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Editor

Annual Update in Intensive Care and Emergency Medicine 2022

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Abbreviations

AKI	Acute kidney injury
APACHE	Acute physiology and chronic health evaluation
ARDS	Acute respiratory distress syndrome
COVID	Coronavirus disease
CRP	C-reactive protein
CRRT	Continuous renal replacement therapy
CT	Computed tomography
DAMP	Damage-associated molecular pattern
DO_2	Oxygen delivery
ECMO	Extracorporeal membrane oxygenation
EHR	Electronic health record
GCS	Glasgow Coma Scale
ICU	Intensive care unit
IFN	Interferon
IL	Interleukin
LV	Left ventricular
MAP	Mean arterial pressure
NO	Nitric oxide
NOS	Nitric oxide synthase
PAMP	Pathogen-associated molecular pattern
PEEP	Positive end-expiratory pressure
RBC	Red blood cell
RCT	Randomized controlled trial
RRT	Renal replacement therapy
RV	Right ventricular
SARS-CoV-2	Severe acute respiratory syndrome coronavirus-2
SOFA	Sequential organ failure assessment
TBI	Traumatic brain injury
TNF	Tumor necrosis factor
VILI	Ventilator-induced lung injury
V_T	Tidal volume

Part I

Sepsis and the Immune Response

The Role of Mitochondria in the Immune Response in Critical Illness

1

Y. Wang and A. S. McLean

1.1 Introduction

Immune dysregulation, characterized by an imbalance between a systemic inflammatory response syndrome and a compensatory anti-inflammatory response syndrome, is often observed in critically ill patients [1, 2]. This imbalance between the pro- and anti-inflammatory responses frequently leads to immunoparalysis in critically ill patients, rendering them more susceptible to further infections, and is associated with increased mortality [3]. Currently, no effective treatments are available to restore immune homeostasis and reduce mortality in these patients, largely due to the heterogeneity in patients' immune status and more importantly the lack of understanding of the underlying cause of such immune dysfunction [2, 4]. Immune response is not a standalone process but is interconnected with other cellular activities, a very important one of which is cellular metabolism. Metabolic pathways and immune response are tightly intertwined both in health and in disease [5]. The link between immune cell function and mitochondrial function is now well recognized and a field known as "immunometabolism" is dedicated to understanding the relationship between immune and metabolic pathways [6–8]. Mitochondria play a crucial role in regulating not only the growth, but also the function, of immune cells. In addition to providing energy to support the synthesis of the macromolecules essential for immune cell proliferation, mitochondria also act as signaling organelles, driving

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activation of immune cells via metabolic intermediates, mitochondrial DNA (mtDNA), and reactive oxygen species (ROS). In addition, mitochondrial dynamics (fusion and fission), biogenesis (synthesis of new mitochondria), and mitophagy (degradation of damaged mitochondria) also play important roles in regulating immune cell functions. Knowledge in immunometabolism in critical illness, in particularly sepsis, opens up a new paradigm in patient care. Potential therapies targeting metabolic pathways, instead of solely immune-related pathways, might be the way to repair cellular function and restore immune homeostasis [4]. The other aspect of immunometabolism—looking at how immune responses influence metabolic pathways—is equally important, but beyond the scope of this review. Interaction between metabolism and immune response at the organ level has been reviewed elsewhere [6].

1.2 Mitochondrial Machinery That Mediates and Regulates Immune Responses in Critical Illness

Apart from being the powerhouse of the cell, the mitochondrion has emerged as a signaling hub that shapes and modulates how the immune system responds to infection or trauma. Mitochondrial dysfunction is evident in leukocytes from critically ill patients, and is believed to be the underlying cause of immunoparalysis and may account for the development of organ dysfunction [7–9]. Early recovery of mitochondrial function correlates with improved recovery in critically ill patients [10].

1.2.1 Metabolic Reprogramming

The immune-regulating mitochondrial machinery is a complex network involving many pathways and mechanisms that diverge and converge at various levels. Metabolic reprogramming is one mechanism that has been well studied in both innate and adaptive immune cells. Immune cells at different activation states (quiescent vs. activated), or with different functions (pro-inflammatory vs. anti-inflammatory), and different cell types (granulocytes, macrophages, dendritic cells, T- and B-lymphocytes), make use of different metabolic pathways (e.g., glycolysis, oxidative phosphorylation, fatty acid metabolism) to produce ATP [11]. The choice of different metabolic pathways, supports the energy demand of cells at different activation state. For example, upon infection or stimulation, immune cells become activated and produce cytokines and hence tend to favor glycolysis over oxidative phosphorylation for fast turnaround of ATP. Although the same amount of starting material, such as glucose, is used, oxidative phosphorylation generates 18 times more ATP than glycolysis, although is a lot slower. On the other hand, the choice of metabolic pathway determines the fate of the immune cells, i.e., naïve or memory, effector or regulatory, etc. However, the environment that the cells are in in the first place, triggers the changes in the metabolic pathways. The overall trend is that

neutrophils, inflammatory macrophages (M1 macrophages), activated effector T cells, and dendritic cells rely more on aerobic glycolysis, whereas alternatively polarized macrophages (M2 macrophages), regulatory T cells (Tregs), and memory T cells prefer oxidative phosphorylation and fatty acid oxidation for energy production [8, 11, 12]. Metabolic reprogramming serves an important role in catering for the immune cells' energy demand at different phases of their activation and proliferation. However, imbalance across the metabolic pathways could have serious pathological impact. One example may be the hyperlactatemia often seen in critically ill patients. Increased aerobic glycolysis in the activated immune cells during the initial hyper-inflammatory response is believed to contribute to the increase in blood lactate levels in sepsis [13, 14].

1.2.2 Mitochondrial ROS and mtDNA

Metabolic reprogramming sets the scene for the immune response, which is then subjected to many more modifications and regulations by factors that are directly or indirectly related to mitochondrial metabolism. Two important mitochondria-related immune regulators that have been well studied are mitochondrial ROS and mtDNA. Mitochondrial ROS are produced in healthy mitochondria, as a by-product of oxidative phosphorylation. At low dose, mitochondrial ROS serve important signaling functions, especially in the innate immune response. They are known to mediate NLRP3 inflammasome activation, leading to production of the pro-inflammatory cytokines, interleukin (IL)-1β and IL-18 [8, 15]. Mitochondrial ROS also induce a type-I interferon (IFN) response via mitochondrial antiviral-signaling (MAVS) and the IFN regulatory factor 3 (IRF3) pathway [16]. However, the level of mitochondrial ROS needs to be tightly regulated by the antioxidant system. Excessive mitochondrial ROS can cause oxidative damage to proteins/enzymes involved in oxidative phosphorylation and create mutations in mtDNA, contributing to the immune dysregulations as seen in critical illness [17]. Like mitochondrial ROS, mtDNA also plays an important role in innate immunity [12]. In healthy cells, mtDNA is located in the matrix of mitochondria, encoding 13 proteins, all of which are components of oxidative phosphorylation. mtDNA is released to the cytosol upon mitochondrial dysfunction which involves changes to the integrity or permeability of the mitochondrial membrane. mtDNA, released into the cytosol, can activate the NLRP3 inflammasome with release of IL-1β and IL-18. Due to its bacterial origin, cytosolic mtDNA also serves as a damage-associated molecular pattern (DAMP), which can be recognized by intracellular pattern recognition receptors (PRRs), such as Toll-like receptor 9 (TLR9), and initiate the nuclear factor-kappa B (NF-κB)-dependent pro-inflammatory signaling pathway. In addition, cytosolic mtDNA can also be sensed by cyclic GMP-AMP synthase (cGAS) and activate the cGAS/stimulator of IFN genes (cGAS/STING) pathway and its downstream IFN response [18]. mtDNA can also be released into the circulation and cause systemic inflammation. Circulating mtDNA has been associated with mortality in critically ill patients [19].

1.2.3 Succinate and Itaconate

In addition to mitochondrial ROS and mtDNA, metabolites such as succinate and itaconate have also emerged as part of immune-regulating mitochondrial machinery [4, 20]. Both succinate and itaconate are intermediates from the tricarboxylic acid (TCA) cycle with opposite effects on the immune response. The TCA cycle generates nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide ($FADH_2$), providing electrons to fuel oxidative phosphorylation. Succinate accumulation occurs under conditions such as hypoxia or inflammation. It can be released from mitochondria into the cytosol and functions as a signal transducer promoting pro-inflammatory gene expression via hypoxia-inducible factor 1α (HIF-1α) activation. Accumulation and oxidation of succinate by succinate dehydrogenase (SDH) in the mitochondria also leads to increased production of mitochondrial ROS via a process called reverse electron transport. This further enhances the pro-inflammatory effect of succinate. Like ROS, the level of succinate needs to be carefully regulated due to its inflammation aggravating effect. Plasma succinate has been proposed as a predictor of mortality for critically ill patients who are severely injured [21]. Itaconate, which is derived from cis-aconitate of the TCA cycle, is a succinate-regulating factor. It is shown to counteract the pro-inflammatory effect of succinate by inhibiting SDH. Itaconate can also be released into the cytosol and activate transcription factor NF-E2 p45-related factor 2 (Nrf2), a master regulator of antioxidant and anti-inflammatory responses [22]. Recently, itaconate has also been shown to inhibit the inflammatory response in macrophages through activating transcription factor 3 (ATF3).

1.2.4 Mitochondrial Dynamics

The above mentioned immune-regulating mitochondrial factors are centered around the biochemical aspect of mitochondrial biology. Another important aspect of immune-regulating mitochondrial machinery is mitochondrial dynamics, which is to maintain and provide infrastructural support for the immune response. The size and shape of mitochondria undergo constant change through fusion and fission, which is important for maintaining the health and function of mitochondria. First, fusion incorporates newly synthesized mitochondria (from mitochondrial biogenesis) into the current mitochondrial network. Second, fusion also allows for mixing of proteins and/or mtDNA between the existing mitochondria, which on one hand enhances the metabolic capacity of the mitochondria, and on the other enables the damaged proteins and/or mutated mtDNA to be segregated from the healthy ones. Finally, segregation is achieved via fission and the damaged mitochondria can be destroyed through a process known as mitophagy. The proportion of mitochondria with damaged proteins or mutated mtDNA is kept below a critical threshold level through this process to maintain mitochondrial function [23, 24]. In addition to quality control, mitochondrial fusion and fission also participate in immune regulation. In activated T cells, there is an increase in fission, which creates round and

fragmented mitochondria with loose cristae, favoring aerobic glycolysis. And in memory T cells, increased fusion generates elongated mitochondria which favors oxidative phosphorylation and fatty acid oxidation [8, 25].

1.3 Immunometabolism: The Perfect World Scenario vs. the Critical Illness Scenario

So far, we have presented a list of mitochondrial components that are thought to play important roles in regulating the immune response. Our list is far from complete, but does highlight a few mechanisms that could relate to the development of immune dysregulation in critical illness. Figure 1.1 illustrates what we think would

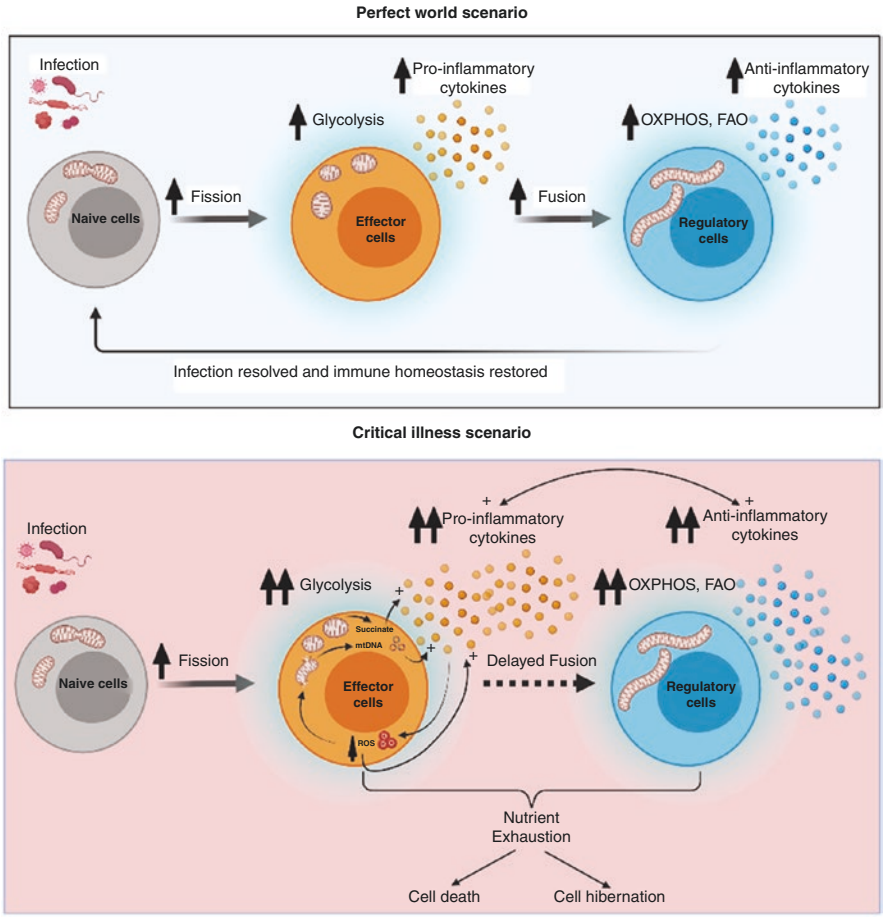

Fig. 1.1 Immunometabolism in the 'perfect world scenario' vs. the 'critical illness scenario'. *OXPHOS* oxidative phosphorylation, *FAO* fatty acid oxidation

happen to the immune response when metabolism was in perfect control (the perfect world scenario) and when it became inconsistent and changeable (the critical illness scenario). In the perfect world scenario, the presence of an insult (e.g., infection or a trauma-related stress signal), would trigger metabolic reprogramming, switching from oxidative phosphorylation to glycolysis. This would enable activation of immune cells and production of pro-inflammatory cytokines and other mediators. At the same time, mitochondrial fission would increase to keep up with the metabolic reprogramming. The slightly elevated mitochondrial ROS and succinate in response to initial insult or cytokines would promote the pro-inflammatory response. Once the insult was eliminated, mitochondrial fusion would increase to create fused elongated mitochondria that favor oxidative phosphorylation and fatty acid oxidation. This would allow activation of regulatory immune cells and production of anti-inflammatory cytokines and other mediators. And itaconate would counteract the effect of succinate, activate the Nrf2-mediated antioxidant pathway to dampen down mitochondrial ROS, and activate ATF3 to inhibit the inflammatory response in macrophages. Immune homeostasis would be achieved as a result.

In the critical illness scenario, initial metabolic reprogramming from oxidative phosphorylation to glycolysis would go on for longer than necessary, generating excessive lactate (hyperlactatemia) and pro-inflammatory cytokines and mediators. A disrupted mitochondrial fusion/fission cycle could be to blame, one which could not support the timely switch to oxidative phosphorylation and fatty acid oxidation. The anti-inflammatory response would eventually kick in but by then damage would already have occurred to mitochondria and mtDNA because of excessive production of ROS in response to stress or cytokines. Excessive ROS and released mtDNA would aggravate the pro-inflammatory response, which in turn would trigger a more aggressive anti-inflammatory response to try and salvage the situation. The competition between pro- and anti-inflammatory responses would exhaust the nutrients and lead to shutdown of the whole metabolic system. Cells would either die or go into hibernation to preserve energy [26]. This scenario is an over-simplified version of what might happen in the actual disease setting, without considering the crosstalk between cells and organs and many other factors that are not included here. It is designed to shed light on the interaction between the immune response and metabolism.

1.4 Potential of Mitochondria-Targeting Therapy in Critical Care

Our understanding thus far leads us to think that targeting mitochondria could perhaps correct the underlying cause of immune dysfunction in critical illness and lead to better recovery of the patients. The central role of mitochondrial dynamics in supporting and initiating metabolic reprogramming would make it the perfect therapeutic target. To get the fusion/fission cycle going, the mitochondrial network needs to be replenished by newly synthesized mitochondria via biogenesis. Therapies that could potentially boost mitochondrial biogenesis are mitochondrial

transplantation, metformin, nitric oxide (NO), and carbon monoxide. Mitochondrial transplantation has been used successfully in pediatric patients with myocardial ischemia-reperfusion injury [27]. Metformin can activate peroxisome proliferator-activated receptor (PPAR)-gamma coactivator-1α (PGC-1α), and Nrf2, the master regulator of mitochondrial biogenesis and antioxidant systems [28]. Premorbid use of metformin is associated with lower mortality in sepsis [29]. NO and carbon monoxide can also enhance mitochondrial biogenesis [30–32]. Dietary nitrite has been trialed in patients with coronary artery disease (ClinicalTrials.gov Identifier: NCT00069654). Other therapies, such as mitochondria-targeted antioxidant (MitoQ) [33], could also be beneficial in protecting mtDNA and oxidative phosphorylation from oxidative damage. MitoQ has been trialed in people with Parkinson's disease (ClinicalTrials.gov Identifier: NCT00329056).

1.5 Challenges of Applying Mitochondria-Targeting Therapy in Critical Care

There are challenges to overcome before mitochondria-targeting therapy would be possible. First, how do we assess mitochondrial dysfunction in the clinic and identify patients who would benefit from such therapy? A few possible ways could be considered. Non-invasive assessment of mitochondrial oxygen metabolism using a novel device called the COMET monitor was tested on 40 patients during the acute phase of sepsis. This device is based on the protoporphyrin IX-triplet state lifetime technique (PpIX-TSLT) and has been shown to be feasible [33]. This technology is still in its early phase of clinical application but does offer some hope. Another possible biomarker that could potentially be used for assessing mitochondrial dysfunction is plasma mtDNA, but its sensitivity and specificity need further investigation [19, 34, 35]. Furthermore, we could consider using immune response markers as a surrogate markers, one such example could be IFNα inducible protein 27 (IFI27) [36]. If we could overcome the first challenge, the second would be how to deliver mitochondria-targeting therapies to the right organ at the right time.

1.6 Conclusion

In this chapter, we have demonstrated the important role of mitochondria in regulating the immune response and proposed a scenario that explains immune–metabolism crosstalk in the context of critical illness. We have highlighted the role of mitochondrial dynamics in overseeing and supporting metabolic reprogramming during immune cell activation. Mitochondrial ROS can be friend or foe when it comes to immune regulation. Two TCA intermediates—succinate and itaconate—with opposite effects have emerged as important players of the immune-regulating mitochondrial machinery. Our understanding in immunometabolism could take us to the next era of critical care: mitochondria-targeting therapy.

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Immunomodulation by Tetracyclines in the Critically Ill: An Emerging Treatment Option?

2

A. Sauer, C. Putensen, and C. Bode

2.1 Introduction

Sepsis and acute respiratory distress syndrome (ARDS) are still the most common causes of death in critically ill patients. Although our knowledge of the underlying immunopathogenesis has grown tremendously and we have made substantial advances in supportive care, the overall mortality for sepsis and ARDS remains high [1, 2]. In 2016, sepsis was redefined as life-threatening organ dysfunction caused by a dysregulated host response to infection. Hyperinflammation occurring concurrently with immunosuppression puts patients at risk for developing fatal secondary infections and chronic critical illness syndrome [1]. An extension of sepsis in terms of pathogenesis has made ARDS similarly resistant to therapy and the prognosis for patients with this syndrome remains equally dismal [2]. Despite over 30 years of preclinical and clinical trials, no effective pharmacotherapies exist to improve outcomes in patents with sepsis or ARDS [1, 2]. New therapeutic agents are desperately needed, even more so in the light of the ongoing coronavirus disease 2019 (COVID-19) pandemic.

Tetracyclines are a family of bacteriostatic antibiotics that inhibit protein synthesis by reversibly binding to the bacterial ribosome. Upon binding they allosterically prevent the binding of the amino acyl-tRNA to the mRNA ribosome complex. They exhibit broad-spectrum antibacterial activity against a wide-range of Gram-positive and Gram-negative bacteria as well as atypical pathogens, such as chlamydiae, spirochaetes, and rickettsiae [3]. Additionally, tetracyclines exert pleiotropic immunomodulatory effects that may be able to rebalance immune homeostasis in critically ill patients. Their beneficial anti-inflammatory effects have been reported for chronic

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pulmonary diseases, chronic inflammatory skin diseases, autoimmune disorders, as well as neurodegenerative diseases, and they have become a standard of care in the treatment of periodontitis, acne, and rosacea [3, 4]. Recently, evidence has emerged that tetracyclines could potentially be beneficial in ARDS and sepsis [5–7].

In this chapter, we provide an overview of the current preclinical and clinical studies on the immunomodulatory effects of tetracyclines in the critical care setting (Tables 2.1 and 2.2). We elucidate the underlying mechanisms of the immunomodulatory properties of tetracyclines that may have therapeutic effects in sepsis and ARDS. Finally, we discuss future research perspectives including the role of non-antibiotic tetracyclines.

2.2 Immunopathogenesis of Sepsis

Sepsis is a heterogenous syndrome characterized by an unbalanced hyperinflammatory state and profound immunosuppression. Pathogen-associated molecular patterns (PAMPs) released by sepsis-inducing microorganisms activate pattern recognition receptors (PRRs) expressed by various immune cells and trigger a strong innate immune response. The best known PAMPs include lipopolysaccharide (LPS), lipoteichoic acid (LPA), and microbial DNA. PRRs can also sense cell injury-associated endogenous molecules referred to as damage-associated molecular patterns (DAMPs), such as ATP, mitochondrial DNA, hyaluronan, heat shock proteins, and fibrinogen [1, 8]. Upon ligand binding, activation of downstream signalling pathways (e.g., nuclear factor kappa-B [NF-κB] and mitogen-activated protein kinase [MAPK]) leads to the transcription of genes encoding pro-inflammatory cytokines and chemokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-1ß, IL-8, IL-18 and interferons (IFN). The expression of IL-1ß and IL-18 is tightly regulated by inflammasomes, which execute a unique form of programmed cell-death called pyroptosis. In most cases, these processes aid in neutralizing invading pathogens and apoptotic cells. However, in sepsis they can lead to an unbalanced host response that can potentially trigger a life-threatening "cytokine storm" [1, 9]. Another hallmark of the innate immune response in sepsis is the activation of the complement system, which results in the recruitment of leukocytes, endothelial cells, and platelets and ultimately in sepsis-induced endothelial barrier dysfunction. Loss of vascular integrity leads to tissue edema and reduced microvascular perfusion. Coagulation activation is tightly interconnected with complement activation and predisposes patients to disseminated intravascular coagulation (DIC), microvascular immunothrombosis, and hemorrhage.

The initial hyperinflammatory state is counterbalanced by immunosuppression which involves both innate and adaptive immunity. One key phenomenon is the apoptosis of B and CD4+ and CD8+ T cells and dendritic cells causing an acquired immune deficiency syndrome linked to an unfavorable prognosis. Depletion of T cells is further augmented by increased expression of programmed cell death 1 (PD1) and upregulation of its ligand (PDL1) on various immune and epithelial cells. The reprogramming of antigen-presenting cells results in reduced human leukocyte

Table 2.1 Immunomodulatory effects of tetracyclines in preclinical and clinical models of acute respiratory distress syndrome (ARDS)

Author	Year	Tetracycline	Model	Stimulants or pathogens	Immune response
Peukert et al. [5]	2021	Tetracycline	Mouse, human (*ex vivo*)	LPS, H1N1 influenza virus (mouse); viral, bacterial, and non-pulmonary ARDS (human)	IL-1ß, IL-18, caspase-1 activation, neutrophil influx ↓, survival ↑
Zhang et al. [20]	2019	Doxycycline	Mouse	Paraquat	MMP-9, MPO, neutrophil influx ↓
Wang et al. [24]	2014	Doxycycline	Rat	Cardiopulmonary bypass	TNF-α, IL-1ß, MMP-9 ↓
Zhang et al. [17]	2014	Doxycycline	Dog	Cardiopulmonary bypass	MMP-9, MPO, neutrophil influx ↓
Roy et al. [16]	2012	CMT-3	Pig	Ischemia by clamping of SMA, placement of fecal clot in peritoneum	TNF-α, IL-1ß, Il-6, IL-8, IL-10 ↓, MMP-2, -9, NE, survival ⇔
Ng et al. [27]	2012	Doxycycline	Mouse	H3N2 influenza virus	MMP-2, MMP-9, T1-α, thrombomodulin, neutrophil influx ↓
Moon et al. [22]	2012	Doxycycline	Mouse	LPS	Syndecan-1 (MMP-7 substrate), neutrophil influx ↓
Zhou et al. [25]	2010	CMT-3	Sheep	3rd degree burn, smoke inhalation, barotrauma	MMP-2 ↓, MMP-9 ⇔, survival ↑
Sochor et al. [21]	2009	Doxycycline	Rat	Acute pancreatitis (intraductal glycodeoxycholic acid, cerulein)	MMP-9, neutrophil influx ↓
Fujita et al. [26]	2007	Doxycycline	Mouse	LPS or *Streptococcus pneumoniae*	MMP-2, -9, neutrophil influx ↓, survival ↑
Kim et al. [14]	2006	CMT-3	Rat	Ventilation	MMP-9, MPO, neutrophil influx ↓
Fujita et al. [28]	2006	Doxycycline	Mouse	Bleomycin	MMP-2, -9, neutrophil influx ↓
Steinberg et al. [19]	2005	CMT-3	Pig	Ischemia by clamping of SMA, placement of fecal clot in peritoneum	IL-6, IL-8, IL-10, NE ↓, IL-1, MMP-2, -9, neutrophil influx ⇔, survival ↑
Steinberg et al. [15]	2003	CMT-3	Rat	Cecal ligation and puncture	MMP-2, MMP-9 ↓, survival ↑
Carney et al. [23]	2001	CMT-3	Pig	LPS	MMP-2, MMP-9, neutrophil influx ↓
McCann et al. [29]	1999	CMT-3	Pig	Cardiopulmonary bypass, LPS	Neutrophil influx ↓
Carney et al. [13]	1999	CMT-3	Pig	Cardiopulmonary bypass, LPS	MMP-2, MMP-9, NE, neutrophil influx ↓, survival ↑

↑ significant increase, ↓ significant decrease, ⇔ no significant difference, *CMT-3* chemically modified tetracycline 3, *IL* interleukin, *LPS* lipopolysaccharide, *MMP* metalloproteinase, *MPO* myeloperoxidase, *NE* neutrophil elastase, *SMA* superior mesenteric artery, *TNF-α* tumor necrosis factor alpha, *T1-α* membrane protein of alveolar type I epithelium

Table 2.2 Immunomodulatory effects of tetracyclines in preclinical and clinical models of sepsis

Author	Year	Tetracycline	Model	Stimulants or pathogens	Immune response
Colaço et al. [6]	2021	Doxycyline	Mouse	*E. coli*, H1N1 influenza virus, *C. albicans*, *Plasmodium berghei*	Liver, lung, kidney injury ↓, mitochondrial protein synthesis ↓; FAO, steroid sensitivity, survival ↑
Patel et al. [7]	2020	Doxycycline	Mouse	Cecal ligation and puncture	TNF-α, IL-1ß, IL-6, MPO ↓, survival ↑
Sun et al. [35]	2020	Minocycline	Human THP-1 monocytes	LPS	TNF-α, IL-8, MIP-1α, MIP-1ß ↓, modulated NF-κB-, p38-, ERK1/2-pathways
Sun et al. [34]	2015	Minocycline, tigecycline, doxycycline	Human THP-1 monocytes	LPS	Autophagy ↑ by inhibiting mTOR; TNF-α, IL-8 ↓
Nukarinen et al. [48]	2015	Doxycycline	RCT	Severe sepsis or septic shock	MMP-8, -9, TIMP-1 ⇔
Fredeking et al. [47]	2015	Doxycycline	RCT	Dengue virus	IL-6, TNF-α, mortality ↓
Bode et al. [45]	2014	Doxycycline	Human THP-1 monocytes, PBMCs (*ex vivo*)	LPS, E. coli	Phagocytosis, IL-1ß, IL-6 ↓, TLR-1, TLR-4, TLR-6 ↓
Tai et al. [41]	2013	Minocycline	Human THP-1 monocytes	LPS	TNF-α, IL-6, IFN-γ, IL-8, IP-10, MCP-1, MIP-1α, MIP-1ß, RANTES, eotaxin ↓, IKKα/β phosphorylation inhibited
Pang et al. [33]	2012	Minocycline	Human monocytes (*ex vivo*)	LPS	TNF-α, IL-1ß, IL-6, COX-2, PGE_2 ↓, LOX-1, NF-κB, LITAF, Nur77, PI3K/Akt-, p38-MAPK pathway ↓
Castro et al. [46]	2011	Tetracycline, doxycycline	RCT	Dengue virus	IL-6, Il-1ß, TNF-α ↓, IL-1ra ↑, TNF-R1 ⇔
Maitra et al. [40]	2005	CMT-3	Rat	Cecal ligation and puncture	Liver injury, MMP-9, MMP-2, TGF-ß1, caspase-3 ↓, survival ↑
Maitra et al. [38]	2004	CMT-3	Rat	Cecal ligation and puncture	TNF-α ↓, p38-, p42/44–MAPK activation inhibited, survival ↑
Maitra et al. [39]	2003	CMT-3	Rat	Cecal ligation and puncture	Liver injury, NO, MMP-9 ↓, survival ↑

Table 2.2 (continued)

Author	Year	Tetracycline	Model	Stimulants or pathogens	Immune response
D'Agostino et al. [44]	2001	CMTs,	Murine J774 macrophages	LPS	TNF-α, IL-10 ⇔, iNOS, nitrite, NO, Il-12 ↓, cytotoxity ↑
Patel et al. [42]	1999	CMTs, minocycline	Murine RAW 264.7 cells, human A 549 cells	LPS	PGE_2, nitrite ↓ (CMT-3)
D'Agostino et al. [36]	1998	Doxycycline	Mouse, murine macrophages	LPS	NO ↓, survival ↑
Amin et al. [43]	1997	CMTs, doxycycline	Murine macrophages	LPS	iNOS mRNA accumulation and protein expression ↓
Milano et al. [37]	1997	Tetracycline	Mouse, murine macrophages	LPS	TNF-α, IL-1α, nitrate, iNOS activity ↓, macrophages: NO ↓, TNF-α, IL-1α ⇔, survival ↑

↑ significant increase, ↓ significant decrease, ⇔ no significant difference, *C. albicans Candida albicans*, *CMT-3* chemically modified tetracycline 3, *COX-2* cyclooxygenase 2, *E. coli Escherichia coli, ERK* extracellular-signal regulated kinases, *FAO* fatty acid oxidation, *IFN* interferon, *IKK* inhibitor of nuclear factor kappa B kinase, *IL* interleukin, *IL-1ra* interleukin-1 receptor antagonist, *iNOS* inducible nitric oxide synthase, *IP-10* interferon gamma induced protein 10, *LITAF* lipopolysaccharide induced TNF factor, *LPS* lipopolysaccharide, *LOX-1* lectin-like oxidized low density lipoprotein receptor-1, *MMP* metalloproteinase, *MAPK* mitogen-activated protein kinase, *MCP* monocyte chemoattractant protein, *MIP* macrophage inflammatory protein, *MPO* myeloperoxidase, *mTOR* mammalian target of rapamycin, *NF-κB* nuclear factor kappa-light-chain-enhancer of activated B-cells, *NO* nitric oxide, *PBMCs* peripheral blood mononuclear cells, PGE_2 prostaglandin E_2, *PI3k* phosphatidylinositol-3-kinase, *RANTES* regulated upon activation, normal T cell expressed and presumably secreted, *RCT* randomized controlled trial, *TGF-β1* transforming growth factor beta 1, *TIMP-1* tissue inhibitor of metalloproteinase-1, *TLR* toll-like receptor, *TNF-α* tumor necrosis factor alpha, *TNF-R1* tumor necrosis factor receptor 1

antigen-antigen D related (HLA-DR) expression on monocytes and impaired production of pro-inflammatory mediators, including TNF-α, IL-6, Il-1ß, and IFN-γ referred to as "immunoparalysis". Although these compensatory mechanisms attempt to restore immune homeostasis, a subtype of patients develops persistent inflammation, immunosuppression, and catabolism syndrome (PICS), which is predictive of a poor outcome. It is most likely caused by persistent inflammation through a constant release of DAMPs driving organ injury [1].

2.3 Immunopathogenesis of ARDS

Sepsis and ARDS have similar underlying mechanisms: ARDS, defined as a life-threatening form of respiratory failure, is driven by an uncontrolled inflammatory host response induced by direct (pulmonary) or indirect (extrapulmonary) insults.

The most common causes include sepsis, viral and bacterial pneumonia, aspiration of gastric contents, and major trauma [2].

Inflammasome activation plays a central role in the development of ARDS [5]. In general, inflammasomes are multiprotein complexes that consist of a sensor NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3), an adaptor apoptosis-associated speck-like protein containing a CARD (ASC), and an effector (caspase-1) [10]. Inflammasome activation generates IL-1ß and IL-18 which both drive the inflammatory cascade forward and are linked to an unfavorable prognosis [5]. This process involves two signals. Inflammasomes assemble downstream of PRRs in response to PAMPs and DAMPs. For example, LPS binding to Toll-like receptor 4 (TLR4) leads to the translocation of NF-κB into the nucleus and the transcription of pro-inflammatory mediators and inflammasome components including pro-caspase-1, pro–IL-1β, and pro-IL-18 (signal 1). Various stimuli such as ATP, viral RNA, and pore-forming toxins activate the sensor NLRP3, resulting in inflammasome assembly via ASC oligomerization (signal 2). Active capase-1 converts pro-IL-1β and pro-IL-18 into their mature forms causing pyroptotic cell death [5, 10, 11]. Inflammation and pyroptosis mediate substantial epithelial and endothelial injury with a subsequent loss of the alveolar-capillary barrier integrity, leading to influx of protein-rich edema fluid and immune cells into the alveoli [2]. This exudative edema causes dysfunctional surfactant and atelectasis, which in turn can predispose patients to biophysical injury of the lungs [2].

The influx of immune cells (especially neutrophils) triggered by the activation of TLRs on alveolar type II cells and resident macrophages is a salient feature of ARDS [12]. As neutrophils begin their transepithelial migration into the lungs, they become primed to phagocytose invading microbes and release toxic mediators including reactive oxygen species (ROS), neutrophil elastase, proteases, and nitric oxide (NO). Proteases such as metalloproteinases (MMPs) contribute to the disruption of the barrier integrity and lung parenchyma by degrading collagen [12–15]. Both neutrophil elastase and MMPs are known to promote lung injury in patients with ARDS [15]. Lastly, persistent inflammation and unbalanced immune homeostasis can further intensify existing lung damage and cause lasting injury and fibrosis [2].

2.4 Mechanisms of Action of Tetracyclines in ARDS

In vitro and *in vivo* studies have highlighted the wide-range of immunomodulatory effects of tetracyclines in models of ARDS, pneumonia, and sepsis [7, 16, 17]. They improve survival and organ injury by modulating a plethora of inflammatory pathways that become dysregulated in critically ill patients [1, 5, 6, 18]. In ARDS, tetracyclines decrease a variety of inflammatory mediators, including inflammasome-dependent IL-1ß and IL-18 secretion, which drives ARDS development [5, 16, 19]. Furthermore, they impair the breakdown of extracellular matrix components and inhibit neutrophil infiltration [14, 17, 20–23] (Fig. 2.1).

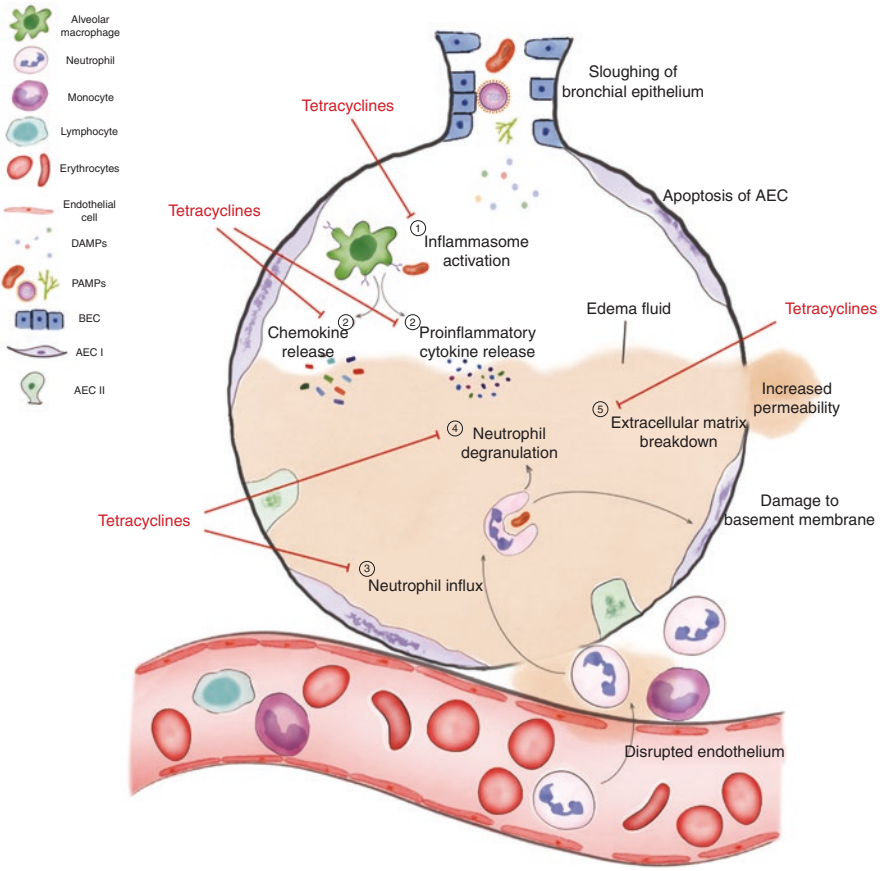

Fig. 2.1 The immunomodulatory effects of tetracyclines in ARDS. ① By sensing pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs), Toll-like receptors (TLRs) become activated, thereby triggering the activation of the NLRP3 inflammasome. Tetracyclines inhibit the activation of nuclear factor-kappa B (NF-κB) and the NLRP3 inflammasome and subsequent ② release of proinflammatory cytokines and chemokines causes impaired ③ chemotaxis of immune cells including neutrophils. Tetracyclines further block ④ neutrophil degranulation and ⑤ extracellular matrix breakdown. ⊥ inhibition, *AEC I* type I alveolar epithelial cell, *AEC II* type II alveolar epithelial cell, *BEC* bronchial epithelial cell

2.4.1 *In Vivo* Models

2.4.1.1 Effects on Inflammatory Cytokines and NLRP3 Inflammasome Caspase-1 Signaling

Tetracyclines significantly reduce the secretion of pro-inflammatory cytokines, including TNF-α, IL-1ß, IL-6, and IL-8, thereby improving survival and lung injury in indirect models of ARDS [16, 19, 24].

Current research suggests that a key mechanism underlying the immunomodulatory effects of tetracyclines is the inhibition of the secretion of the pro-inflammatory

cytokines IL-1ß and Il-18 via the NLRP3 inflammasome pathway. In a recent study, tetracycline significantly reduced both LPS- and influenza-induced lung injury in mice by inhibiting inflammasome-caspase-1 dependent IL-1ß and IL-18 production. This effect was mediated by direct inhibition of caspase-1 activation by tetracycline [5].

2.4.1.2 Effects on MMPs

The best described property of tetracyclines is the inhibition of MMPs in ARDS. MMPs are a family of zinc-dependent endopeptidases that degrade the basement membrane as well as extracellular matrix components and are involved in numerous pathological conditions including inflammation, tissue remodeling, and tumorigenesis. They are produced by a variety of cells including stromal, epithelial, and inflammatory cells. Tetracyclines directly inhibit MMP activity by chelating Zn^{2+} ions from their active site and by inhibiting their transcription [3].

The potential role of tetracyclines as MMP inhibitors in the pathogenesis of ARDS has been investigated in several animal studies. Carney et al. [23] showed that pigs pretreated with chemically modified tetracycline 3 (CMT-3) 12 h prior to intravenous LPS developed less lung injury, less edema and hypoxia by inhibiting MMP-9 and MMP-2. Additionally, plateau airway pressure was decreased [23]. Similar results were achieved by the same group through the inhibition of gelatinases and neutrophil elastase by CMT-3 in a porcine cardiopulmonary bypass and LPS-induced lung injury model. The survival rate was increased from 60 to 100% by CMT-3 treatment [13]. Steinberg et al. [15] demonstrated that blockage of MMP-2 and MMP-9 by CMT-3 was associated with less edema and histological lung injury as well as increased survival in an indirect model of ARDS in rats subjected to cecal ligation and puncture. Of note, CMT-3 prevented all the histopathological changes seen in ARDS [15]. Although not statistically significant, the authors observed a 64% reduction in MMP-2 activity and a 34% reduction in MMP-9 activity in bronchoalveolar lavage (BAL) fluid in a porcine model of ARDS [19]. Furthermore, administration of CMT-3 improved hemodynamics, gas exchange, lung histology, and survival through the inhibition of MMP-2 in an ovine ARDS model induced by third-degree burns, smoke inhalation and barotrauma injuries [25]. MMP-9 levels were not affected by CMT-3 but levels were only measured in plasma and not in BAL fluid as opposed to in the studies described earlier [25]. Levels of MMP-2, MMP-9, and neutrophil elastase measured in plasma were also not affected by CMT-3 in a cecal ligation and puncture-induced ARDS model [16]. The authors concluded that this might be due to the use of ketamine, which has been shown to weaken the effects of cecal ligation and puncture in rats via the inhibition of NF-κB.

Doxycycline has also been described as another potent MMP inhibitor in various animal models of primary and secondary ARDS [17, 20, 21, 24, 26, 27]. In a pancreatitis-induced ARDS model, doxycycline reduced MMP-9 levels which correlated with decreased pulmonary edema and hemorrhage [21]. Similar results were reproduced in cardiopulmonary bypass-induced ARDS models [20, 24]. The positive influence of doxycycline on endothelial barrier integrity was also demonstrated

by decreased levels of endothelial protein. Not only were MMP-2 and MMP-9 levels in BAL fluid reduced in a H3N2 influenza-induced ARDS model, so were concentrations of endothelial protein thrombomodulin and T1-α, a membrane protein of alveolar type I epithelium, indicating less alveolar capillary membrane damage [27]. Furthermore, doxycycline might attenuate the development of pulmonary fibrosis in ARDS through the inhibition of gelatinases [28].

2.4.1.3 Effects on Neutrophil Transmigration

One of the hallmarks of ARDS is the influx of neutrophils into the lungs. Tetracyclines attenuate neutrophil infiltration and thereby prevent ARDS, an effect possibly linked to the concomitant decrease of MMP levels [13, 14, 23, 26]. In a ventilation-induced lung injury (VILI) model, pretreatment with CMT-3 decreased neutrophil infiltration and myeloperoxidase levels, which correlated significantly with MMP-9 activity. The role of MMP-9 during neutrophil migration is, however, not well defined. As a proteinase, MMP-9 could potentially degrade the basement membrane and thereby facilitate migration [14]. In an *in vitro* experiment, neutrophil transmigration across Matrigel and MMP-9 levels in the Matrigel invasion chamber were reduced by doxycycline [21].

Pretreatment with CMT-3 inhibited neutrophil influx in models of bacterial- and cardiopulmonary bypass-induced ARDS [13, 23, 29]. Additionally, doxycycline prevented neutrophil infiltration in models of viral-, bacterial-, cardiopulmonary bypass- and pancreatitis-induced ARDS [17, 21, 22, 27, 30].

2.4.2 Human Data

Recently, Peukert et al. described the effect of tetracycline on the NLRP3 inflammasome pathway in patients with direct ARDS (Fig. 2.1). Human alveolar leukocytes were isolated within 24 h of onset of direct ARDS. Cultured leukocytes continued to produce IL-1ß and IL-18 suggesting that the NLRP3 inflammasome pathway remained intact. Tetracycline inhibited the production of IL-1ß and IL-18 by alveolar leukocytes in a dose-dependent manner. This study indicates that the inhibition of caspase-1-dependent IL-1ß and IL-18 by tetracyclines might be a new therapeutic approach in patients with direct ARDS [5].

A randomized clinical trial is currently investigating whether doxycycline can limit the NF-κB dependent release of pro-inflammatory cytokines and thereby prevent evolution towards ARDS in patients with COVID-19 (ClinicalTrials.gov Identifier: NCT04371952). In 89 high-risk COVID-19 patients living in long-term care facilities, it was recently shown that the administration of doxycycline within 12 h after symptom onset was associated with early clinical recovery, decreased hospitalization and reduced mortality [31]. These findings contradict the results of a randomized controlled trial which suggested doxycycline was not effective for suspected COVID-19 [32]. In this study, 798 participants received doxycycline compared to 994 participants randomized to standard care. However, the trial had several limitations: first, the trial included participants recruited

within 14 days after symptom onset. Second, almost half of the participants were accrued without PCR-confirmed severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection [32]. Because the inflammasome-caspase-1 pathway is activated early in ARDS [5, 8], this might explain why doxycycline was not beneficial.

2.5 Mechanisms of Action of Tetracyclines in Sepsis

Tetracyclines exert their pleiotropic immunomodulatory effects via several inflammatory pathways such as NF-κB and MAPKs downstream of PRRs whereby they inhibit the secretion of inflammatory mediators including cytokines, chemokines, MMPs, prostaglandin E_2 (PGE_2), and NO [7, 33–38]. They also ameliorate sepsis-induced liver injury by inhibiting apoptotic pathways [39, 40]. Mild perturbation of mitochondrial function by tetracyclines can install disease tolerance mechanisms like tissue repair and metabolic reprogramming [6].

2.5.1 *In Vitro* Models

2.5.1.1 Effects on Cytokine and Chemokine Production

An uncontrolled host response to infection can trigger a so-called cytokine storm which is one of the main characteristics of sepsis [1]. Mounting evidence has identified autophagy as an important regulator of excessive inflammation. Sun et al. [35] have shown that minocycline, which induces autophagy by inhibiting the mammalian target of rapamycin (mTOR) signaling pathway, suppresses cytokine production and cell proliferation, and protects human THP-1 cells from LPS-toxicity. Additionally, the study suggests that the IKK/NF-κB signal pathway was linked to minocycline-induced autophagy [35]. A previous study also demonstrated that minocycline decreased cytokine and chemokine production by inhibiting IKKα/β phosphorylation in LPS-stimulated THP-1 cells [41]. The influence of tetracyclines on certain signaling pathways was further characterized by Sun et al. [34]. The modulated phosphorylation of the NF-κB-, p38- and ERK1/2-pathways by doxycycline, minocycline, and tigecycline significantly inhibited the expression of TNF-α, IL-8, macrophage inflammatory protein 1α (MIP-1α) and MIP-1ß by LPS-stimulated THP-1 cells [34].

2.5.1.2 Effects on Arachidonic Acid Metabolites and NO Production

Metabolites of arachidonic acid, such as PGE_2 and NO, are inhibited by tetracyclines and play a role in inflammatory processes [42]. CMT-3 inhibited both nitrite and the cyclooxygenase 2 (COX-2) mediated PGE_2 accumulation in murine macrophages stimulated with LPS [42]. Moreover, tetracyclines regulate inducible NO synthase (iNOS) at the post-transcriptional level, thereby decreasing NO levels in LPS-stimulated murine macrophages [36, 37, 43, 44].

2.5.2 *In Vivo* Models

2.5.2.1 Effects on MAPK Signaling Pathways and Inflammatory Mediators

Doxycycline ameliorated systemic and pulmonary inflammation in a murine sepsis model induced by cecal ligation and puncture [7]. By decreasing levels of IL-1ß, IL-6, TNF-α, myeloperoxidase (MPO), and the antioxidant glutathione in plasma and lung homogenates, doxycycline improved survival. The anti-inflammatory effect of CMT-3 is possibly mediated through the inhibition of MAPKs. In rats subjected to cecal ligation and puncture, pretreatment with CMT-3 inhibited TNF-α secretion and activation of p38 and p42/44–MAPK pathways, thereby preventing the progression to septic shock [38].

Tetracyclines also act as inhibitors of NO synthesis. In mice injected intraperitoneally with LPS, doxycycline prevented septic shock by inhibiting nitrate production by an IL-10 independent mechanism [36]. Furthermore, tetracyclines caused a decrease in iNOS activity in a similar sepsis model [37].

2.5.2.2 Effects on Organ Dysfunction

Maitra et al. [39] showed that CMT-3 improved survival and was hepatoprotective in rats subjected to sepsis by cecal ligation and puncture. They demonstrated that the underlying mechanisms by which CMT-3 improved survival and hepatic injury were the CMT-3 induced reduction of MMP-9 and NO. The hepatoprotective effect of CMT-3 was further characterized by the same group. Administration of CMT-3 in septic rats caused decreased levels of MMP-9 and increased the expression of tissue inhibitor of metalloproteinase-1 (TIMP-1), which is an *in vivo* inhibitor of MMP-9. Furthermore, transforming growth factor beta-1 (TGF-ß1) and caspase-3 were reduced, thereby preventing liver injury and increasing survival in septic rats [40]. Recently, Colaço et al. [6] demonstrated how doxycycline can protect against sepsis by inducing disease tolerance without diminishing bacterial load in a mouse model of bacterial sepsis. In this study, tissue damage of the liver, lungs, and kidneys on a molecular and histopathological level was reduced by treatment with doxycycline. Furthermore, the authors demonstrated similar protective effects in influenza-induced sepsis in contrast to fungal- or cerebral malaria-induced infection models. Bulk RNA sequencing showed that doxycycline altered the expression of genes involved in epithelial cell differentiation suggesting more effective lung repair without the development of lung fibrosis. Functional analysis found a cluster of down-regulated genes related to decreased liver collagen production indicating that doxycycline potentially plays a role in limiting liver fibrosis. During infection, livers of septic mice accumulated acylcarnitines and steroids. Administration of doxycycline partially decreased this accumulation suggesting that it might reverse the block in mitochondrial import of fatty acids during sepsis. It also increased the activation of glucocorticoid receptors through serine phosphorylation. Furthermore, it was shown that mild perturbation of mitochondrial function, like the electron transport chain, by doxycycline can activate disease tolerance mechanisms, such as