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Phagocytic cell functions in sepsis – role and potential therapies of sepsis-induced immunosuppression

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PHAGOCYTIC CELL FUNCTIONS IN SEPSIS – ROLE AND POTENTIAL THERAPIES OF SEPSIS-INDUCED IMMUNOSUPPRESSION

by

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PHAGOCYTIC CELL FUNCTIONS IN SEPSIS – ROLE AND POTENTIAL THERAPIES OF SEPSIS-INDUCED IMMUNOSUPPRESSON

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ABSTRACT

Globally, sepsis is one of the largest causes of death in acute care units. Even with adequate treatment, severe sepsis affects over 49 million people worldwide and is responsible for approximately 11 million deaths each year. People respond differently to infection, which is evidence that individuals have varying degrees of immune suppression. As a result of a pathogen triggering the Systemic Inflammatory Syndrome (SIRS), sepsis is an immunological condition that is diagnosed based on patterns of temperature, heart rate, respiration, and white blood cell (WBC) count.

Although the pathogenesis of sepsis remains unclear, there is growing evidence that oxidants and antioxidants play a major role. Oxidative stress is defined by elevated intracellular levels of reactive oxygen species (ROS) that cause damage to lipids, proteins, and DNA. Sepsis pathophysiology includes infection by gram negative or positive bacteria, an elevated inflammatory reaction; lower blood pressure that may result in vasodilatory shock; and reduction of oxygen delivery to organs as a response to cell dysfunction. It has been demonstrated that free radicals such as nitric oxide (NO), peroxynitrite (ONOO-), and hydrogen peroxide are involved in these effects. Specifically, it has been demonstrated that hydroxyl radicals play a role.

An extensive network of complicated phagocytic mechanisms is implicated in the development of sepsis. As a result, phagocytosis plays an important function in sepsis

and presumably contributes to most clinical phases of the disease. Only a few studies, however, have particularly investigated and defined the effects of immunosuppression on phagocytic activity during sepsis because of the infection. In this paper, I explain the immune response's phagocytic mechanisms that occurs prior to the development of sepsis and identify the features of phagocytosis that may act as a predictive marker for the outcome of the disease. To begin with, I describe some of the most important characteristics of phagocytic processes such as phagocytic main receptors and signaling characteristics. After that, I address the role of phagocytic cell functions during sepsis-induced immunosuppression, as well as some of the research that's been done on the subject recently. Finally, I discuss some of the potential immunosuppressive therapies for septic patients.

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LIST OF ABBREVIATIONS

APC……..….....Antigen Presenting Cell

ATP…….....Adenosine Triphosphate

CARS…….….....Compensatory Anti-inflammatory Response Syndrome

CLP…….....Cecal Ligation and Puncture

DC…….....Dendritic Cell

EPIC II……..... European Prevalence of Infection in Intensive Care

GM-CSF…….....Granulocyte Macrophage Stimulating Factor

GSH…….…….....Glutathione

ICU…….....Intensive Care Unit

IL-1…….....Interleukin-1

IL-10…….....Interleukin-10

IL-4…….....Interleukin-4

IL-6…….....Interleukin-6

IL-7…….....Interleukin-7

IL-8…….....Interleukin-8

LPS…….....Lipopolysaccharides

MHC-II…….....Major Histocompatibility Complex Molecules Class 2

MODS…….....Multiple Organ Dysfunction Syndrome

NO…….....Nitric Oxide

NOX…….....NADPH Oxidase

ONOO-…….…….....Peroxynitrite

PAMPS…….....Pathogen-Associated Molecular Patterns

PIRO……...Predisposition, Infection, Response, and Organ Failure

PMN…….....Polymorphonuclear Leukocytes

PRR…….....Pattern Recognition Receptor

qSOFA…….....Quick SOFA

RNS…….....Reactive Nitrogen Species

ROS…….....Reactive Oxygen Species

SIRS……..... Systemic Inflammatory Response Syndrome

SOFA…….....Sequential Organ Failure Assessment Score

TLR-4…….....Toll Like Receptor 4

TNF…….....Tumor Necrosis Factor

WBC…….....White blood cell

CHAPTER ONE: GENERAL INTRODUCTION

1.1: Introduction

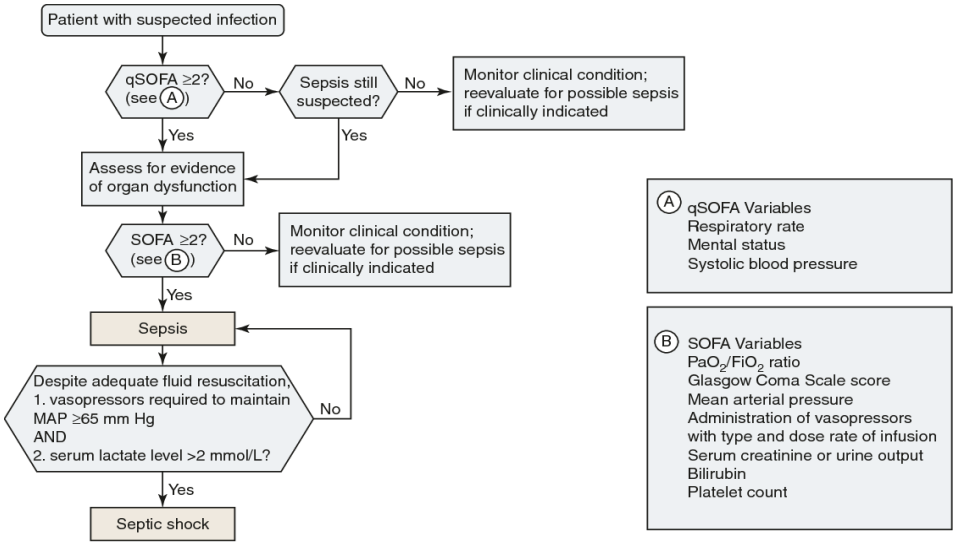
Sepsis is a "life-threatening organ dysfunction caused by dysregulated host response to infection." [1] People of all ages are susceptible to sepsis, which is the greatest cause of death and serious illness among those hospitalized at the intensive care units (ICU). It is also the tenth most common cause of mortality in the United States, according to the Centers for Disease Control and Prevention. [2] Prevalence of sepsis is expected to rise by 1.5 percent per year and could reach more than 1,110,000 cases. [2] Many people who survive sepsis experience a decrease in their quality of life as a result of the illness. According to Namas et al, Sepsis, in itself, is a Greek word thought to have originated more than 2700 years ago and is defined as the breakdown of organic materials when bacteria is present in plants or animals.

Sepsis is a medical condition that is not only difficult to diagnose but even more difficult to treat. Today, it is classified as a Systemic inflammatory response syndrome (SIRS) rather than a disease. [3] In contrast to a typical infection, the term Sepsis is used to describe patients who are extremely ill, have some degree of organ dysfunction, and who must be hospitalized in the ICU for close observation. [3] Sepsis diagnosis is made by ruling out the possibility of other conditions presenting themselves in a similar manner by following the universal clinical criteria for identifying patients with Sepsis and Septic shock. (Table 1.) Diagnosis is made based on several discoveries, including general uneasiness, which can show up in several different ways, including high body temperature, trouble breathing, lower blood pressure, and a high heart rate. Some patients

show signs of confusion and obtundation, dyschromia, and prolonged capillary refill time. High lactate and abnormal arterial blood gases are common laboratory findings, as are defective kidney and liver function tests. In addition, the levels of C-reactive protein and procalcitonin in the blood are typically increased, and while they can aid diagnose the condition, they are not tailored to the condition. [4]

Table 1. Operationalization of Clinical Criteria Identifying Patients with Sepsis and Septic Shock

Adapted from Singer et al, 2016



A score for the Sequential Organ Failure Assessment (SOFA) was detailed by Singer et al 2016 in accordance with the standard set at the Third International Consensus Definitions for Sepsis and Septic Shock, which entailed parameters used to assess the respiratory system, coagulation, hepatic system, cardiovascular system, central nervous system, and the renal system. (Table 2.) In addition, a newly described measure, qSOFA

(quick SOFA), was developed that concentrated on altered mentation, breaths of 22 minutes or greater, and systolic blood pressure of 100 Hg or less. [1] It is possible to identify adult individuals with probable infection who could mostly be associated with poor results closely related to sepsis if they meet at least two of the diagnostic criteria listed in the quick SOFA. [1], [4] It is also possible to demonstrate organ dysfunction by a two point or greater increase in the SOFA score, which correlates to a 10% or greater mortality in hospitals. [4]

Table 2: **Sequential [Sepsis-Related] Organ Failure Assessment Score[a]**

Adapted from Singer et al, 2016 [1]

Table 1. Sequential [Sepsis-Related] Organ Failure Assessment Score[a]

	Score				
System	0	1	2	3	4
Respiration					
Pao_2/Fio_2, mm Hg (kPa)	≥400 (53.3)	<400 (53.3)	<300 (40)	<200 (26.7) with respiratory support	<100 (13.3) with respiratory support
Coagulation					
Platelets, $\times 10^3/\mu L$	≥150	<150	<100	<50	<20
Liver					
Bilirubin, mg/dL (μmol/L)	<1.2 (20)	1.2-1.9 (20-32)	2.0-5.9 (33-101)	6.0-11.9 (102-204)	>12.0 (204)
Cardiovascular	MAP ≥70 mm Hg	MAP <70 mm Hg	Dopamine <5 or dobutamine (any dose)[b]	Dopamine 5.1-15 or epinephrine ≤0.1 or norepinephrine ≤0.1[b]	Dopamine >15 or epinephrine >0.1 or norepinephrine >0.1[b]
Central nervous system					
Glasgow Coma Scale score[c]	15	13-14	10-12	6-9	<6
Renal					
Creatinine, mg/dL (μmol/L)	<1.2 (110)	1.2-1.9 (110-170)	2.0-3.4 (171-299)	3.5-4.9 (300-440)	>5.0 (440)
Urine output, mL/d				<500	<200

Abbreviations: Fio_2, fraction of inspired oxygen; MAP, mean arterial pressure; Pao_2, partial pressure of oxygen.

[a] Adapted from Vincent et al.[27]

[b] Catecholamine doses are given as μg/kg/min for at least 1 hour.

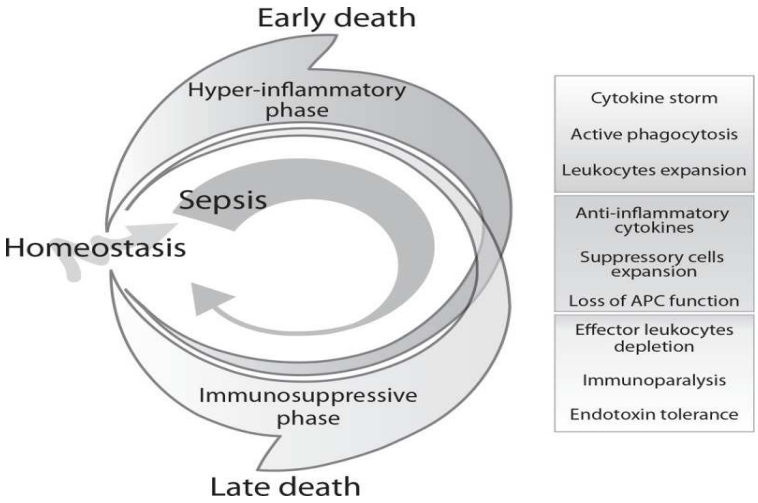
[c] Glasgow Coma Scale scores range from 3-15; higher score indicates better neurological function.

Patients suffering from sepsis have an increased inflammatory response to a microbial infection, which may be caused by a lack of the ability to phagocytize the bacteria and get rid of the insult in the body. When it comes to sepsis, the existence of an early high inflammation phase followed by an immunosuppressive phase distinguishes it. [5] According to Kohoutkova 2020 al et, an extensive range of cytokines, chemotactic

mediators, and other effector molecules are produced during the initial phase, and these molecules have an impact on phagocytic events as well as the signaling events that follow. In the later immunosuppressive phase, reduced phagocyte activity, restructuring of monocytes, diminished functioning of APCs linked with lowered MHC-II presentation, increased myeloid-driven suppressor cells, and reduction of effector cells are all immune response features. (Figure 1.)

Figure 1. Dynamic Changes in the immune system during sepsis progression.

Adapted from Kohoutkova et al ,2020 [5]



Phagocytes are highly effective defense system from invading microorganisms that pose a threat to our lives. The ability of activated polymorphonuclear leukocytes to generate and release large amounts of reactive oxygen species is a distinguishing characteristic of these cell types. [6] A great deal has been written about the critical role played by these molecules in the killing of microorganisms and the subsequent contributions they make to tissue damage during injury inflammation. According to

Prauchner, 2017, activated phagocytic cells are directly exposed to their own toxic metabolites during activation and the potential damage that could occur as a result of the excessive radical formation could have a significant impact on the vital functions of the cells. This, therefore, illustrates oxidative stress plays a role in both direct and indirect effects on phagocyte functions during sepsis.

1.2: Septic Shock

A greater death rate is related with septic shock, a kind of sepsis that results from severe abnormalities in circulation or cellular metabolism. [4] Common signs of septic shock include continuous low blood pressure that is characterized by the necessity for vasopressor treatment to sustain arterial blood pressure of 65mm HG and lactate levels of the serum greater than 18 mg/dL irrespective of the volumes of resuscitation. [4]. Septic shock is characterized by a high level of oxidative stress in several ways. Toxic effects of ROS include the promotion of oxidative degradation of lipids, inhibiting respiratory chain enzymes in the mitochondria, silencing of glyceraldehyde-3-phosphate dehydrogenase, suppression of sodium-potassium ATP-ase activity, sodium channel down-regulation, and modification of protein processes that are not oxidative.[7] Furthermore, ROS are powerful inducers of breaking DNA strands, which leads to the initiation of the poly-ADP enzyme ribosyl synthase and, as a result, the depletion of the cells' energy reserves. [7]

A higher incidence of septic shock is observed in neonates, older people, and pregnant women. [4] Currently, there is no definitive explanation for the pathogenesis of

septic shock. According to Forrester, 2021, the production of inflammatory mediators including the tumor necrosis factor (TNF), interlukin-1 (IL-1), as well as other cytokines are triggered by an inflammatory stimulus such as bacteria. The cytokines promote the attachment of neutrophils to endothelial cells, the activation of the coagulation process, and induce the formation of microthrombi in the body. There are numerous other mediators that are released including leukotrienes, lipoxygenase, histamine, bradykinin, serotonin, and interlukin-2 (IL-2.) [8] As a result of anti-inflammatory mediators such as interlukin-4 (IL-4) and interlukin-10 (IL-10) working against them, a negative feedback mechanism is created. The arteries and arterioles widen initially reducing the resistance of the arteries leading to "warm shock." [4] In the following hours, cardiac output decreases, hypotension starts, and the classic signs and symptoms of shock manifest themselves. The presence of vasoactive mediators causes the blood to flow around the capillary exchange vessels even when the heart rate is elevated. Poor capillary flow as a result of this shift, combined with obstruction of the capillaries caused by microthrombi, reduces oxygen distribution and affects carbon dioxide and waste products from being removed from the bloodstream. [9] Reduced perfusion results in the dysfunction and, in some cases, some key organs such as kidneys, lungs, liver, brain, and heart, among other organ systems fail due to oxidative stress.

1.3 Epidemiology of sepsis

Sepsis is a life-threatening illness that affects nearly one million people in the United States every year, with the number of cases increasing year after year. Over a two-

decade period, an increase of approximately 8.7 percent in the occurrence of sepsis in patients in the hospitals in the United States has been observed. Additionally, according to Paoli, Reynolds, Sinha, Gitlin, and Crouser (2018), sepsis is responsible for a significant portion of all hospital deaths, and mortality increases in direct proportion to the stage of sepsis a patient is in. Sepsis has a rate of death of approximately 10–20 percent, severe sepsis has a mortality rate of 20–40 percent, and septic shock has a death rate of 40–80 percent. [10] The total costs for treating patients suffering from Sepsis nationally is approximately $16.7 billion and this cost might increase "due to the continued aging of the population, increasing burden of chronic health conditions, and increased use of immunosuppressive therapy, transplantation, chemotherapy, and invasive procedures." [10, 11]

Gram-positive infections have become virtually as frequent as gram-negative infections as a result of a gradual increase in their incidence over time. [12,13] This is most likely as a result of the increased use of invasive operations performed as well as an increase in the percentage of opportunistic infections acquired in hospitals. [14] Because of the increased usage of broad-spectrum antibiotics in sick patients in the intensive care units, it is probable that bacterial resistance will increase in the coming years. [15]

Table 3: Types of organisms in culture-positive infected patients and associated risk of hospital mortality

Adapted from Mayr, Yende, &Angus, 2013 [10]

Bacteria	Frequency	OR (95% CI)
Gram-positive	46.8	
Staphylococcus aureus	20.5	0.8 (0.6-1.1)
MRSA	10.2	1.3(0.9-1.8)
Enterococcus	10.9	1.6(1.1-2.3)
S. epidermidis	10.8	0.9(0.7-1.1)
S. pneumoniae	4.1	0.8(0.5-1.4)
Other	6.4	0.9 (0.7-1.2)
Gram-negative	62.2	
Pseudomonas species	19.9	1.4(1.2-1.6)
Escherichia coli	16	0.9(0.7-1.1)
Klebsiella species	12.7	1.0 (0.8-1.2)
Acinetobacter species	8.8	1.5(1.2-2.0)
Enterobacter	7.0	1.2(0.9-1.6)
Other	17	0.9(0.7-1.3)
Anaerobes	4.5	0.9(0.7-1.3)
Fungi	17.0	1.1(0.9-1.3)

According to Mayr, Yende & Angus, 2013, organisms that cause severe sepsis are a significant factor in determining the outcome. In spite of the fact that current studies have indicated an increase in occurrence of gram- positive pathogens, the most recent European Prevalence of Infection in Intensive Care (EPIC II) examination found a higher frequency of gram-negative organisms in the study population (62.2 percent vs 46.8 percent.) [10] According to table 3, it was found that there were patterns of similarity in infecting organisms when compared to previous studies, with the most common organisms being Staphylococcus aureus (20.5 percent), Pseudomonas species (19.9 percent), Enterobacteriacae (E. coli, 16.0 percent), and fungi (19 percent). The Enterococcus, Pseudomonas, and Acinetobacter species were the only organisms identified as being related to deaths in hospitals. [10]

2: Pathophysiology of Sepsis

According to recent works, sepsis is caused by a traumatic or infectious event which leads to an abnormal inflammatory response**.** Early research on this approach was carried out with the endotoxin lipopolysaccharide (LPS), which is a component of the cell wall of gram-negative bacteria. [16] To recognize pathogen-associated molecular patterns (PAMPs), Toll like receptor -4, a Pattern Recognition Receptor (PRR), which is found on mammalian cells, mostly on macrophages, binds to LPS, a pathogen-associated molecular pattern (PAMP.) Following the activation of TLR-4, pro-inflammatory cytokines and chemokines are released into the environment, which initiates an inflammatory response and recruits additional immune cells, such as neutrophils (PMNs),

to help eradicate the infection. [17] A disproportionate amount of PAMP/PRR signaling, on the other hand, may be harmful to the host.

2:1 Role of cytokines

Many white blood cells, including macrophages, release a group of mediators. TNF-a, and interleukins are all common cytokines in sepsis, as is the production of cytokines by the immune system. Normally, an anti-inflammatory response, which includes the release of anti-inflammatory mediators, counterbalances the inflammatory response. While sepsis and severe acute respiratory syndrome (SARS) have traditionally been thought to be caused by an overabundance of inflammatory mediators, anti-inflammatory mediators may also play a significant role in the outcome of certain patients. CARS (compensated anti-inflammatory response syndrome) is a new syndrome that has been identified and described. CARS patients may experience immune system paralysis as a result of an imbalance in their helper T cell ratios. [18] Endotoxin is released by bacteria when they are lysed by white blood cells (WBCs) in a gram-negative infection. Endotoxin is a lipopolysaccharide component of the bacteria's cell wall. In response to endotoxin, the release of a variety of inflammatory mediators (Table 4) is induced, which serves to activate the host's cellular defenses. [18]

Table 4: Inflammatory and Anti-inflammatory Mediators.

Adapted from (Jacobi, 2002) [18]

Pro-inflammatory	Anti-inflammatory
TNF-α	IL-1Ra
IL1β, IL-2, IL-6, IL-8, IL-15	IL-4
Neutrophil elastase	IL-10
IFN-γ	IL-13
Thromboxane	Type II IL-1 receptor
Platelet-activating factor	Transforming growth factor-β
Vasoactive neuropeptides	Epinephrine
Phospholipase A_2	Soluble TNF-α receptors
Plasminogen activator inhibitor-1	Leukotriene B_4-receptor antagonist
Prostaglandins	LPS binding protein
Prostacyclin	Soluble recombinant CD-14
Free radical generation	
Soluble adhesion molecules	
Tyrosine kinase	
Protein kinase	
H_2S	
NO	
HMGB1 protein	

In patients with sepsis, pro-inflammatory cytokines [interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-18 (IL-18), and tumor necrosis factor-alpha (TNF-alpha)] and anti-inflammatory cytokines (IL-10) increased in response to pathogen infection. The researchers also discovered that a decrease in the production of IL-6 was associated with a better prognosis, and that overproduction of IL-10 was found to be the most significant predictor of severity and fatal outcome. [19] Pro-inflammatory cytokines, such as interleukin-1, interleukin-6, and TNF-a, act as endogenous pyrogens, up-regulate the synthesis of secondary mediators and other pro-inflammatory cytokines by both macrophages and mesenchymal cells, such as fibroblasts, epithelial and endothelial cells, and stimulate the production of acute phase proteins, or attract inflammatory cells to the site of inflammation. Patients with sepsis who die later than

expected may have suffered from host immunosuppression. [20, 21] The apoptosis of circulating and tissue lymphocytes such as B-cells, CD4+ T-cells, and dendritic cells (DCs) has been shown to cause immunosuppression, according to research. [22]

2:2 Coagulation Dysregulation

The coagulation system and the cells that control the coagulation system undergo considerable changes as a result of the inflammatory process during sepsis. Pathogen-associated molecular patterns (PAMP), such as lipopolysaccharide (LPS) and exotoxins, activate coagulation, causing microvascular thrombosis, hypoperfusion, and finally Multiple Organ Dysfunction Syndrome (MODS), which leads to mortality. Platelets, monocytes, and macrophages are thought to play a role in the coagulation cascade by expressing tissue factor (TF) and by producing TF-expressing microparticles. Damage to the vascular endothelial cells causes leakage and disseminated intravascular coagulation (DIC.) In septic patients, DIC with platelet consumption and prolonged clotting times is a common occurrence. [11,23,24]

Blood loss occurs only in certain areas, despite the fact that coagulopathy is systemic and broad. With regard to the clotting mechanism and white blood cells and endothelial cells in general, coagulopathy is complex. A diminished supply of oxygen-rich blood to important organs is seen in patients suffering from Virchow's triad of irregular blood flow, coagulation abnormalities, and endothelial damage. Endothelial cell anticoagulant components are decreased in sepsis. Antithrombin, protein C, and the tissue factor pathway inhibitor are all reduced in the blood. When they are reduced, they have a bad outlook. [11,23]

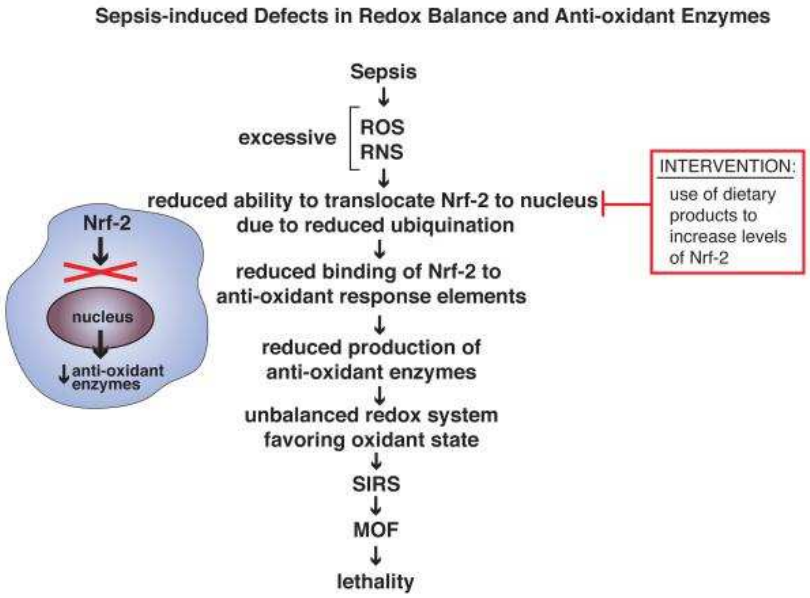
2:3 Hyperinflammatory Response

Sepsis appears to intensify SIRS and its subsequent events as a result of biochemical changes disrupting the redox system, which leads to the formation of an oxidant state. [25] To compensate for this imbalance, the body produces less antioxidants like glutathione (GSH) and thioredoxins (which produce GSH), as well as less mitochondrial ATP and oxidized lipids, all of which contribute to an overly reactive state.

As illustrated in figure 2, a lack of redox balance and antioxidant enzymes in the body is caused by sepsis. Reduced Nrf2 ubiquitination and nuclear translocation leads to decreased activation of genes that encode antioxidant enzymes in sepsis as a result of increased levels of ROS and RNS. [26]. The SIRS response is heightened as a result of this imbalance, and the resulting MOF and lethality are both enhanced. Studies in CLP mice suggest that restoring the redox balance with inducers of Nrf2 may lessen the severity of SIRS and its downstream events. Sulforaphane, a dietary product, may be able to reverse the redox imbalance in sepsis by inducing Nrf2. [27] Overall, it appears that activation of PARP (poly-ADP ribose polymerase) compromises homeostasis by significantly depleting mitochondrial ATP in inflammatory responses to sepsis and ischemia-reperfusion. Endothelial cells are more susceptible to oxidative stress when PARP is blocked.

Figure 2: Sepsis- Induced Defects in Redox Balance and Antioxidant Enzymes

Adapted from Bosmann, & Ward, 2014 [26]



2:4 Cellular Impairment

Cellular dysfunction occurs in sepsis and can be characterized as either overactivation or underactivity. It is possible for cells to be activated excessively, which means they will respond to a second stimulus in a disproportionately strong manner. Neutrophils are an example of a cell that is overactivated, causing damage to other cells in the vicinity. A neutrophil's inability to phagocytize and remove foreign pathogens is an example of a dysfunctional cell. [11]

Severe sepsis necessitates the involvement of lymphocytes, which play an increasingly important role in the immune response. [11] It has been shown that sepsis

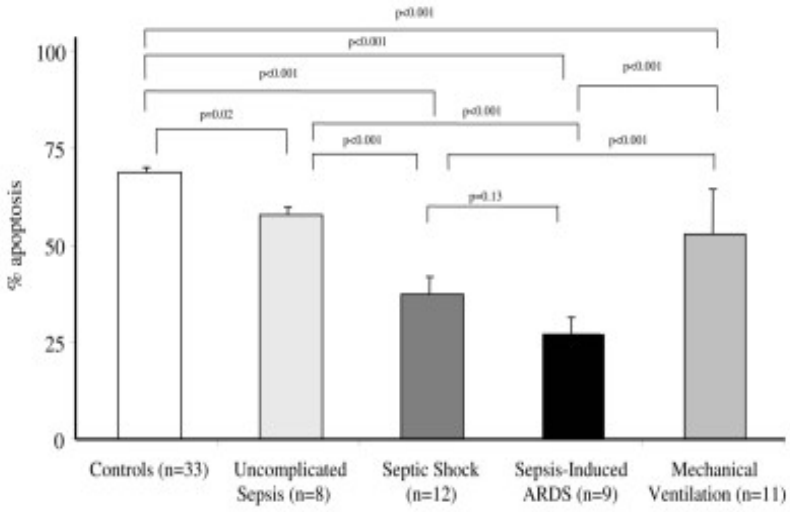
affects immune function by causing widespread apoptosis of lymphocytes in organs such as the thymus, the liver, and the spleen. [28] Apoptosis may be responsible for the decreased lymphocyte function observed in septic patients previously as a result of the occurrence of both apoptotic and necrotic cell death. [11]

Additionally, neutrophil recruitment in some septic patients shows am excessive response while others have a blunted response. Studies have shown that neutrophil apoptosis is linked to the "severity and/or outcome of the disease." [28] In a study done by Fialkow et al 2006, medical patients with uncomplicated sepsis, shock, and ARDS had significantly lower levels of apoptotic death in their neutrophils than did healthy controls. In addition, as the severity of sepsis increased, there was a noticeable decrease in neutrophil apoptosis. (Figure 3) [29]

Neutrophils from Septic patients exhibit decreased chemotaxis towards IL-8 as well as lower CXCR2 expression. They also display higher levels of surface integrins, which aid in the formation of solid adhesions with endothelial cells. This results in neutrophils remaining more firmly attached to the endothelial cells hence failing to migrate correctly into the site of bacterial infection. [11]

Figure 3: Mean percentage of neutrophil apoptosis in medical patients

Adapted from Fialkow, et al, 2006 [29]



3:1 Treatment of Sepsis

Treatment for sepsis currently includes antibiotics, eradication of infectious sources, aggressive fluid resuscitation, vasopressors and other procedures to keep stabilized hemodynamics. [30]. Although 28-day mortality has been reduced, it is not clear how this is accomplished [31]. In-hospital mortality increased by 7.6 percent for every hour that passed between the onset of hypotension and the administration of antibiotics [32], highlighting the significance of bacterial clearance. Complications such as acute respiratory failure, acute renal failure, shock, coagulopathy, and multiple organ failure frequently occur. [33] Tumor necrosis factor (TNF) and anti-interleukin-1 therapy were developed based on the hypothesis that excessive inflammation is a cause of death

(IL-1). Despite promising results in animals, these therapies failed to improve human outcomes. [11]

After early goal-directed therapy has been started, lung-protective ventilation should be considered. [31] Respiratory ventilation, or using low lung volumes to secure the lungs, is an essential part of managing sepsis when it occurs. In addition, lung-protective ventilation reduces mortality and improves outcomes in septic acute lung injury. [34] The risk of lengthy ventilation and nosocomial pneumonia necessitates acceptable but not extreme sedation for patients undergoing ventilation. [35]

Until a patient's immune status can be determined, it is imperative that cultures be collected as well as intravenous broad-spectrum antibiotics be administered as soon as possible in cases of sepsis. Fungi, gram-positive bacteria, highly resistant gram-negative bacteria, methicillin-resistant Staphylococcus aureus, vancomycin resistant enterococcus, and penicillin resistant pneumococcus, as well as regional patterns of antibiotic susceptibility should all be considered when selecting antibiotics. Septic shock and sepsis are more severe if the bacteria responsible are resistant to the initial antibiotic regimen. [31]

Patients with hyperglycemia and who are critically ill suffer from insulin insensitivity. In a clinical trial by Berghe., et al, 2001, intensive insulin therapy was found to reduce mortality in critically ill patients. [11] Although current guidelines for patients in the ER's trauma unit recommend that they maintain strict glycemic control, there are concerns with hypoglycemia and the costs of constant monitoring. [11,36]

Another possible treatment of sepsis is the recombinant activated protein C (APC.) Clinical trials of APC (an inhibitor of coagulation), however, experienced failure. [37] The treatment of sepsis is likely to be complicated by the existence of heterogeneity within and between the various classifications. There is no consideration for variables such as the pathogen, comorbidities, or patient demographics when determining the severity of the illness. Patients have been stratified based on their mortality risk using PIRO (predisposing factors, infection, response, and organ failure) as a means of addressing this issue. [38] Because there is still a lot of questions as to why patients die from sepsis, more research is needed to find out what causes it, which is further complicating treatment.

4:1 Phagocyte Mechanisms in Sepsis

Neutrophils and monocyte-macrophages are examples of phagocytes, which are cells specialized for microbial breakdown. These cells have been given the name "phagocytes" because they are capable of ingesting large particles. Phagocytosis, the process by which bacteria are ingested or integrated into the phagocyte within a cytoplasmic vesicle known as the phagocytic vesicle, is used to destroy bacteria. There are many types of phagocytes, and all of them have the ability to kill microorganisms. Pathogens are killed by the phagocytes in a variety of ways. As well as destroying microorganisms, neutrophils and macrophages also remove dead tissue and play a role in tissue repair when dealing with bacterial and fungal infections. [39]

In the process of phagocytosis, there are multiple stages: (i) the identification of the particle to be ingested, (ii) the initiation of the internalization process, (iii) the development of a specialized vacuole known as a phagosome, and (iv) the maturation of the phagosome membrane. [40] Phagocytes are capable of identifying a wide range of particles that could potentially be consumed, including apoptotic cells and pathogenic bacteria. Discrete receptors are responsible for mediating this recognition by identifying the particle as a target and then activating signaling pathways that promote phagocytosis. The receptors on the plasma membrane of phagocytes are classified as either non-opsonic or opsonic receptors. Non-opsonic receptors recognize various molecular patterns on the particle that is to be eaten without the need of opsonizing signals while opsonic receptors are capable of detecting host-derived proteins that have been bound to target particles. [5, 40], (Table 5.)

The most well-known example of phagocytosis mechanism is that of Fc receptors that is able to recognize particles that are coated with IgG. [41] Surface receptors bind ligand molecules on the pathogens. The cell membrane gradually engulfs the target as receptors bind more ligand molecules. At this point, a phagosome forms that combine with lysosomes to digest the target. [42]

Table 5: Phagocytic Receptors that recognize opsonized and nonopsonized targets

Adapted from Adapted from Kohoutkova., et al, 2020 [5]

Receptor	Receptor type	Target	Main adaptive molecules	Associated pathways	Cell type	References
FcR	Opsonic	IgG-, IgE-opsonized pathogens	Src, Syk, ITAM	Ras, MEK, ERK, PKC	Mast cells (h)	(14)
Dectin-1	Nonopsonic	β-glucans	Hemi-ITAM, Syk	PKC, PI3-K	BMDM (m)	(118, 119)
MARCO	Nonopsonic	Bacteria	Src, MerTK, PLC-γ2	Poorly defined	AM (h), BMDM (m), PM (m), and J774A.1 (m)	(15, 16, 22)
SR-A	Nonopsonic	Apoptotic cells	TLRs	Poorly defined	Thymic macrophages (m), PM (m), and BMDC (m)	(120, 121)
CD36	Nonopsonic	Exposed phosphatidyl serine Erythrocytes *(P Falciparum* infected)	TLRs, Integrins, FcR	ERK, p38 MAPK, JNK	PM (m) and MDM (h)	(17, 18)
CR3	Opsonic/ nonopsonic	Zymosan, C3bi, apoptotic cells	Rap1, Talin, Arp2/3, Rac1	PKC, PI3-K, RhoA	Macrophages from PBMCs (h) and ovary cells (Chinese hamster)	(122, 123)

(h) indicates human; (m), murine; AM, alveolar macrophages; BMDC, bone marrow dendritic cells; BMDM, bone marrow derived macrophages; MDM, monocytic derived macrophages; PM, peritoneal macrophages.

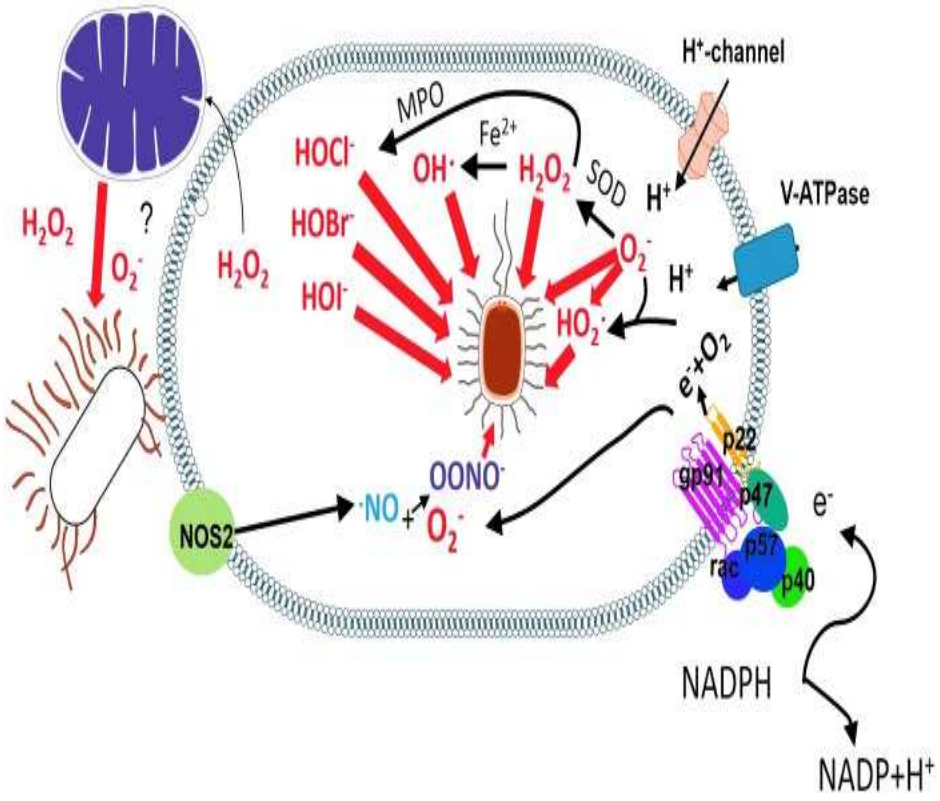
By opsonizing microorganisms, antibodies or complement can activate the Fc-Receptor (FcR) or complement receptor (CR) pathways. Conversely, phagocytes have scavenger receptors (SR) that can bind microorganisms without opsonin. Both polymorphonuclear neutrophils (PMNs) and macrophages phagocytize pathogens, although they have distinct bactericidal mechanisms. NADPH Oxidase (NOX) and different granules are at the center of PMN antimicrobial activities. They can be Azurophilic, Specific, Tertiary or Secretory PMN granules. Azurophilic granules contain lysosomal proteases that are activated by phagocytosis and considered the antimicrobial compartment. Specific granules include supplementary antimicrobial substances, tertiary granules that carry enzymes for the release of PMN into tissues, and secretory granules

that contain different types of receptors that are transported to the surface when activated. [43]

NADPH Oxidase (NOX), which is an enzyme that catalyzes the transfer of electrons to O_2 leading to the formation of superoxide (H_2O_2) with NADPH as an electron donor, is the fundamental Reactive Oxygen Species (ROS) that leads to a variety of additional ROS. [44] It is thought to be involved in the production of nitric oxide. Because NOX is found all across the plasma membrane of PMNs, this puts it in a good position to pump ROS into the extracellular environment, as well as intracellularly. Granules then combine with the phagosomes. After phagosome development is completed, NOX is triggered, resulting in a significant increase in ROS production. [45] When superoxide converts to peroxide, it comes into contact with myeloperoxidase enzyme that catalyzes the generation of HOCl, which is regarded to be effective in killing the bacteria. [46] In addition, it is believed that the significant ROS influx causes an ionic gradient to be established, which leads in a K+ influx, which allows granule proteases to be released, and digest the bacteria. [47] Ros overproduction could, however, come with consequences such as oxidative stress which has been thought to play a role is mortality of critically ill septic patients. [48] (Figure 4.)

Figure 4: ROS promote pathogen elimination by oxidative mechanisms

Adapted from Paiva, Bozza, 2020 [48]



CHAPTER TWO: THE ROLE OF PHAGOCYTIC CELLS IN SEPSIS-INDUCED IMMUNOSUPPRESSION

Introduction

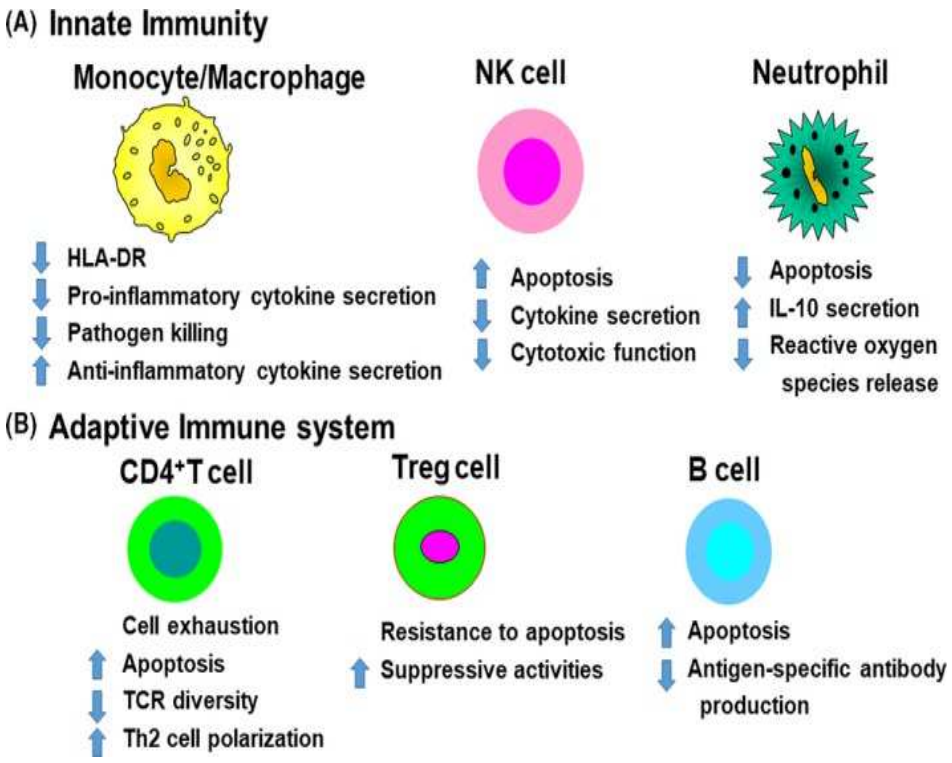
In septic patients, systemic inflammation is a major contributor to Oxidative stress. [49] Oxidative stress is defined as "generation of reactive nitrogen or oxygen species (RNS and ROS) in excess of the antioxidant capacity of the body." [49] Apart from being cytotoxic, reactive oxygen species (ROS) can start a number of signaling pathways that affect macrophage function and are important for controlling the body's reaction to infections. [50] Macrophages play an important role in all stages of sepsis, influencing both immunological and inflammatory processes, and macrophage impairment is thought to be a significant source of sepsis-induced immunosuppression.

As a result of significant advancements in medicine, some individuals with sepsis can now be treated earlier, allowing them to regain immunological balance, clear their infection, and make a full recovery. [51] Without effective treatment for sepsis-induced immunosuppression, patients progress into late stage sepsis and suffer from severe immunosuppression, described as an impaired activation of the immune response and a hypo-inflammatory response. [52] Sepsis-induced immunosuppression is caused by a multifactorial immunopathogenesis involving both innate and adaptive lymphocytes, and in fact, macrophage impairment has a role to play. [50] Also, oxidative stress has been shown to not only affect the functions of macrophages both intracellularly and extracellularly, but also alter their functions in septic patients. [8]

In septic patients, apoptosis results in the significant loss of immune cells, including natural killer (NK) and CD4+ and CD8+ T cells and B cells, which results in immunosuppression. Antigen presenting cells, such as monocytes and macrophages, have decreased expression of the human leukocyte antigen DR (HLA) on their surface, which may interfere with optimal presentation of microbial antigens to T cells. [53] (Figure 5)

Figure 5: Sepsis-Induced immunosuppression

Adapted from Ono, Tsujimoto, Hiraki, & Aosasa, 2018 [53]



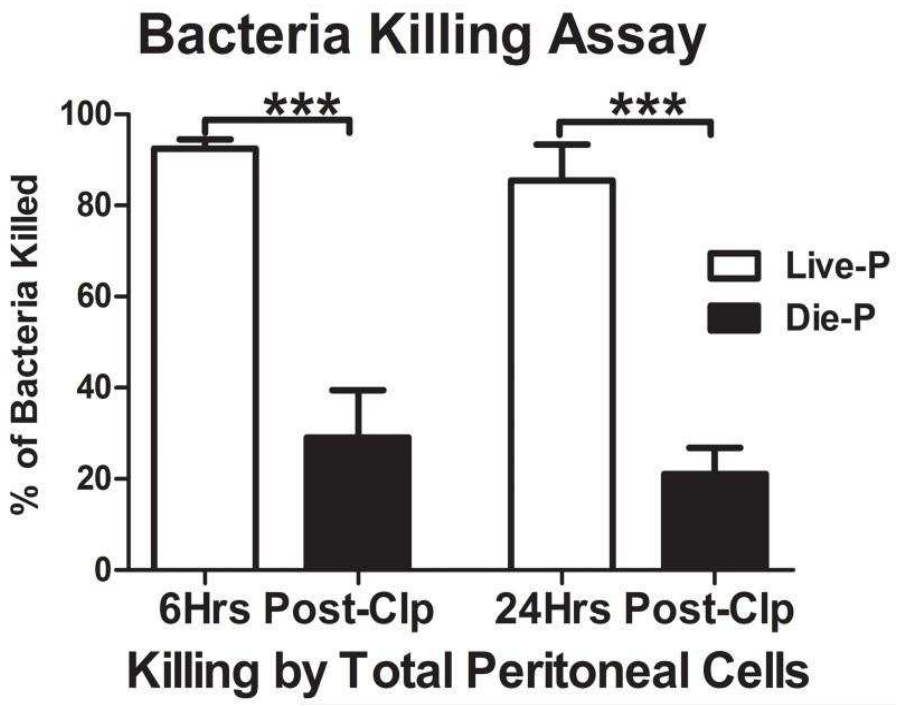
2:1 Immunosuppression by Macrophages in Sepsis

As a result of their widespread presence and extensive impact on immunological balance and response to inflammation, macrophages are critical players in the progression of sepsis through all stages. In response to infection, the Toll-like receptor (TLR) on the surface of the macrophage recognizes pathogen-associated molecular patterns (PAMPs) that is produced by an invading pathogen such as lipopolysaccharide (LPS) in Gram-negative bacteria and lipoteichoic acid (LTA)/peptidoglycan (PGN) in Gram-positive bacteria.[51] The early stages of sepsis are characterized by the maturation of macrophages into M1 cells, which aid in the defense of the host by removing invading pathogens and delivering large amounts of pro-inflammatory cytokines such as tumor necrosis factor alpha, interleukin-1, interleukin-6, interleukin-8, and interleukin-10.[50,54]

In the early phase of Sepsis, however, macrophages may become abnormally activated and release excessive amounts of pro-inflammatory cytokines, which have been identified as one of the key reasons for the high death rate during the early stage of the disease. [55] The fact that active pro-inflammatory macrophages either undergo apoptosis or polarize to the M2 phenotype, which lessens the pro-inflammatory response, suggests that they may play a role in immunosuppression. The existence of an excessive degree of macrophage apoptosis has been demonstrated in previous investigations, both in human autopsies and in animal models of sepsis. It has been shown that the buildup of LPS can alter inflammatory responses from activation to suppression, leading to decreased production of pro-inflammatory cytokines when an infection persists over long periods of

time [56] LPS-tolerance may be present in both the afflicted host and macrophages. The death of T cells is accelerated and Th1 responses are suppressed by macrophages of the M2 phenotype. [50]

Figure 6: Die-P Mice Exhibit Impaired Killing of E. coli at 6 and 24hr post CLP Adapted from Chiswick, Mella, Bernado, & Remick, 2015 [57]



In an experiment by Chiswick, Mella, Bernado, & Remick, 2015, a Cecal Ligation and Puncture mice model of sepsis with a 50% mortality was used to investigate innate immunosuppression in septic mice. In their work, mice were stratified as either P-live or P-Die based on their IL-6 levels which is an important aspect in efficacy assessment and "provides a window of time in which to observe the detrimental divergence between survivors and non-survivors." [57] In their findings, Die-P mice

exhibited a reduction in bacteria killed when compared to Live-P mice within 6 hours of CLP, and this reduction maintained for the next 24 hours. [57] (Figure 6) In addition, substantial difference was seen between Die-P and Live-P cells in terms of total bacterial phagocytization at both the early and late time points hence suggesting suppression of the Die-P peritoneal cells. (Figure 7.)

Figure 7: Die-P Phagocytes Are Suppressed in Phagocytosis.

Adapted from Chiswick, Mella, Bernado, & Remick, 2015 [57]

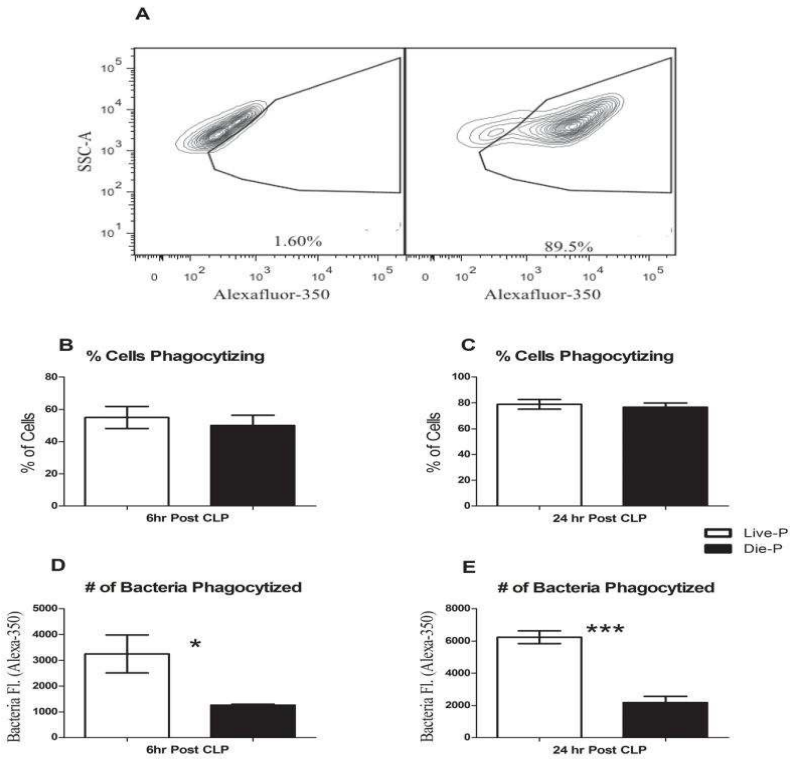
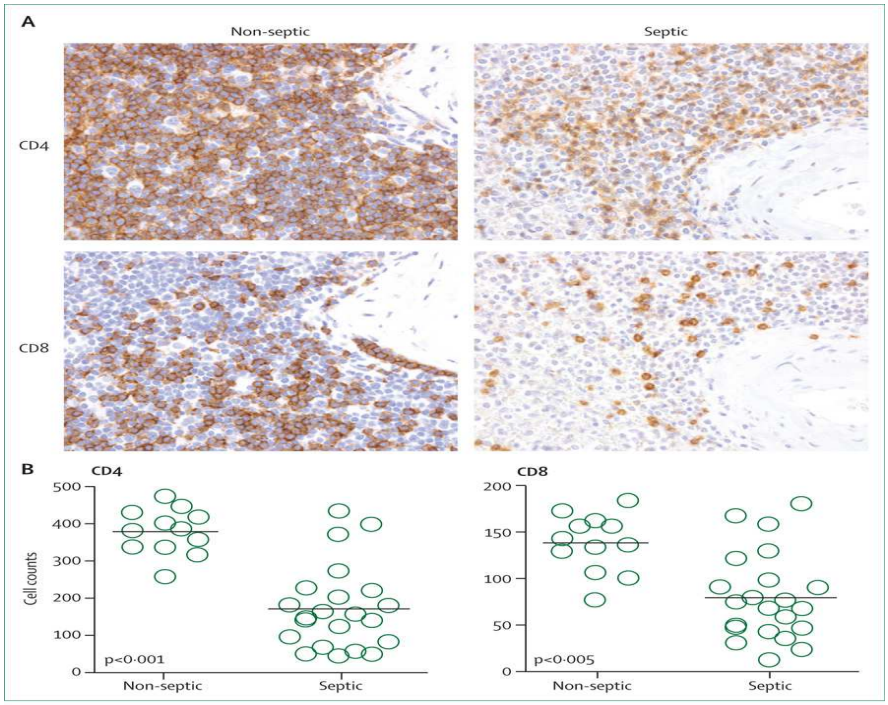


Figure 8: Depletion of Splenic Lymphocytes in septic patients

Adapted from Hotchkiss, Monneret, & Payen, 2013 [52]



In another experiment by Hotchkiss, Monneret, & Payen, 2013, quick tissue collection at the bedsides of patients who were dying of sepsis was performed. Their findings showed that patients experienced a significant loss of cells from both innate and adaptive immune cells, including CD4 and CD8 T cells, B cells, and dendritic cells, as a result of apoptosis. (Figure 8.) Lymphocyte apoptosis increases during sepsis resulting in a significant decrease of lymphocytes and hence immunosuppression.

2:2 Role of Regulatory T cells in Sepsis- Induced Immunosuppression

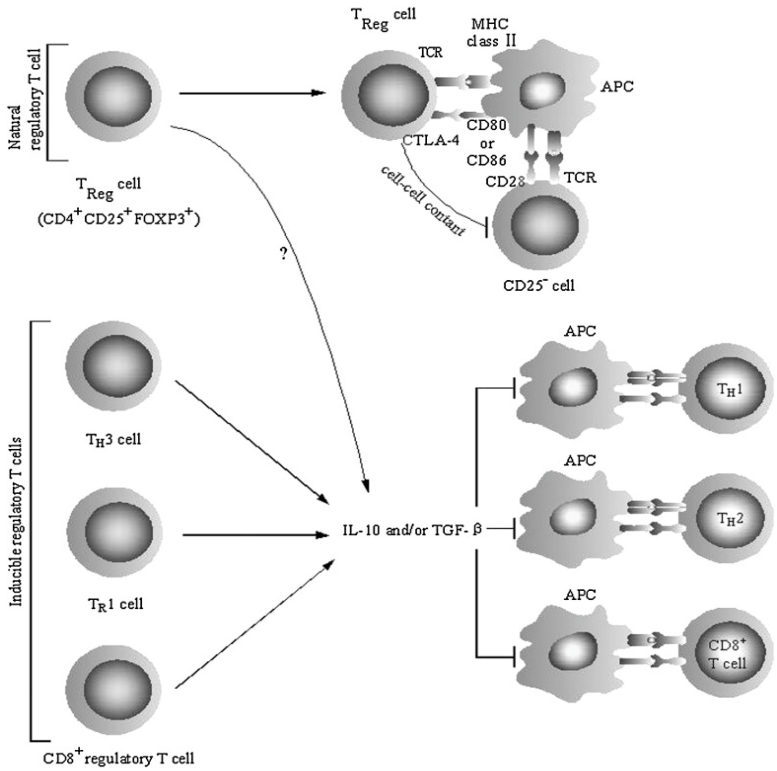
Increasing evidence has revealed that regulatory T cells (Tregs) play a significant role in the suppression of immune response, as evidenced by the increase in the number of T-regs and the improvement in their function following the start of severe sepsis or septic shock. [58] Regulatory T cells (Tregs) have been shown to be important for regulating host immune responses in animal models and humans of not only autoimmune disorders, tumors, allergies, contagious diseases, but also in transplant rejection. T-regulatory cells can suppress the immune response by competing with effector cells for IL-2, immunosuppression through cAMP, and by producing adenosine through CD39 and CD79. [59] Since Treg cells have a stronger affinity for the growth factor IL-2, they compete for it with proliferative cells for its synthesis. Proliferating effector cells are deprived of an essential cytokine, triggering metabolic stoppage and cell death, when Treg cells deprive proliferating cells of IL-2. [59]

Two-way signaling in cells is initiated by Cyclic AMP (cAMP), which acts as a second messenger and stimulates additional signaling pathways. Modifications inside the cellular environment and function are reflected in changes in intracellular cAMP levels. The cAMP/protein kinase A (PKA) signaling pathway in T cells is a significant negative regulator of T-cell immunological activity. Homeostasis in the T cell is regulated by the cAMP/PKA inhibitory signaling pathway modulating TCR signaling. Nevertheless, any imbalance in TCR regulation may result in T cell malfunction and have significant functional implications. T cells with high cAMP concentrations, such as in cancer and

persistent infections, are more likely to downregulate TCR signaling, which can lead to disease progression. [60]

Tregs have a critical function in regulating immunological responses, both adaptive and innate. The ability of these cells to grow in vivo and reduce the activity of CD4+ T and CD8+ T cells, as well as natural killer (NK) cells, has previously been demonstrated. [58]

Figure 9: Targets of Tregs and mechanisms underlying immunosuppression. Adapted from. (Jiang, Ming Yao, & Sheng, 2012.) [58]



Peripheral immunity is affected by two types of Tregs. Two types of T cells that have been researched extensively for their involvement in autoimmunity and tolerance

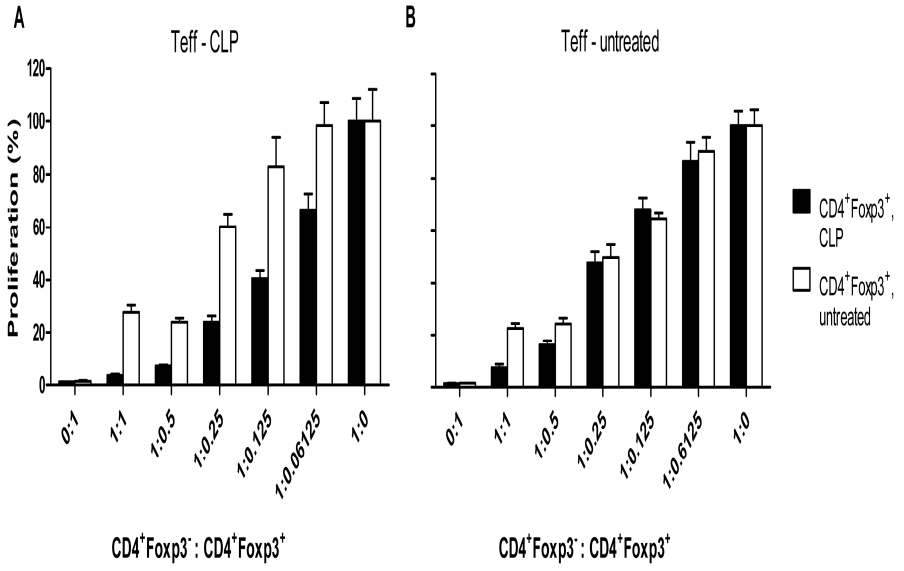
are naturally occurring CD4+CD+Foxp3+ T cells that originate in the thymus. In the second category, which includes Treg1 and helper T cells, there is an adaptive or induced group of Treg cells that arise from the activation and differentiation of mature CD4+CD25 T cells in the periphery. [61] CD25-expressing T cells are inhibited by a variety of ways, including cell–cell interaction, by TReg cells (natural Tregs). To prevent T cell activation, natural Tregs express CTLA-4 on the surface of lymphocytes, which interacts with CD80 and CD86. Naturally occurring Tregs are thought to have a role in immunosuppression through the release of TGF- or IL-10 from cells or their surface. IL-10 and TGF- can be produced by a variety of Treg populations, including TR1, TH3, and CD8+ Tregs. Direct inhibition of the proliferation and production of effector T cells, especially TH1, TH2, and CD8+ CD8+ lymphocytes, could be achieved by these immunosuppressive cytokines. These cytokines execute their immunosuppressive action by decreasing antigen-presenting cell maturation and activation. [62] (Figure 9)

In an experiment by Kuhlhorn., et al 2013, sepsis was induced on DEREG (Depletion of Regulatory T cells) mice using CLP and CD4+ Foxp32 Teffs were isolated 24hr after CLP. Their findings show a significant enhancement in suppressor Treg activity 24h after CLP. (Figure 10.) In another observational study of 30 septic shock patients by Venet., et al 2008, it was found that an increased proportion of CD4+CD25+CD127 cells in septic shock patients, compared to healthy volunteers (12% 1.0% as opposed to 6.8% 0.3%), was closely associated with a decrease in CD4+ lymphocytes (41054 as opposed to 77963 cells/L, P0.001). Peripheral blood mononuclear cell proliferation in response to mitogen stimulation was also considerably reduced in

these septic patients when compared to healthy persons. The increased fraction of CD25+ subpopulation among CD4+ T cells was not related to a proliferation of Tregs but rather to a selective reduction of CD4+CD25 T cells. [63] (Figure 11).

Figure 10: Tregs maintained their suppressive capacity after CLP

Adapted from Kuhlhorn., at al 2013 [73]

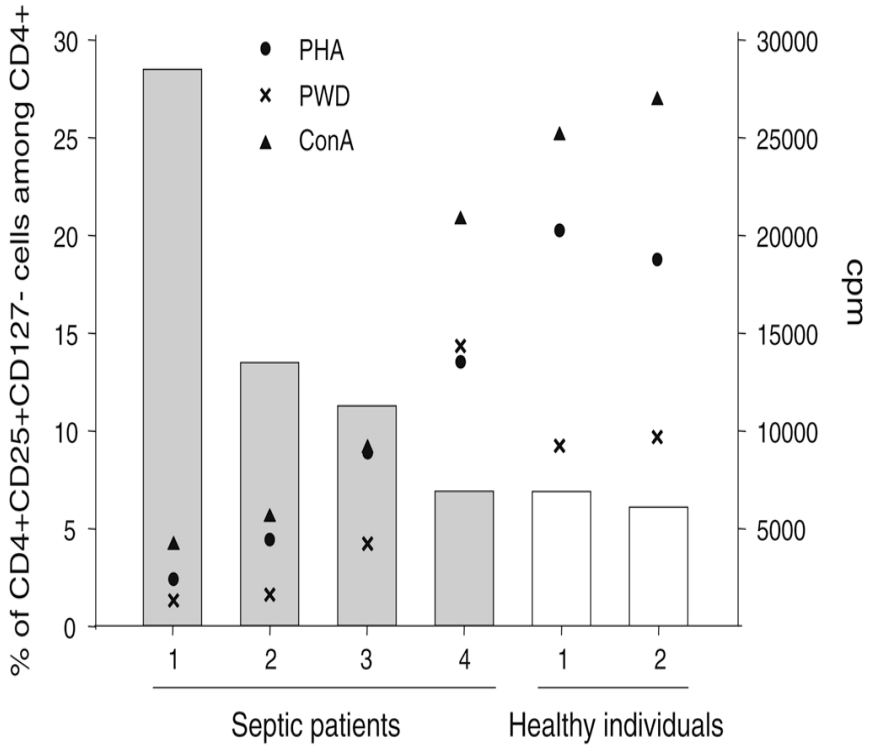


The above studies show that the level of immunosuppression in sepsis is directly linked to the presence of Tregs, a key regulatory T cell subset. Additional clinical studies by Monneret., et al 2003 of small cohort of individuals who had septic shock showed that the number of circulating Tregs was higher during the onset of the infection. Furthermore, they discovered that non-survivors had a larger fraction of T cells known to block T cell proliferation (CD4+ and CD25+) and hence a protracted and severe

immunoparalysis, linked with bad prognosis, may occur in some septic patients as a result.

Figure 11: Increase in $CD4^+CD25^+CD127^-$ lymphocytes is correlated with a decreased lymphocyte proliferation in septic shock patients

Adapted from Venet., et al 2008 [63]



CHAPTER THREE: POTENTIAL THERAPIES

Introduction

Given the high mortality rates of Sepsis, there is need for development of new therapeutic approaches. Prior sepsis drug trials used compounds that inhibited the body's ability to fight off pathogens or reduced inflammation. Drugs that block inflammatory cytokines may play a role in sepsis, but they should only be used in patients with extremely elevated levels of proinflammatory cytokines and be administered early in the course of the disease. A number of proinflammatory cytokines and mediators have been targeted in an attempt to improve outcomes, but none of these attempts have been successful. [64] Immunosuppression sets in quickly for the majority of patients. Aside from improving antibiotic administration timing and devising clinical practices to avoid infection, efforts should be directed toward enhancing host immunity. [52]

Antibiotics, surgical procedures to remove the source of infection, intravenous fluid administration to maintain adequate intravascular volume, and norepinephrine or vasopressin and mechanical ventilation are all current strategies for symptomatic treatment of sepsis. The effectiveness of numerous drug candidates, including corticosterone, toll-like receptor 4 antagonist, anti-LPS [65], anti-interleukin (IL)-1, and anti-tumor necrosis factor (TNF)- antibodies has not been demonstrated in clinical trials [66,67]. The only FDA-approved treatment for sepsis, Xigris (a systemic anticoagulant known as "activated protein C"); was withdrawn in 2010 due to hemorrhagic side effects and no improvement in mortality as a result. [68] Some of the current immunotherapies

targeting immunosuppression in sepsis include, IFN-γ, Granulocyte macrophage colony stimulating factor (GM-CSF), IL-7, and PD-1 and PD-L1.

3:1 IFN-γ

Reduced production of IFN-γ, a cytokine required for innate immunity activation, is a major immunological malfunction in sepsis. Sepsis has been the focus of a few small clinical trials involving recombinant IFN-γ. Recombinant IFN-γ treatment reversed monocyte dysfunction in patients with sepsis whose monocytes had decreased HLA-DR expression and produced reduced levels of TNF in response to LPS. By upregulating costimulatory and HLA molecules, Interferon-y promotes the production of proinflammatory cytokines by monocytes in response to LPS, increasing their ability to present antigens hence it's could play a major role in sepsis treatment.
Furthermore, in a research that involved staphylococcus aureus sepsis patients who received IFN-γ treatment, there was a significant increase in monocyte HLA-DR expression, an increase in CD4+ T cell IL-17 expression, and the bacteria was eliminated. [69] Patients with chronic granulomatous disease who develop fungal sepsis respond well to IFN-γ treatment. [70]

In an experiment by Kingsley, Elizabeth, & Haichao, CLP was used to induce sepsis on Sprague-Dawley rats and treated with interferon gamma antibody (1.2mg/kg) via IV after surgery. Their findings showed that there was a decrease in mortality over a 10-day period in the CLP rats given interferon gamma antibody 24 hours after surgery when they were compared with the control rats. (Figure 12). This was further supported by a study by Docke., et al 1997 where nine patients received interferon gamma

subcutaneously. The study showed that interferon gamma restored monocytic HLA-DR expression in septic patients. [71] (Figure 13)

Figure 12: Decrease in mortality over 10days in CLP rats given IFN-γ antibody (1.2 mg/kg, i.v.) 24 h after surgery compared with CLP rats that received irrelevant antibody

Adapted from Kingsley, Elizabeth, & Haichao, 2005 [74]

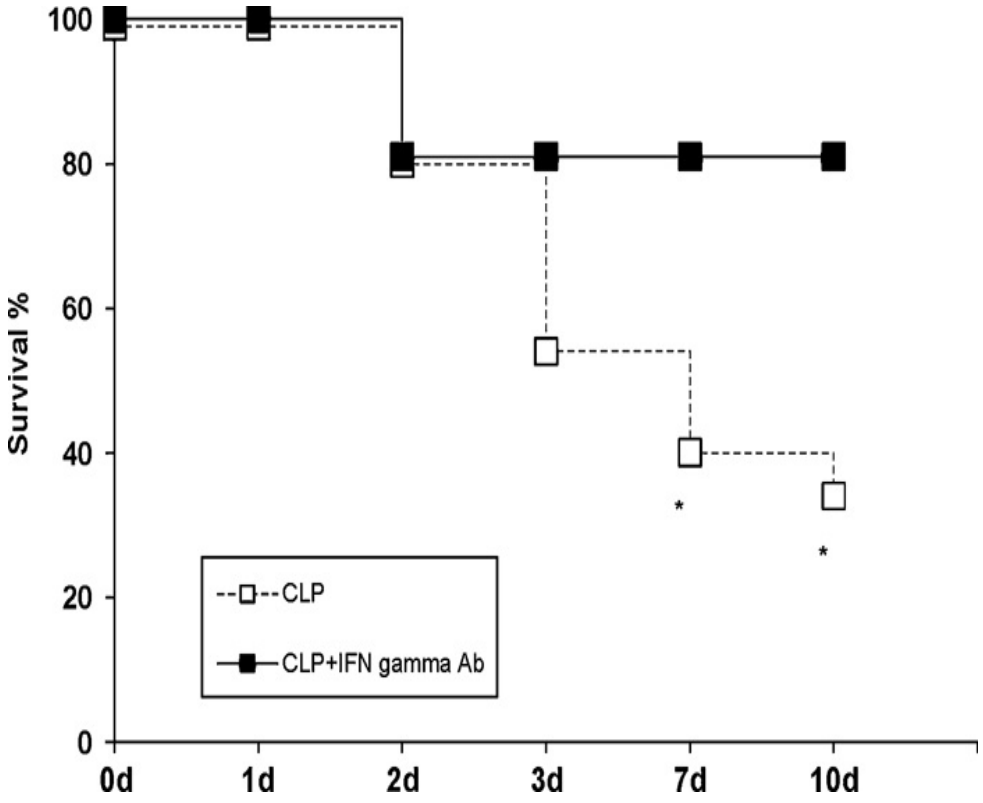
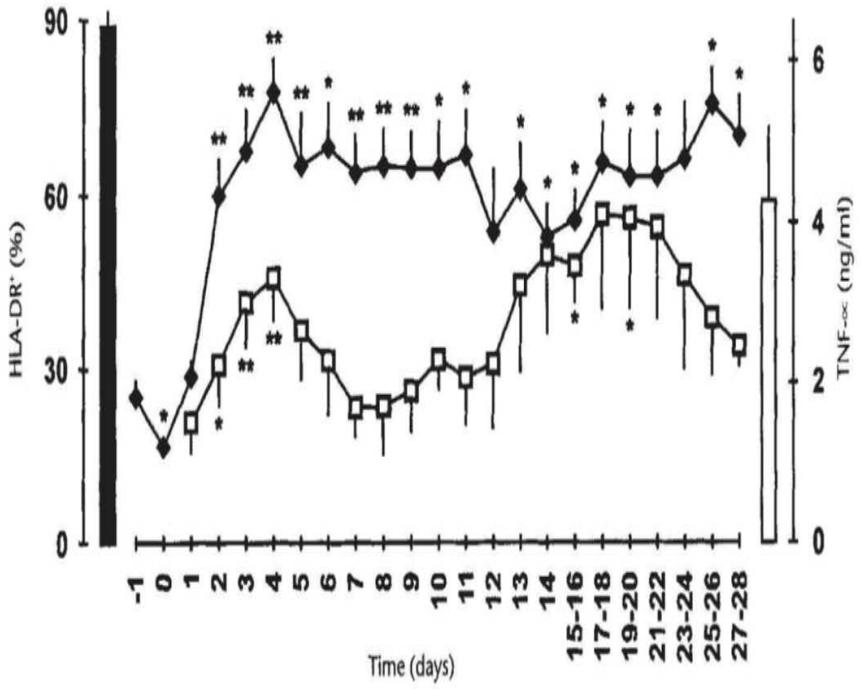


Figure 13: Treatment of patients with IFN-y restores monocytic HLA-DR expression.

Adapted from Docke., et al 1997 [71]



3:2 IL-7

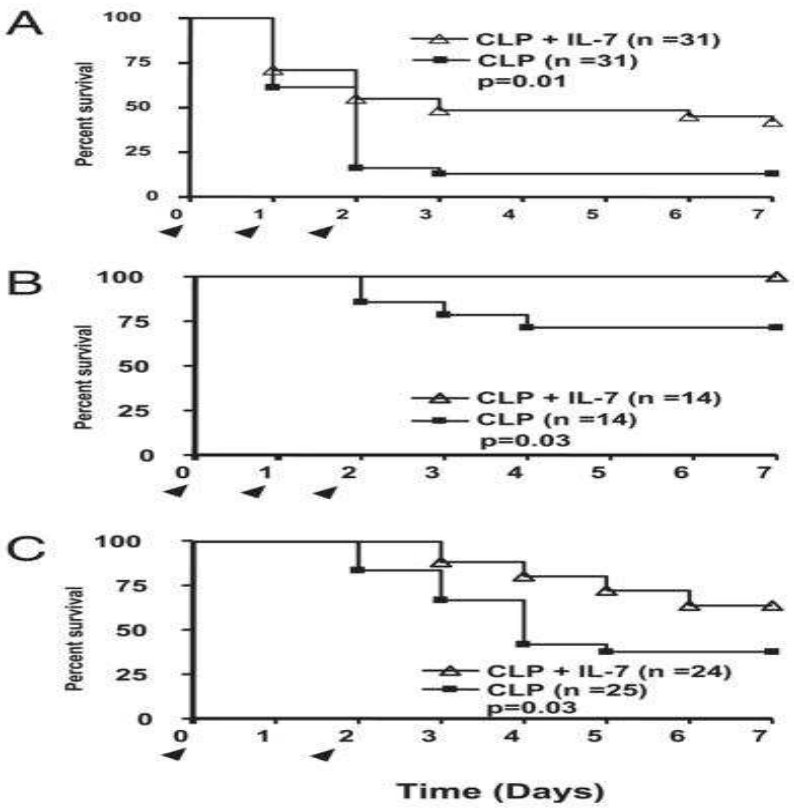
Human T cells express the receptor for the 25-kD glycoprotein, which is produced by stromal cells in response to IL-7. T cells that are either naive or memory express high levels of IL-7, whereas T cells that are Tregs express low levels of IL-7. T cell development, proliferation, and homeostasis require IL-7 receptor-mediated signaling. IL-7 may be a promising treatment option for sepsis, given the severe impairments in T cells that occur during the condition. Immune modulation in sepsis can benefit from IL-7

because it increases T cell repertoire diversity in circulation while decreasing the proportion of Tregs in circulation. [68]

In an experiment by Unsinger., et al 2010, CLP was used to induce sepsis in mice and treated with recombinant human 1IL-7 (rhIL-7). Their studies showed that restoring IFN- y production, preventing CD4 and CD8 T cell apoptosis, and improving the recruitment of immune effector cells to the infected site were all significant outcomes of rhIL-7 treatment and hence an improved mortality in the septic mice. (Figure 14)

Figure 14: RhIL-7 improves survival in Sepsis

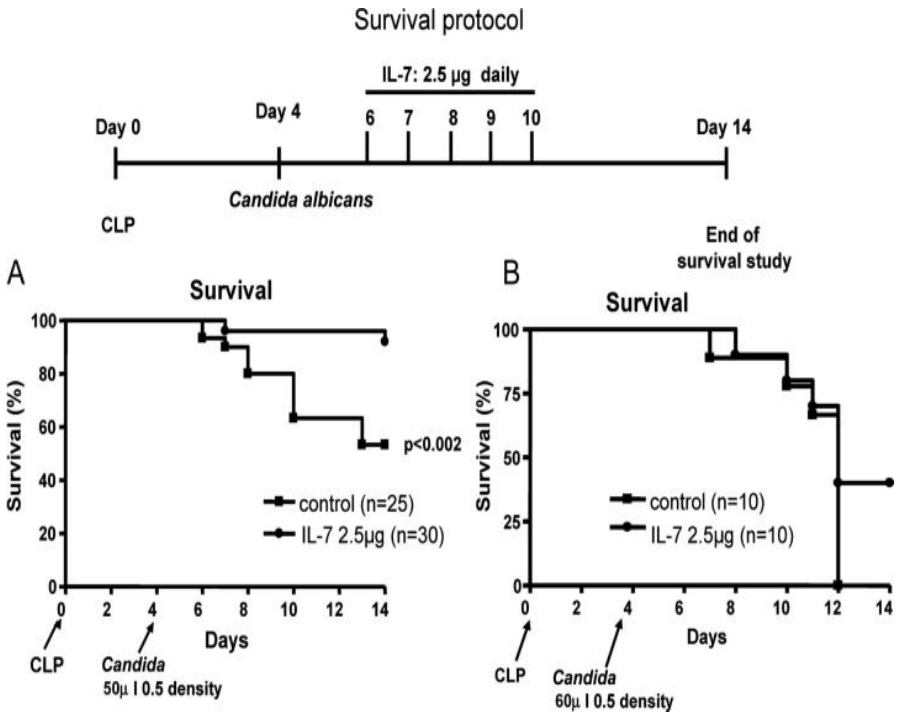
Adapted from Unsinger., et al 2010 [72]



Furthermore, in another experiment by Unsinger., et al 2012, a 2-hit fungal model of sepsis whereby CLP was used to induce sepsis in mice and the surviving mice had an intravenous injection with Candida albicans and treated with IL-7. Their findings add to the first experiment by improving survival in septic (figure 15) since IL-7 not only increased splenic CD4 and CD8 T-cell counts but also improved their proliferation and activation as well as reversing the T-cell defect in cytokine production in the septic mice. [75]

Figure 15: IL-7 improves survival in 2-hit Candida sepsis

Adapted from Unsinger., et al 2012[75]



3:3 GM-CSF (GRANULOCYTE MACROPHAGE COLONY STIMULATING FACTOR)

Granulocyte macrophage colony stimulating factor (GM-CSF) is "a cytokine that activates and induces production of neutrophils and monocytes or macrophages." [52] Systemic GM-CSF significantly promotes the levels of monocytes and neutrophils from the bone marrow during inflammation. It also plays a big role in the growth and development of myeloid cells.

In a randomized blinded study by Meisel., et al 2009, 38 septic patients were treated with GM-CSF or placebo for 8 days and followed for 28 days. The number of neutrophils in the treated individuals rose significantly compared to those in the control group after just one dosage of GM-CSF. On Day 4, the GM-CSF group had more monocytes than the control group. In patients treated with GM-CSF, CD4+ and CD8+ T-lymphocyte subsets rose over time, but not in control patients and T-cell counts in the intervention group were significantly higher at Day 9 after therapy. (Figure 16.) These results were further supported by another experiment by Frenck, Sarman, Harper, & Buescher, 1990 whereby recombinant human GM-CSF was given to neonatal rats in order to examine its ability to reduce sepsis-induced death due to Staphylococcus aureus. In their experiment, S. aureus LD90 was administered to control mice intraperitoneally, followed six hours later by an injection of HBSS, which resulted in a 70% mortality rate within 24 hours and 90% mortality rate during the first 96 hours. Test animals fared better than controls across the full dose range of rmGM-CSF tested with significant differences at all rmGM-CSF dosages in survival. However, the protection provided by rmGM-CSF was dependent on the dose

with animals that received a higher dose of rm GM-CSF being protected to the maximum extent, with 52% of the animals surviving the lethal dose of the bacteria. (Figure 17.)

Figure 16: Impact of granulocyte-macrophage colony-stimulating factor (GM-CSF) on leukocyte counts and leukocyte subpopulations

Adapted from Meisel., et al 2009 [76]

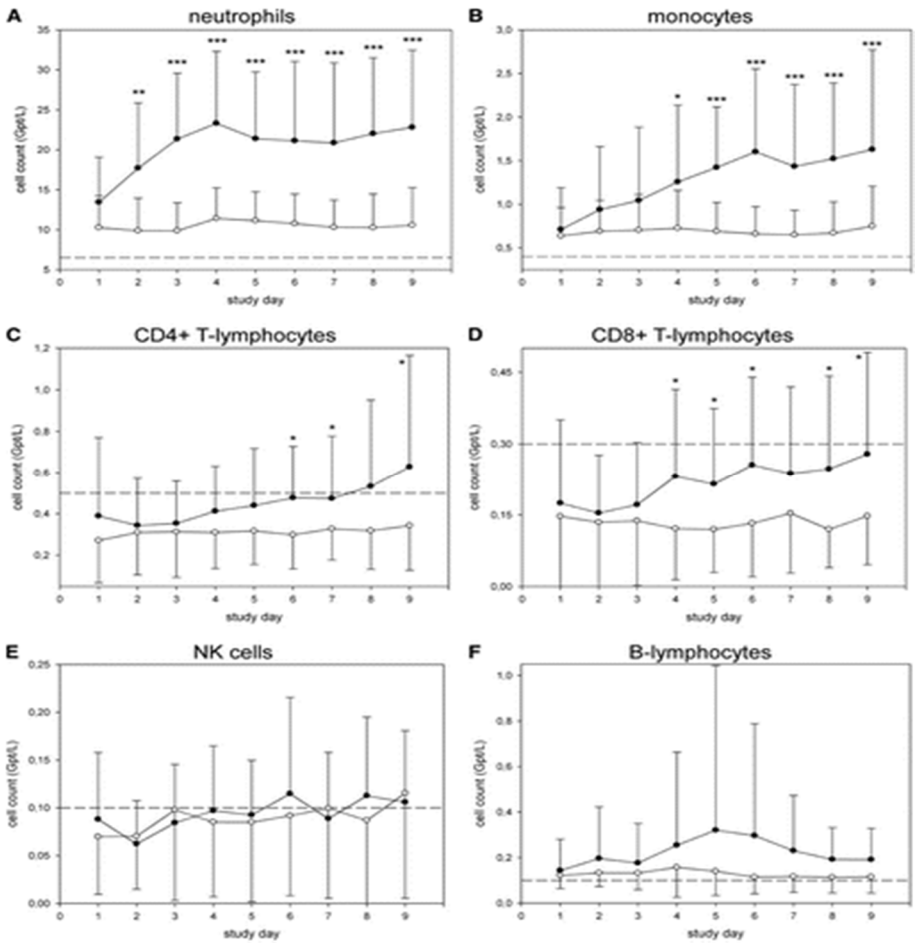
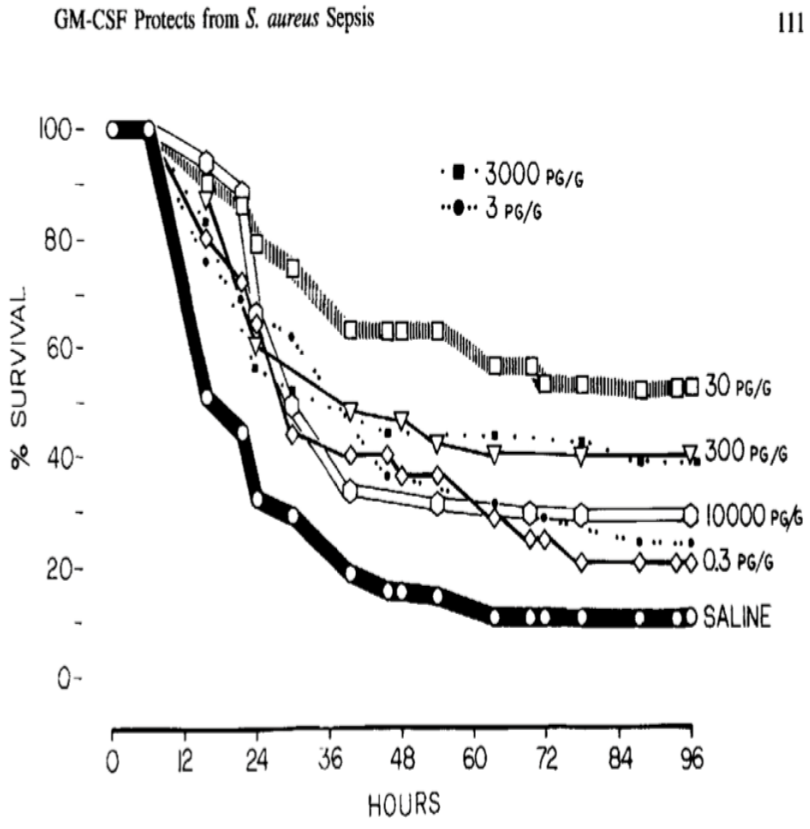


Figure 17: Dose-Response effects of GM-CSF pretreatment on survival of neonatal rats

Adapted from Frenck, Sarman, Harper, & Buescher, 1990 [77]



CHAPTER FOUR: DISCUSSION

In the past, sepsis has been suspected to be caused by a huge pro-inflammatory storm, and people die as a result of the hyperinflammation as a result of the ensuing inflammation. After surviving the hyper-inflammatory response, those who succeeded to do so were drawn into a corrected anti-inflammatory response. Patients who died during this time period were assumed to have suffered from immunosuppressive diseases. According to the findings of Cohen, Opal, and Calandra (2012), multiple therapies aiming at lowering hyperinflammation with the intention of preventing organ failure were found to be ineffective. Several factors are likely to have played a role in the failure of these treatments, with poor research design likely to be at the top of the list. One of the most plausible reasons for this was that sepsis was treated as a single, unchanging disease, characterized clinically as SIRS in conjunction with an infection, rather than as a dynamic disorder, and as a result, only one treatment method was likely to be effective. [57]. There are still immunosuppressive clinical trials being conducted at this time, and some are being terminated because of increased mortality in some of them. There is evidence that boosting immunity rather than neutralizing it is preferable, and there are efforts to identify those people who are more at risk of progressing from SIRS to sepsis. [73]

It is believed that apoptosis occurs at some time throughout the course of sepsis, resulting in a large loss of immune cells, such as natural killer cell(s) (NK cells), T cells (CD4+ and CD8+), and B cells in the patient, resulting in immunosuppression. Antigen presentation cells, such as monocytes and macrophages, are responsible for presenting

microbial antigens to T lymphocytes. These cells have reduced expression of the human leukocyte antigen DR (HLA) on their surfaces, which makes them more susceptible to infection. This may prevent T cells from presenting antigens in the most effective way possible. Being ubiquitous and having a substantial impact on immunological balance and response to inflammation, microphages are crucial in the advancement of sepsis at every stage of the disease's evolution. When a cell is inflamed, toll-like receptors (TLRs) on the cell surface of macrophages are activated, allowing them to recognize pathogen-associated molecular patterns (PAMPs) produced by an invading pathogen, such as lipopolysaccharide (LPS) in Gram-negative bacteria and lipoteichoic acid/peptidoglycan (PGN) in Gram-positive bacteria.

During the early stages of Sepsis, which is associated with abnormally activated macrophages that generate excessive quantities of pro-inflammatory cytokines, a high death rate is found. According to research, this is one of the primary reasons for the high fatality rate observed during this stage of the disease. Because active pro-inflammatory macrophages undergo either apoptosis or polarize to the M2 phenotype, which suppresses the pro-inflammatory response, it has been hypothesized that they may play a role in immunosuppressive immunity. The presence of an excessive degree of macrophage apoptosis has previously been demonstrated in both human autopsy and animal models of sepsis, according to the findings of previous investigations. Infections that are allowed to persist for a prolonged period of time can cause an inflammatory response to shift from activation to suppression, resulting in a reduction in the production of pro-inflammatory cytokines. It has been demonstrated that increasing levels of LPS can cause an

inflammatory response to shift from activation to suppression, resulting in a reduction in the production of pro-inflammatory cytokines.

Regulation of T cells in Sepsis-Induced Immunosuppression is discussed in detail in this paper. Regulatory T cells (Tregs) are thought to play a key role in the inhibition of the immune response following the development of severe sepsis or septic shock, according to growing data. T-regs are increased in number and function improves after the start of severe sepsis or septic shock, as indicated by the rise in T-regs and improvement in their function. Regulatory T cells (Tregs) have been shown to be crucial in the regulation of host immune responses in animal models of autoimmune diseases, malignancies, allergies, infectious diseases, and transplant rejection, among other conditions. When T-regulatory cells compete for IL-2 with effector cells, they can suppress the immune response through the production of cAMP and the production of adenosine through the expression of CD39 and CD79 on their surface. As a result, Treg cells compete with proliferative cells for the production of the growth factor IL-2, which Treg cells have a higher affinity for than proliferative cells. A metabolic stop and cell death occur as a result of the deprivation of an essential cytokine that stimulates cell proliferation by Treg cells when they deprive developing effector cells of IL2. When cyclic AMP (cAMP) is present in cells, it acts as a second messenger and stimulates the activity of other signaling pathways, allowing for two-way communication. Changes in intracellular cAMP levels reflect changes in the cellular environment and function that are taking place within the cell. Evidence suggests that the cAMP/protein kinase A (PKA) signaling pathway in T cells serves as a major negative regulator for the function

of T cells. The cAMP/PKA inhibitory signaling pathway, which controls TCR signaling, is responsible for keeping the T cell in a state of homeostasis. To be sure, any imbalance in TCR regulation can result in T cell malfunction, which can have serious consequences for the body's ability to operate properly. When T cells have high cAMP concentrations, such as those observed in cancer and persistent infections, the downregulation of TCR signaling is more likely to occur, which can result in disease progression.

As a result of the high mortality rates linked with sepsis, it is critical that new therapeutic approaches be developed. Previous sepsis therapy research relied on either antagonist of the body's ability to fight infections or anti-inflammatory medicines to alleviate the symptoms of the condition. The use of medications that inhibit the production of proinflammatory cytokines in the treatment of sepsis may be beneficial; however, they should only be used in individuals who have exceptionally high levels of proinflammatory cytokines and who are treated early in the course of the disease, as these individuals are more likely to survive. Attempts have been made to target a variety of proinflammatory cytokines and mediators in an attempt to improve outcomes, but none of these projects has been successful. Immunosuppression sets in fairly quickly for the great majority of people who are treated with it. In addition to optimizing antibiotic delivery timing and formulating therapeutic guidelines to prevent infection, efforts should be directed toward increasing host immunity to help prevent infection. Antibiotics, surgical procedures to remove the source of the infection, intravenous fluid administration to maintain adequate intravascular volume, norepinephrine or vasopressin administration, and mechanical ventilation are currently available treatments for sepsis to alleviate the

signs and symptoms of the condition. The efficacy of a number of drug options has been demonstrated in clinical trials, including corticosterone, toll-like receptor 4 antagonist, anti-LPS antibodies [65], anti-interleukin-1 antibodies, and anti-tumor necrosis factor (TNF) antibodies, among others [66,67]. Some of the currently available potential immunotherapies for sepsis that target immunosuppression include interferon-gamma (IFN-gamma), Granulocyte macrophage colony stimulating factor (GM-CSF), interleukin-7 (IL-7), and anti-PD-1 and anti-PD-L1 antibodies.

CHAPTER FIVE: CONCLUSION

The primary cause of deaths that occur after a person has recovered from an initial episode of sepsis has been debated for many years. Long-term morbidity and mortality from sepsis are thought to be primarily caused by immunosuppression rather than high- or low-grade local inflammation, according to recent findings by Hotchkiss., et al 2013. Apoptosis, anergy, fatigue, and other dysfunctional phenotypes are all caused by sepsis, which impairs both innate and adaptive immune cells. Due to the projected increase in not only mortality but also the cost of treating sepsis, it is imperative to come up with new treatment options, and therapies that target immunosuppression have shown promising results.

The majority of researchers and doctors agree that patients with sepsis should be treated using individualized precision medicine strategies because of the complex immunological dysfunction that sepsis creates during sickness. There must be a complete understanding of how medicinal substances affect the immune system in order to do this, hence the importance of stratification of patients to better enhance their treatments. Patients with immune dysfunction should be identified using innovative biomarkers. For the best results, modern molecular biotechnology such as omics and system biology should be used to further refine the phenotyping of a patient's immune status, and a combination of biomarkers can be established for goal-directed application of immunomodulatory therapies such as GM-CSF or IFN- trials. [68] Based on previous studies, we already know that a single medicine used to treat sepsis would be useless. A

novel mix of medicines to treat a wide range of problems in people over time is critically essential.

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CURRICULUM VITAE