein related to platelet alpha-granule proteins. J Biol Chem 1989; 264:4133-7.

95. Chishti AD, Shenton BK, Kirby JA, Baudouin SV. Neutrophil chemotaxis and receptor expression in clinical septic shock. Intensive Care Med 2004; 30:605-11.

96. Ness TL, Hogaboam CM, Strieter RM, Kunkel SL. Immunomodulatory role of CXCR2 during experimental septic peritonitis. J Immunol 2003; 171:3775-84.

97. Zhang S, Rahman M, Qi Z, Thorlacius H. Simvastatin antagonizes CD40L secretion, CXC chemokine formation, and pulmonary infiltration of neutrophils in abdominal sepsis. J Leukoc Biol 2011; 89:735-42.

98. Ley K. Molecular mechanisms of leukocyte recruitment in the inflammatory process. Cardiovasc Res 1996; 32:733-42.

99. Montoya MC, Holtmann K, Snapp KR, Borges E, Sanchez-Madrid F, Luscinskas FW, et al. Memory B lymphocytes from secondary lymphoid organs interact with E-selectin through a novel glycoprotein ligand. J Clin Invest 1999; 103:1317-27.

100. Vestweber D, Blanks JE. Mechanisms that regulate the function of the selectins and their ligands. Physiol Rev 1999; 79:181-213.

101. Tangelder GJ, Arfors KE. Inhibition of leukocyte rolling in venules by protamine and sulfated polysaccharides. Blood 1991; 77:1565-71.

102. Fox-Robichaud A, Kubes P. Molecular mechanisms of tumor necrosis factor alpha-stimulated leukocyte recruitment into the murine hepatic circulation. Hepatology 2000; 31:1123-7.

103. Mizgerd JP, Peschon JJ, Doerschuk CM. Roles of tumor necrosis factor receptor signaling during murine Escherichia coli pneumonia. Am J Respir Cell Mol Biol 2000; 22:85-91.

104. Mansson P, Zhang XW, Jeppsson B, Johnell O, Thorlacius H. Critical role of P-selectin-dependent rolling in tumor necrosis factor-alpha-induced leukocyte adhesion and extravascular recruitment in vivo. Naunyn Schmiedebergs Arch Pharmacol 2000; 362:190-6.

105. Klintman D, Li X, Thorlacius H. Important role of P-selectin for leukocyte recruitment, hepatocellular injury, and apoptosis in endotoxemic mice. Clinical and Diagnostic Laboratory Immunology 2004; 11:56-62.

106. Awla D, Abdulla A, Zhang S, Roller J, Menger MD, Regner S, et al. Lymphocyte function antigen-1 regulates neutrophil recruitment and tissue damage in acute pancreatitis. Br J Pharmacol 2011; 163:413-23.

107. Thorlacius H, Vollmar B, Guo Y, Mak TW, Pfreundschuh MM, Menger MD, et al. Lymphocyte function antigen 1 (LFA-1) mediates early tumour necrosis factor alpha-induced leucocyte adhesion in venules. Br J Haematol 2000; 110:424-9.

108. Smith CW. Adhesion molecules and receptors. J Allergy Clin Immunol 2008; 121:S375-9; quiz S414.

109. Laudes IJ, Guo RF, Riedemann NC, Speyer C, Craig R, Sarma JV, et al. Disturbed homeostasis of lung intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 during sepsis. Am J Pathol 2004; 164:1435-45.

110. Ding ZM, Babensee JE, Simon SI, Lu H, Perrard JL, Bullard DC, et al. Relative contribution of LFA-1 and Mac-1 to neutrophil adhesion and migration. J Immunol 1999; 163:5029-38.

111. Childs EW, Smalley DM, Moncure M, Miller JL, Cheung LY. Effect of LFA-1beta antibody on leukocyte adherence in response to hemorrhagic shock in rats. Shock 2000; 14:49-52.

112. Hentzen ER, Neelamegham S, Kansas GS, Benanti JA, McIntire LV, Smith CW, et al. Sequential binding of CD11a/CD18 and CD11b/CD18 defines neutrophil capture and stable adhesion to intercellular adhesion molecule-1. Blood 2000; 95:911-20.

113. George JN. Platelets. Lancet 2000; 355:1531-9.

114. Aarts PA, Heethaar RM, Sixma JJ. Red blood cell deformability influences platelets--vessel wall interaction in flowing blood. Blood 1984; 64:1228-33.

115. Zarbock A, Polanowska-Grabowska RK, Ley K. Platelet-neutrophil-interactions: linking hemostasis and inflammation. Blood Rev 2007; 21:99-111.

116. Vincent JL, Yagushi A, Pradier O. Platelet function in sepsis. Crit Care Med 2002; 30:S313-7.

117. Flad HD, Brandt E. Platelet-derived chemokines: pathophysiology and therapeutic aspects. Cell Mol Life Sci 2010; 67:2363-86.

118. Zarbock A, Singbartl K, Ley K. Complete reversal of acid-induced acute lung injury by blocking of platelet-neutrophil aggregation. J Clin Invest 2006; 116:3211-9.

119. Rendu F, Brohard-Bohn B. The platelet release reaction: granules' constituents, secretion and functions. Platelets 2001; 12:261-73.

120. Power CA, Clemetson JM, Clemetson KJ, Wells TN. Chemokine and chemokine receptor mRNA expression in human platelets. Cytokine 1995; 7:479-82.

121. Brandt E, Petersen F, Ludwig A, Ehlert JE, Bock L, Flad HD. The beta-thromboglobulins and platelet factor 4: blood platelet-derived CXC chemokines with divergent roles in early neutrophil regulation. J Leukoc Biol 2000; 67:471-8.

122. Pitchford SC, Momi S, Giannini S, Casali L, Spina D, Page CP, et al. Platelet P-selectin is required for pulmonary eosinophil and lymphocyte recruitment in a murine model of allergic inflammation. Blood 2005; 105:2074-81.

123. Laschke MW, Dold S, Menger MD, Jeppsson B, Thorlacius H. Platelet-dependent accumulation of leukocytes in sinusoids mediates hepatocellular damage in bile duct ligation-induced cholestasis. Br J Pharmacol 2008; 153:148-56.

124. Li Z, Yang F, Dunn S, Gross AK, Smyth SS. Platelets as immune mediators: their role in host defense responses and sepsis. Thromb Res 2011; 127:184-8.

125. Stout RD, Suttles J. The many roles of CD40 in cell-mediated inflammatory responses. Immunol Today 1996; 17:487-92.

126. 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 2003; 9:61-7.

127. Winning J, Reichel J, Eisenhut Y, Hamacher J, Kohl M, Deigner HP, et al. Anti-platelet drugs and outcome in severe infection: clinical impact and underlying mechanisms. Platelets 2009; 20:50-7.

128. Hollenbaugh D, Grosmaire LS, Kullas CD, Chalupny NJ, Braesch-Andersen S, Noelle RJ, et al. The human T cell antigen gp39, a member of the TNF gene family, is a ligand for the CD40 receptor: expression of a soluble form of gp39 with B cell co-stimulatory activity. EMBO J 1992; 11:4313-21.

129. Zirlik A, Maier C, Gerdes N, MacFarlane L, Soosairajah J, Bavendiek U, et al. CD40 ligand mediates inflammation independently of CD40 by interaction with Mac-1. Circulation 2007; 115:1571-80.

130. Grewal IS, Flavell RA. CD40 and CD154 in cell-mediated immunity. Annu Rev Immunol 1998; 16:111-35.

131. Phipps RP. Atherosclerosis: the emerging role of inflammation and the CD40-CD40 ligand system. Proc Natl Acad Sci U S A 2000; 97:6930-2.

132. Fernandez Bello I, Alvarez MT, Lopez-Longo FJ, Arias-Salgado EG, Martin M, Jimenez-Yuste V, et al. Platelet soluble CD40L and matrix metalloproteinase 9 activity are proinflammatory mediators in Behcet disease patients. Thromb Haemost 2012; 107:88-98.

133. Lievens D, Eijgelaar WJ, Biessen EA, Daemen MJ, Lutgens E. The multi-functionality of CD40L and its receptor CD40 in atherosclerosis. Thromb Haemost 2009; 102:206-14.

134. Andre P, Nannizzi-Alaimo L, Prasad SK, Phillips DR. Platelet-derived CD40L: the switch-hitting player of cardiovascular disease. Circulation 2002; 106:896-9.

135. Choi WS, Jeon OH, Kim DS. CD40 ligand shedding is regulated by interaction between matrix metalloproteinase-2 and platelet integrin alpha(IIb)beta(3). J Thromb Haemost 2010; 8:1364-71.

136. Jin Y, Nonoyama S, Morio T, Imai K, Ochs HD, Mizutani S. Characterization of soluble CD40 ligand released from human activated platelets. J Med Dent Sci 2001; 48:23-7.

137. Henn V, Steinbach S, Buchner K, Presek P, Kroczek RA. The inflammatory action of CD40 ligand (CD154) expressed on activated human platelets is temporally limited by coexpressed CD40. Blood 2001; 98:1047-54.

138. Henn V, Slupsky JR, Grafe M, Anagnostopoulos I, Forster R, Muller-Berghaus G, et al. CD40 ligand on activated platelets triggers an inflammatory reaction of endothelial cells. Nature 1998; 391:591-4.

139. Vishnevetsky D, Kiyanista VA, Gandhi PJ. CD40 ligand: a novel target in the fight against cardiovascular disease. Ann Pharmacother 2004; 38:1500-8.

140. Danese S, Sans M, Fiocchi C. The CD40/CD40L costimulatory pathway in inflammatory bowel disease. Gut 2004; 53:1035-43.

141. Berner B, Wolf G, Hummel KM, Muller GA, Reuss-Borst MA. Increased expression of CD40 ligand (CD154) on CD4+ T cells as a marker of disease activity in rheumatoid arthritis. Ann Rheum Dis 2000; 59:190-5.

142. Inwald DP, McDowall A, Peters MJ, Callard RE, Klein NJ. CD40 is constitutively expressed on platelets and provides a novel mechanism for platelet activation. Circ Res 2003; 92:1041-8.

143. Furman MI, Krueger LA, Linden MD, Barnard MR, Frelinger AL, 3rd, Michelson AD. Release of soluble CD40L from platelets is regulated by glycoprotein IIb/IIIa and actin polymerization. J Am Coll Cardiol 2004; 43:2319-25.

144. Ra HJ, Parks WC. Control of matrix metalloproteinase catalytic activity. Matrix Biol 2007; 26:587-96.

145. Stamenkovic I. Extracellular matrix remodelling: the role of matrix metalloproteinases. J Pathol 2003; 200:448-64.

146. Vanlaere I, Libert C. Matrix metalloproteinases as drug targets in infections caused by gram-negative bacteria and in septic shock. Clin Microbiol Rev 2009; 22:224-39, Table of Contents.

147. Mitchell PG, Magna HA, Reeves LM, Lopresti-Morrow LL, Yocum SA, Rosner PJ, et al. Cloning, expression, and type II collagenolytic activity of matrix metalloproteinase-13 from human osteoarthritic cartilage. J Clin Invest 1996; 97:761-8.

148. Churg A, Wang RD, Tai H, Wang X, Xie C, Dai J, et al. Macrophage metalloelastase mediates acute cigarette smoke-induced inflammation via tumor necrosis factor-alpha release. Am J Respir Crit Care Med 2003; 167:1083-9.

149. Albert J, Radomski A, Soop A, Sollevi A, Frostell C, Radomski MW. Differential release of matrix metalloproteinase-9 and nitric oxide following infusion of endotoxin to human volunteers. Acta Anaesthesiol Scand 2003; 47:407-10.

150. Pugin J, Widmer MC, Kossodo S, Liang CM, Preas HLn, Suffredini AF. Human neutrophils secrete gelatinase B in vitro and in vivo in response to endotoxin and proinflammatory mediators. Am J Respir Cell Mol Biol 1999; 20:458-64.

151. Awla D, Abdulla A, Syk I, Jeppsson B, Regner S, Thorlacius H. Neutrophil-derived matrix metalloproteinase-9 is a potent activator of trypsinogen in acinar cells in acute pancreatitis. J Leukoc Biol 2011.

152. Kassim SY, Gharib SA, Mecham BH, Birkland TP, Parks WC, McGuire JK. Individual matrix metalloproteinases control distinct transcriptional responses in airway epithelial cells infected with Pseudomonas aeruginosa. Infect Immun 2007; 75:5640-50.

153. Opdenakker G, Van den Steen PE, Dubois B, Nelissen I, Van Coillie E, Masure S, et al. Gelatinase B functions as regulator and effector in leukocyte biology. J Leukoc Biol 2001; 69:851-9.

154. Woessner JF, Jr. The family of matrix metalloproteinases. Ann N Y Acad Sci 1994; 732:11-21.

155. Sawicki G, Salas E, Murat J, Miszta-Lane H, Radomski MW. Release of gelatinase A during platelet activation mediates aggregation. Nature 1997; 386:616-9.

156. Fernandez-Patron C, Martinez-Cuesta MA, Salas E, Sawicki G, Wozniak M, Radomski MW, et al. Differential regulation of platelet aggregation by matrix metalloproteinases-9 and -2. Thromb Haemost 1999; 82:1730-5.

157. Galt SW, Lindemann S, Allen L, Medd DJ, Falk JM, McIntyre TM, et al. Outside-in signals delivered by matrix metalloproteinase-1 regulate platelet function. Circ Res 2002; 90:1093-9.

158. Murate T, Yamashita K, Isogai C, Suzuki H, Ichihara M, Hatano S, et al. The production of tissue inhibitors of metalloproteinases (TIMPs) in megakaryopoiesis: possible role of platelet- and megakaryocyte-derived TIMPs in bone marrow fibrosis. Br J Haematol 1997; 99:181-9.

159. Radomski A, Jurasz P, Sanders EJ, Overall CM, Bigg HF, Edwards DR, et al. Identification, regulation and role of tissue inhibitor of metalloproteinases-4 (TIMP-4) in human platelets. Br J Pharmacol 2002; 137:1330-8.

160. Chung AW, Radomski A, Alonso-Escolano D, Jurasz P, Stewart MW, Malinski T, et al. Platelet-leukocyte aggregation induced by PAR agonists: regulation by nitric oxide and matrix metalloproteinases. Br J Pharmacol 2004; 143:845-55.

161. Menchen L, Marin-Jimenez I, Arias-Salgado EG, Fontela T, Hernandez-Sampelayo P, Rodriguez MC, et al. Matrix metalloproteinase 9 is involved in Crohn's disease-associated platelet hyperactivation through the release of soluble CD40 ligand. Gut 2009; 58:920-8.

162. Gresele P, Falcinelli E, Loffredo F, Cimmino G, Corazzi T, Forte L, et al. Platelets release matrix metalloproteinase-2 in the coronary circulation of patients with acute coronary syndromes: possible role in sustained platelet activation. Eur Heart J 2011; 32:316-25.

163. Ohashi Y, Kawashima S, Mori T, Terashima M, Ichikawa S, Ejiri J, et al. Soluble CD40 ligand and interleukin-6 in the coronary circulation after acute myocardial infarction. Int J Cardiol 2006; 112:52-8.

164. Milano S, Arcoleo F, D'Agostino P, Cillari E. Intraperitoneal injection of tetracyclines protects mice from lethal endotoxemia downregulating inducible nitric oxide synthase in various organs and cytokine and nitrate secretion in blood. Antimicrob Agents Chemother 1997; 41:117-21.

165. Maitra SR, Bhaduri S, Valane PD, Tervahartiala T, Sorsa T, Ramamurthy N. Inhibition of matrix metalloproteinases by chemically modified tetracyclines in sepsis. Shock 2003; 20:280-5.

166. Solorzano CC, Ksontini R, Pruitt JH, Auffenberg T, Tannahill C, Galardy RE, et al. A matrix metalloproteinase inhibitor prevents processing of tumor necrosis factor alpha (TNF alpha) and abrogates endotoxin-induced lethality. Shock 1997; 7:427-31.

167. Medina C, Jurasz P, Santos-Martinez MJ, Jeong SS, Mitsky T, Chen R,

et al. Platelet aggregation-induced by caco-2 cells: regulation by matrix metalloproteinase-2 and adenosine diphosphate. J Pharmacol Exp Ther 2006; 317:739-45.

168. Rivers E, Nguyen B, Havstad S, Ressler J, Muzzin A, Knoblich B, et al. Early goal-directed therapy in the treatment of severe sepsis and septic shock. N Engl J Med 2001; 345:1368-77.

169. Wesche-Soldato DE, Swan RZ, Chung CS, Ayala A. The apoptotic pathway as a therapeutic target in sepsis. Curr Drug Targets 2007; 8:493-500.

170. Annane D, Bellissant E, Cavaillon JM. Septic shock. Lancet 2005; 365:63-78.

171. Dellinger RP, Levy MM, Carlet JM, Bion J, Parker MM, Jaeschke R, et al. Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock: 2008. Crit Care Med 2008; 36:296-327.

172. Rice TW, Bernard GR. Therapeutic intervention and targets for sepsis. Annu Rev Med 2005; 56:225-48.

173. Deans KJ, Haley M, Natanson C, Eichacker PQ, Minneci PC. Novel therapies for sepsis: a review. J Trauma 2005; 58:867-74.

174. Sprung CL, Cohen J, Eidelman LA. A plea for caution in the performance of sepsis trials. Intensive Care Med 1995; 21:389-90.

175. Deitch EA. Animal models of sepsis and shock: a review and lessons learned. Shock 1998; 9:1-11.

176. Bernard GR, Vincent JL, Laterre PF, LaRosa SP, Dhainaut JF, Lopez-Rodriguez A, et al. Efficacy and safety of recombinant human activated protein C for severe sepsis. N Engl J Med 2001; 344:699-709.

177. Annane D, Sebille V, Charpentier C, Bollaert PE, Francois B, Korach JM, et al. Effect of treatment with low doses of hydrocortisone and fludrocortisone on mortality in patients with septic shock. JAMA 2002; 288:862-71.

178. van den Berghe G, Wouters P, Weekers F, Verwaest C, Bruyninckx F, Schetz M, et al. Intensive insulin therapy in critically ill patients. N Engl J Med 2001; 345:1359-67.

179. Szabo G, Romics L, Jr., Frendl G. Liver in sepsis and systemic inflammatory response syndrome. Clin Liver Dis 2002; 6:1045-66, x.

180. Rittirsch D, Huber-Lang MS, Flierl MA, Ward PA. Immunodesign of experimental sepsis by cecal ligation and puncture. Nat Protoc 2009; 4:31-6.

181. Buras JA, Holzmann B, Sitkovsky M. Animal models of sepsis: setting the stage. Nat Rev Drug Discov 2005; 4:854-65.

182. Knaus WA, Draper EA, Wagner DP, Zimmerman JE. Prognosis in acute organ-system failure. Ann Surg 1985; 202:685-93.

183. Schierwagen C, Bylund-Fellenius AC, Lundberg C. Improved method for quantification of tissue PMN accumulation measured by myeloperoxidase activity. J Pharmacol Methods 1990; 23:179-86.

184. Bradley PP, Priebat DA, Christensen RD, Rothstein G. Measurement of cutaneous inflammation: estimation of neutrophil content with an enzyme marker. J Invest Dermatol 1982; 78:206-9.

185. He P, Zhang H, Zhu L, Jiang Y, Zhou X. Leukocyte-platelet aggregate adhesion and vascular permeability in intact microvessels: role of activated endothelial cells. Am J Physiol Heart Circ Physiol 2006; 291:H591-9.

186. Yoshida K, Kondo R, Wang Q, Doerschuk CM. Neutrophil cytoskeletal rearrangements during capillary sequestration in bacterial pneumonia in rats. Am J Respir Crit Care Med 2006; 174:689-98.

187. Jilma B, Hergovich N, Stohlawetz P, Stummvoll G, Albinni S, Simak S, et al. Effects of sodium nitroprusside on hemodialysis-induced platelet activation. Kidney Int 1999; 55:686-91.

188. Michelson AD, Barnard MR, Krueger LA, Valeri CR, Furman MI. Circulating monocyte-platelet aggregates are a more sensitive marker of in vivo platelet activation than platelet surface P-selectin: studies in baboons, human coronary intervention, and human acute myocardial infarction. Circulation 2001; 104:1533-7.

189. Hamburger SA, McEver RP. GMP-140 mediates adhesion of stimulated platelets to neutrophils. Blood 1990; 75:550-4.

190. Rinder HM, Bonan JL, Rinder CS, Ault KA, Smith BR. Activated and unactivated platelet adhesion to monocytes and neutrophils. Blood 1991; 78:1760-9.

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

192. Weber C, Springer TA. Neutrophil accumulation on activated, surface-adherent platelets in flow is mediated by interaction of Mac-1 with fibrinogen bound to alphaIIbbeta3 and stimulated by platelet-activating factor. J Clin Invest 1997; 100:2085-93.

193. Deuel TF, Senior RM, Chang D, Griffin GL, Heinrikson RL, Kaiser ET. Platelet factor 4 is chemotactic for neutrophils and monocytes. Proc Natl Acad Sci U S A 1981; 78:4584-7.

194. Wiles ME, Welbourn R, Goldman G, Hechtman HB, Shepro D. Thromboxane-induced neutrophil adhesion to pulmonary microvascular and aortic endothelium is regulated by CD18. Inflammation 1991; 15:181-99.

195. Akbar GK, Mills DC, Kunapuli SP. Characterization of extracellular nucleotide-induced Mac-1 (alphaM beta2 integrin) surface expression on peripheral blood leukocytes. Biochem Biophys Res Commun 1997; 233:71-5.

196. Asaduzzaman M, Lavasani S, Rahman M, Zhang S, Braun OO, Jeppsson B, et al. Platelets support pulmonary recruitment of neutrophils in abdominal sepsis. Crit Care Med 2009; 37:1389-96.

197. Ding Y, Chung CS, Newton S, Chen Y, Carlton S, Albina JE, et al. Polymicrobial sepsis induces divergent effects on splenic and peritoneal dendritic cell function in mice. Shock 2004; 22:137-44.

198. Katsiari CG, Liossis SN, Dimopoulos AM, Charalambopoulo DV, Mavrikakis M, Sfikakis PP. CD40L overexpression on T cells and monocytes from patients with systemic lupus erythematosus is resistant to calcineurin inhibition. Lupus 2002; 11:370-8.

199. Grammer AC, Lipsky PE. CD40-mediated regulation of immune responses by TRAF-dependent and TRAF-independent signaling mechanisms. Adv Immunol 2000; 76:61-178.

200. Li G, Sanders JM, Bevard MH, Sun Z, Chumley JW, Galkina EV, et al. CD40 ligand promotes Mac-1 expression, leukocyte recruitment, and neointima formation after vascular injury. Am J Pathol 2008; 172:1141-52.

201. Tekamp-Olson P, Gallegos C, Bauer D, McClain J, Sherry B, Fabre M, et al. Cloning and characterization of cDNAs for murine macrophage inflammatory protein 2 and its human homologues. J Exp Med 1990; 172:911-9.

202. Kopydlowski KM, Salkowski CA, Cody MJ, van Rooijen N, Major J, Hamilton TA, et al. Regulation of macrophage chemokine expression by lipopolysaccharide in vitro and in vivo. J Immunol 1999; 163:1537-44.

203. Liu Q, Wang Y, Thorlacius H. Dexamethasone inhibits tumor necrosis factor-alpha-induced expression of macrophage inflammatory protein-2 and adhesion of neutrophils to endothelial cells. Biochem Biophys Res Commun 2000; 271:364-7.

204. Rahman M, Zhang S, Chew M, Ersson A, Jeppsson B, Thorlacius H. Platelet-derived CD40L (CD154) mediates neutrophil upregulation of Mac-1 and recruitment in septic lung injury. Ann Surg 2009; 250:783-90.

205. Inwald DP, Faust SN, Lister P, Peters MJ, Levin M, Heyderman R, et al. Platelet and soluble CD40L in meningococcal sepsis. Intensive Care Med 2006; 32:1432-7.

206. Frossard JL, Morel P, Kwak B, Pastor C, Berney T, Buhler L, et al. Soluble CD40 ligand in prediction of acute severe pancreatitis. World J Gastroenterol 2006; 12:1613-6.

207. Ludwiczek O, Kaser A, Tilg H. Plasma levels of soluble CD40 ligand are elevated in inflammatory bowel diseases. Int J Colorectal Dis 2003; 18:142-7.

208. Nolan A, Weiden M, Kelly A, Hoshino Y, Hoshino S, Mehta N, et al. CD40 and CD80/86 act synergistically to regulate inflammation and mortality in polymicrobial sepsis. Am J Respir Crit Care Med 2008; 177:301-8.

209. Cena JJ, Lalu MM, Cho WJ, Chow AK, Bagdan ML, Daniel EE, et al. Inhibition of matrix metalloproteinase activity in vivo protects against vascular hyporeactivity in endotoxemia. Am J Physiol Heart Circ Physiol 2009; 298:H45-51.

210. Steinberg J, Halter J, Schiller HJ, Dasilva M, Landas S, Gatto LA, et al. Metalloproteinase inhibition reduces lung injury and improves survival after cecal ligation and puncture in rats. J Surg Res 2003; 111:185-95.

211. Downey GP, Worthen GS, Henson PM, Hyde DM. Neutrophil sequestration and migration in localized pulmonary inflammation. Capillary localization and migration across the interalveolar septum. Am Rev Respir Dis 1993; 147:168-76.

212. Nolte D, Kuebler WM, Muller WA, Wolff KD, Messmer K. Attenuation of leukocyte sequestration by selective blockade of PECAM-1 or VCAM-1 in murine endotoxemia. Eur Surg Res 2004; 36:331-7.

213. Sikora L, Johansson AC, Rao SP, Hughes GK, Broide DH, Sriramarao P. A murine model to study leukocyte rolling and intravascular trafficking in lung microvessels. Am J Pathol 2003; 162:2019-28.

214. Schramm R, Thorlacius H. Staphylococcal enterotoxin B-induced acute inflammation is inhibited by dexamethasone: important role of CXC chemokines KC and macrophage inflammatory protein 2. Infect Immun 2003; 71:2542-7.

215. Nakamura T, Ebihara I, Shimada N, Shoji H, Koide H. Modulation of plasma metalloproteinase-9 concentrations and peripheral blood monocyte mRNA levels in patients with septic shock: effect of fiber-immobilized polymyxin B treatment. Am J Med Sci 1998; 316:355-60.

216. Yassen KA, Galley HF, Webster NR. Matrix metalloproteinase-9 concentrations in critically ill patients. Anaesthesia 2001; 56:729-32.

217. Stefanidakis M, Koivunen E. Cell-surface association between matrix metalloproteinases and integrins: role of the complexes in leukocyte migration and cancer progression. Blood 2006; 108:1441-50.

218. Van Lint P, Libert C. Chemokine and cytokine processing by matrix metalloproteinases and its effect on

leukocyte migration and inflammation. J Leukoc Biol 2007; 82:1375-81.

219. Delclaux C, Delacourt C, D'Ortho MP, Boyer V, Lafuma C, Harf A. Role of gelatinase B and elastase in human polymorphonuclear neutrophil migration across basement membrane. Am J Respir Cell Mol Biol 1996; 14:288-95.

220. Torrence AE, Brabb T, Viney JL, Bielefeldt-Ohmann H, Treuting P, Seamons A, et al. Serum biomarkers in a mouse model of bacterial-induced inflammatory bowel disease. Inflamm Bowel Dis 2008; 14:480-90.

221. Teng L, Yu M, Li JM, Tang H, Yu J, Mo LH, et al. Matrix metalloproteinase-9 as new biomarkers of severity in multiple organ dysfunction syndrome caused by trauma and infection. Mol Cell Biochem 2012; 360:271-7.

222. Reinboldt S, Wenzel F, Rauch BH, Hohlfeld T, Grandoch M, Fischer JW, et al. Preliminary evidence for a matrix metalloproteinase-2 (MMP-2)-dependent shedding of soluble CD40 ligand (sCD40L) from activated platelets. Platelets 2009; 20:441-4.

223. Schonbeck U, Mach F, Sukhova GK, Murphy C, Bonnefoy JY, Fabunmi RP, et al. Regulation of matrix metalloproteinase expression in human vascular smooth muscle cells by T lymphocytes: a role for CD40 signaling in plaque rupture? Circ Res 1997; 81:448-54.

224. Chai H, Aghaie K, Zhou W. Soluble CD40 ligand induces human coronary artery smooth muscle cells proliferation and migration. Surgery 2009; 146:5-11.

225. Smola-Hess S, Schnitzler R, Hadaschik D, Smola H, Mauch C, Krieg T, et al. CD40L induces matrix-metalloproteinase-9 but not tissue inhibitor of metalloproteinases-1 in cervical carcinoma cells: imbalance between NF-kappaB and STAT3 activation. Exp Cell Res 2001; 267:205-15.

226. Czermak BJ, Breckwoldt M, Ravage ZB, Huber-Lang M, Schmal H, Bless NM, et al. Mechanisms of enhanced lung injury during sepsis. Am J Pathol 1999; 154:1057-65.

227. Renckens R, Roelofs JJ, Florquin S, de Vos AF, Lijnen HR, van't Veer C, et al. Matrix metalloproteinase-9 deficiency impairs host defense against abdominal sepsis. J Immunol 2006; 176:3735-41.

228. Malik M, Bakshi CS, McCabe K, Catlett SV, Shah A, Singh R, et al. Matrix metalloproteinase 9 activity enhances host susceptibility to pulmonary infection with type A and B strains of Francisella tularensis. J Immunol 2007; 178:1013-20.

229. Lee MM, Yoon BJ, Osiewicz K, Preston M, Bundy B, van Heeckeren AM, et al. Tissue inhibitor of metalloproteinase 1 regulates resistance to infection. Infect Immun 2005; 73:661-5.

230. McClellan SA, Huang X, Barrett RP, Lighvani S, Zhang Y, Richiert D, et al. Matrix metalloproteinase-9 amplifies the immune response to Pseudomonas aeruginosa corneal infection. Invest Ophthalmol Vis Sci 2006; 47:256-64.

231. Klintman D, Li X, Sato T, Wang Y, Jeppsson B, Thorlacius H. Staphylococcal enterotoxin A-induced hepatotoxicity is predominantly mediated by Fas ligand (CD95L). Ann Surg 2004; 240:1065-72; discussion 1072-3.

232. Remick DG, Newcomb DE, Bolgos GL, Call DR. Comparison of the mortality and inflammatory response of two models of sepsis: lipopolysaccharide vs. cecal ligation and puncture. Shock 2000; 13:110-6.