

REVIEW

Open Access



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

Andrea Sauer, Christian Putensen and Christian Bode\*

## Abstract

This article is one of ten reviews selected from the Annual Update in Intensive Care and Emergency Medicine 2022. Other selected articles can be found online at <https://www.biomedcentral.com/collections/annualupdate2022>. Further information about the Annual Update in Intensive Care and Emergency Medicine is available from <https://link.springer.com/bookseries/8901>.

## 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 pre-clinical and clinical trials, no effective pharmacotherapies exist to improve outcomes in patients 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 immuno-modulatory effects that may be able to rebalance immune homeostasis in critically ill patients. Their beneficial anti-inflammatory effects have been reported for chronic 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 1 and 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.

\*Correspondence: christian.bode@ukbonn.de  
Department of Anesthesiology and Intensive Care Medicine, University Hospital Bonn, Bonn, Germany

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

$\uparrow$  significant increase,  $\downarrow$  significant decrease,  $\leftrightarrow$  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- $\alpha$  tumor necrosis factor alpha, T1- $\alpha$  membrane protein of alveolar type I epithelium

### Immunopathogenesis of Sepsis

Sepsis is a heterogeneous 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 (LTA), 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- $\kappa$ B] and mitogen-activated protein kinase [MAPK]) leads to the transcription of genes encoding pro-inflammatory cytokines and chemokines such as tumor necrosis factor (TNF)- $\alpha$ , interleukin (IL)-1 $\beta$ , IL-8, IL-18 and interferons (IFN). The expression of IL-1 $\beta$  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

**Table 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	Doxycycline	Mouse	<i>E. coli</i> , H1N1 influenza virus, <i>C. albicans</i> , <i>Plasmodium berghei</i>	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-1β, IL-6, MPO ↓, survival ↑
Sun et al. [35]	2020	Minocycline	Human THP-1 monocytes	LPS	TNF-α, IL-8, MIP-1α, MIP-1β ↓, 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 ( <i>ex vivo</i> )	LPS, <i>E. coli</i>	Phagocytosis, IL-1β, 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-1β, RANTES, eotaxin ↓, IKKα/β phosphorylation inhibited
Pang et al. [33]	2012	Minocycline	Human monocytes ( <i>ex vivo</i> )	LPS	TNF-α, IL-1β, IL-6, COX-2, PGE2 ↓, LOX-1, NF-κB, LITAF, Nur77, PI3K/ Akt-, p38-MAPK pathway ↓
Castro et al. [46]	2011	Tetracycline, doxycycline	RCT	Dengue virus	IL-6, IL-1β, 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-β1, 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 ↑
D'Agostino et al. [44]	2001	CMTs,	Murine J774 macrophages	LPS	TNF-α, IL-10 ↔, iNOS, nitrite, NO, IL-12 ↓, cytotoxicity ↑
Patel et al.	1999	CMTs, minocycline	Murine RAW264.7 cells, human A549 cells	LPS	PGE2, 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 lipopoly- saccharide 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, PGE2 prostaglandin E2, 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

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 antigen-antigen D related

(HLA-DR) expression on monocytes and impaired production of pro-inflammatory mediators, including TNF-α, IL-6, IL-1β, 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].

### 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-1 $\beta$  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- $\kappa$ B into the nucleus and the transcription of pro-inflammatory mediators and inflammasome components including pro-caspase-1, pro-IL-1 $\beta$ , 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 caspase-1 converts pro-IL-1 $\beta$  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].

### 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-1 $\beta$  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. 1).

#### *In Vivo* Models

##### Effects on Inflammatory Cytokines and NLRP3

##### Inflammasome Caspase-1 Signaling

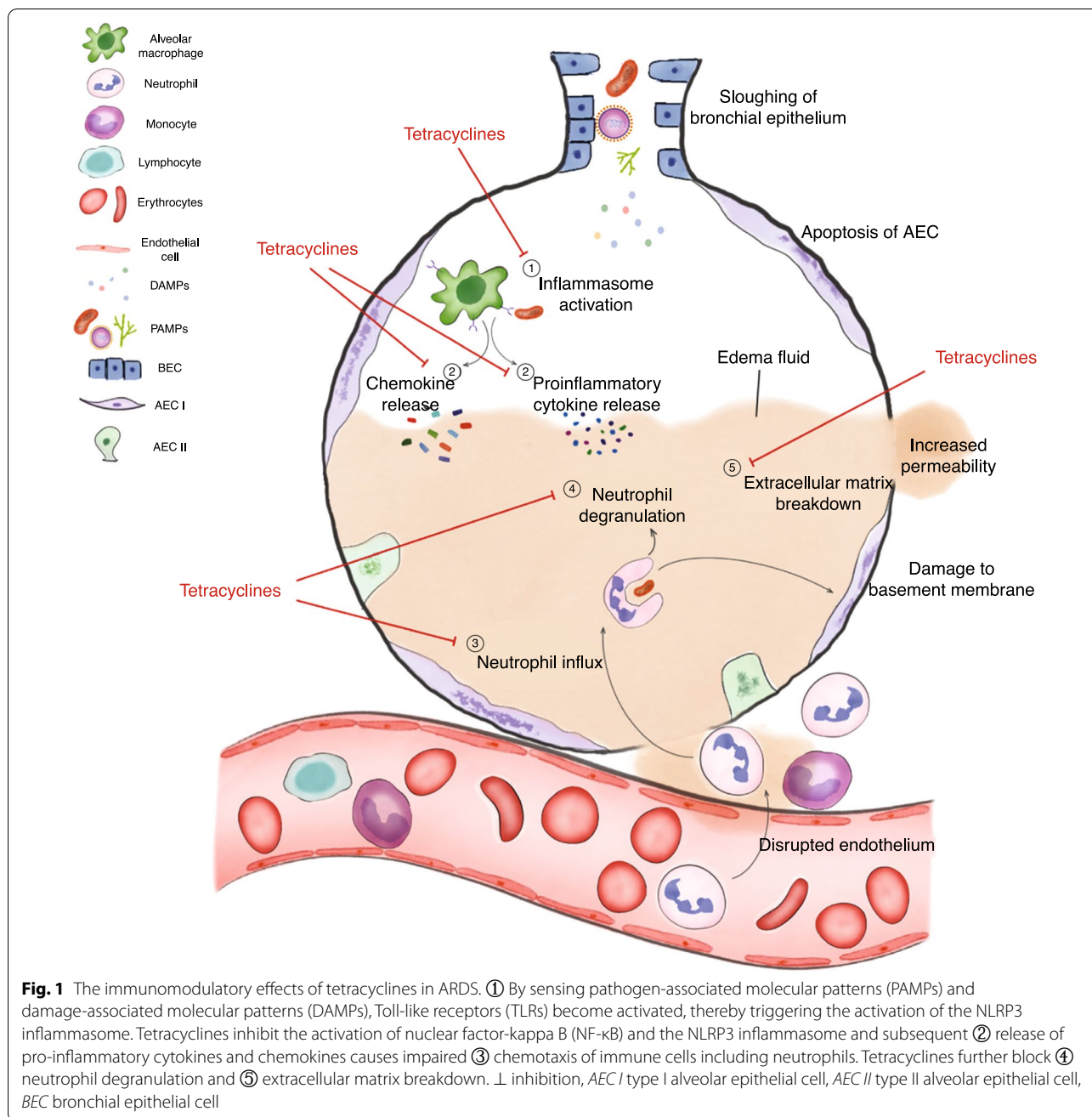
Tetracyclines significantly reduce the secretion of pro-inflammatory cytokines, including TNF- $\alpha$ , IL-1 $\beta$ , IL-6, and IL-8, thereby improving survival and lung injury in indirect models of ARDS [16, 19, 24].

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-1 $\beta$  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-1 $\beta$  and IL-18 production. This effect was mediated by direct inhibition of caspase-1 activation by tetracycline [5].

##### 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<sup>2+</sup> 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- $\kappa$ B.

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- $\alpha$ , 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].

### 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].

### Human Data

Recently, Peukert et al. described the effect of tetracycline on the NLRP3 inflammasome pathway in patients with direct ARDS (Fig. 1). Human alveolar leukocytes were isolated within 24 h of onset of direct ARDS. Cultured leukocytes continued to produce IL-1 $\beta$  and IL-18 suggesting that the NLRP3 inflammasome pathway remained intact. Tetracycline inhibited the production of IL-1 $\beta$  and IL-18 by alveolar leukocytes in a dose-dependent manner. This study indicates that the inhibition of caspase-1-dependent IL-1 $\beta$  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- $\kappa$ B 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 longterm 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 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.

### Mechanisms of Action of Tetracyclines in Sepsis

Tetracyclines exert their pleiotropic immunomodulatory effects via several inflammatory pathways such as NF- $\kappa$ B and MAPKs downstream of PRRs whereby they inhibit the secretion of inflammatory mediators including cytokines, chemokines, MMPs, prostaglandin E2 (PGE2), 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].

## **In Vitro Models**

### **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- $\kappa$ B signal pathway was linked to minocycline-induced autophagy [35]. A previous study also demonstrated that minocycline decreased cytokine and chemokine production by inhibiting IKK $\alpha$ / $\beta$  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- $\kappa$ B-, p38- and ERK1/2-pathways by doxycycline, minocycline, and tigecycline significantly inhibited the expression of TNF- $\alpha$ , IL-8, macrophage inflammatory protein 1 $\alpha$  (MIP-1 $\alpha$ ) and MIP-1 $\beta$  by LPS-stimulated THP-1 cells [34].

### **Effects on Arachidonic Acid Metabolites and NO Production**

Metabolites of arachidonic acid, such as PGE2 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 PGE2 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].

### **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-1 $\beta$ , IL-6, TNF- $\alpha$ , 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- $\alpha$  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].

### **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- $\beta$ 1) 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 tissue repair and metabolic reprogramming in sepsis. One of the underlying mechanisms could be the doxycycline-induced sensitization to adrenergic agonists that reduces lipid accumulation in the liver. This elegant study demonstrates how doxycycline may rebalance immune homeostasis in sepsis [6].

## Human Data

Minocycline significantly ameliorated the LPS-induced inflammatory response in human monocytes obtained from healthy volunteers by decreasing the production of TNF- $\alpha$ , IL-1 $\beta$ , IL-6, COX-2, and PGE2, as well as reducing activation of lectin like oxidized low density lipoprotein receptor-1 (LOX-1), TNF- $\alpha$  factor (LITAF), and Nur77 nuclear receptor. The immunomodulatory effects of minocycline were mediated through the blocked activation of NF- $\kappa$ B, p38 MAPK, and phosphoinositide 3-kinase (PI3K)/Akt pathways [33]. Consistent with this, another study demonstrated that doxycycline reduced the LPS-induced gene expression of IL-1 $\beta$  and IL-6 as well as phagocytosis of heat-inactivated *Escherichia coli* by monocytes [45].

The Fredeking group investigated the impact of tetracyclines on the inflammatory response in patients with dengue hemorrhagic fever in two randomized controlled trials [46, 47]. In the first trial, hospitalized patients received usual care or usual care combined with doxycycline or tetracycline. Serum cytokine and cytokine receptor levels were determined on days 1, 3, and 7 of treatment. Doxycycline was significantly more effective in modulating levels of IL-6, IL-1 $\beta$ , TNF- $\alpha$ , IL-1 receptor antagonist (IL-1ra) and TNF-R1 than tetracycline. In the second trial with 231 participants, doxycycline treatment was associated with lower mortality, which positively correlated with reduced levels of pro-inflammatory cytokines. Patients in the doxycycline arm had a 46% lower mortality than those in the usual care arm [47].

In a prospective randomized placebo-controlled pilot trial, intravenous doxycycline did not have an impact on MMP-8, MMP-9, or TIMP-1 in patients with severe sepsis or septic shock [48]. This finding might be explained by the small sample size and large variance of disease severity that made it nearly impossible to detect a statistical significance. Only 23 patients were included in the analysis of this pilot trial. The studied population was also very heterogeneous in terms of disease severity and disease onset, which was reflected in the detected variation in baseline concentrations and activities of MMP-8, MMP-9, and TIMP-1 [48].

## Future Research Perspectives

ARDS and sepsis are both heterogeneous syndromes characterized by significant variability in the degree of immune dysregulation, disease severity, and prognosis. As described in this chapter, tetracyclines modulate immunity at multiple levels involving the inhibition of concurrent pro- and anti-inflammatory pathways.

Although our knowledge of the underlying mechanisms has increased, we need more clinical trials before

we can adopt tetracyclines as routine treatment in sepsis and ARDS.

To prevent the development of antibiotic resistance, we need to further investigate the use of non-antibacterial tetracycline derivatives such as CMT-3; their effectiveness has already been observed in preclinical trials [5, 16, 25]. Furthermore, research should focus on developing tetracyclines with minimal adverse effects and on augmenting certain immunomodulatory effects.

Most clinical trials of novel sepsis and ARDS therapies have focused on large patient cohorts without considering the heterogeneous nature of both syndromes and the varying immune responses of each patient. This has likely contributed to the fact that immunomodulatory drugs have not been demonstrated to have a clinical benefit in the past. Each patient needs to be longitudinally evaluated by using biomarkers and establishing baseline immune cell responsiveness [1]. In other words, immune profiling is necessary to stratify critical care patients in future trials and to identify biological subphenotypes in ARDS and sepsis. Calfee et al. [49] have described differential responses to treatment according to phenotype in ARDS. Predictive enrichment strategies are recommended to promote the efficiency of clinical trials and to enable precision medicine.

## Conclusion

In summary, tetracyclines have been shown to be potent drugs with pleiotropic immunomodulatory effects inhibiting multiple inflammatory pathways that trigger an uncontrolled immune response in ARDS and sepsis. In preclinical studies, tetracyclines have been described as promising immunomodulatory agents that seem to have the potential to correct the unbalanced immune homeostasis present in critically ill individuals. Future trials need to further investigate tetracyclines in terms of dosing and side effects as well as focus on preventing antibiotic resistance. Finally, the extent of inflammation and immunosuppression varies between each patient explaining why so many trials have failed to show a survival benefit for immunomodulatory drugs in the past. We need to identify subphenotypes of ARDS and sepsis that will respond to adjunctive tetracycline treatment.

## Acknowledgements

None.

## Authors' contributions

Conceptualization: AS, CP, and CB; Writing—original draft: AS and CB; Writing (review and editing): AS, CP and CB. All authors read and approved the final manuscript.

## Funding

Publication costs for this article were funded by the authors' institutions.



## Declarations

### Ethics approval and consent to participate

Not applicable

### Consent for publication

Not applicable

### Availability of data and materials

Not applicable

### Competing interests

The authors declare that they have no competing interests

Published online: 22 March 2022

## References

- Steinhagen F, Schmidt SV, Schewe JC, Peukert K, Klinman DM, Bode C. Immunotherapy in sepsis—brake or accelerate? *Pharmacol Ther*. 2020;208:107476.
- Matthay MA, Zemans RL, Zimmerman GA, et al. Acute respiratory distress syndrome. *Nat Rev Dis Prim*. 2019;5:18.
- Griffin MO, Fricovsky E, Ceballos G, Villarreal F. Tetracyclines: a pleiotropic family of compounds with promising therapeutic properties. Review of the literature. *Am J Physiol Cell Physiol*. 2010;299:C539–48.
- Smith-Norowitz TA, Weaver D, Norowitz YM, et al. Doxycycline suppresses *Chlamydia pneumoniae* induced interferon-gamma responses in peripheral blood mononuclear cells in children with allergic asthma. *J Infect Chemother*. 2018;24:470–5.
- Peukert K, Fox M, Schulz S, et al. Inhibition of caspase-1 with tetracycline ameliorates acute lung injury. *Am J Respir Crit Care Med*. 2021;204:53–63.
- Colaço HG, Barros A, Neves-Costa A, et al. Tetracycline antibiotics induce host-dependent disease tolerance to infection. *Immunity*. 2021;54:53–67.
- Patel A, Khande H, Periasamy H, Mokale S. Immunomodulatory effect of doxycycline ameliorates systemic and pulmonary inflammation in a murine polymicrobial sepsis model. *Inflammation*. 2020;43:1035–43.
- Opitz B, van Laak V, Eitel J, Suttrop N. Innate immune recognition in infectious and noninfectious diseases of the lung. *Am J Respir Crit Care Med*. 2010;181:1294–309.
- Zarrin AA, Bao K, Lupardus P, Vucic D. Kinase inhibition in autoimmunity and inflammation. *Nat Rev Drug Discov*. 2021;20:39–63.
- Guo H, Callaway JB, Ting JPY. Inflammasomes: mechanism of action, role in disease, and therapeutics. *Nat Med*. 2015;21:677–87.
- Hornung V, Latz E. Critical functions of priming and lysosomal damage for NLRP3 activation. *Eur J Immunol*. 2010;40:620–3.
- Zemans RL, Matthay MA. What drives neutrophils to the alveoli in ARDS? *Thorax*. 2017;72:1–3.
- Carney DE, Lutz CJ, Picone AL, et al. Matrix metalloproteinase inhibitor prevents acute lung injury after cardiopulmonary bypass. *Circulation*. 1999;100:400–6.
- Kim JH, Suk MH, Yoon DW, et al. Inhibition of matrix metalloproteinase-9 prevents neutrophilic inflammation in ventilator-induced lung injury. *Am J Physiol Lung Cell Mol Physiol*. 2006;291:L580–7.
- Steinberg J, Halter J, Schiller HJ, et al. Metalloproteinase inhibition reduces lung injury and improves survival after cecal ligation and puncture in rats. *J Surg Res*. 2003;111:185–95.
- Roy SK, Kubiak BD, Albert SP, et al. Chemically modified tetracycline 3 prevents acute respiratory distress syndrome in a porcine model of sepsis + ischemia/reperfusion-induced lung injury. *Shock*. 2012;37:424–32.
- Zhang C, Gong W, Liu H, Guo Z, Ge S. Inhibition of matrix metalloproteinase-9 with low dose doxycycline reduces acute lung injury induced by cardiopulmonary bypass. *Int J Clin Exp Med*. 2014;7:4975–82.
- Halter JM, Pavone LA, Steinberg JM, et al. Chemically modified tetracycline (COL-3) improves survival if given 12 but not 24 hours after cecal ligation and puncture. *Shock*. 2006;26:587–91.
- Steinberg J, Halter J, Schiller H, et al. Chemically modified tetracycline prevents the development of septic shock and acute respiratory distress syndrome in a clinically applicable porcine model. *Shock*. 2005;24:348–56.
- Zhang F, Hu L, Wu YX, et al. Doxycycline alleviates paraquat-induced acute lung injury by inhibiting neutrophil-derived matrix metalloproteinase 9. *Int Immunopharmacol*. 2019;72:243–51.
- Sochor M, Richter S, Schmidt A, Hempel S, Hopt UT, Keck T. Inhibition of matrix metalloproteinase-9 with doxycycline reduces pancreatitis-associated lung injury. *Digestion*. 2009;80:65–73.
- Moon A, Gil S, Gill SE, Chen P, Matute-Bello G. Doxycycline impairs neutrophil migration to the airspaces of the lung in mice exposed to intratracheal lipopolysaccharide. *J Inflamm (Lond)*. 2012;9:31.
- Carney DE, McCann UG, Schiller HJ, et al. Metalloproteinase inhibition prevents acute respiratory distress syndrome. *J Surg Res*. 2001;99:245–52.
- Wang CT, Zhang L, Wu HW, Wei L, Xu B, Li DM. Doxycycline attenuates acute lung injury following cardiopulmonary bypass: involvement of matrix metalloproteinases. *Int J Clin Exp Pathol*. 2014;7:7460–8.
- Zhou X, Wang D, Ballard-Croft CK, Simon SR, Lee HM, Zwischenberger JB. A tetracycline analog improves acute respiratory distress syndrome survival in an ovine model. *Ann Thorac Surg*. 2010;90:419–26.
- Fujita M, Harada E, Ikegame S, et al. Doxycycline attenuated lung injury by its biological effect apart from its antimicrobial function. *Pulm Pharmacol Ther*. 2007;20:669–75.
- Ng HH, Narasaraju T, Phoon MC, Sim MK, Seet JE, Chow VT. Doxycycline treatment attenuates acute lung injury in mice infected with virulent influenza H3N2 virus: involvement of matrix metalloproteinases. *Exp Mol Pathol*. 2012;92:287–95.
- Fujita M, Ye Q, Ouchi H, et al. Doxycycline attenuated pulmonary fibrosis induced by bleomy-cin in mice. *Antimicrob Agents Chemother*. 2006;50:739–43.
- McCann UG, Gatto LA, Searles B, et al. Matrix metalloproteinase inhibitor: differential effects on pulmonary neutrophil and monocyte sequestration following cardiopulmonary bypass. *J Extra Corpor Technol*. 1999;31:67–75.
- Liu J, Zhong X, He Z, et al. Effect of low-dose, long-term roxithromycin on airway inflammation and remodeling of stable noncystic fibrosis bronchiectasis. *Mediat Inflamm*. 2014;2014:708608.
- Alam MM, Mahmud S, Rahman MM, Simpson J, Aggarwal S, Ahmed Z. Clinical outcomes of early treatment with doxycycline for 89 high-risk COVID-19 patients in long-term care facilities in New York. *Cureus*. 2020;12:e9658.
- Butler CC, Yu LM, Dorward J, et al. Doxycycline for community treatment of suspected COVID-19 in people at high risk of adverse outcomes in the UK (PRINCIPLE): a randomised, controlled, open-label, adaptive platform trial. *Lancet Respir Med*. 2021;9:1010–20.
- Pang T, Wang J, Benicky J, Saavedra JM. Minocycline ameliorates LPS-induced inflammation in human monocytes by novel mechanisms including LOX-1, Nur77 and LITAF inhibition. *Biochim Biophys Acta*. 2012;1820:503–10.
- Sun J, Shigemami H, Tanaka Y, Yamauchi T, Ueda T, Iwasaki H. Tetracyclines downregulate the production of LPS-induced cytokines and chemokines in THP-1 cells via ERK, p38, and nuclear factor- $\kappa$ B signaling pathways. *Biochem Biophys Res*. 2015;4:397–404.
- Sun J, Shigemami H, Cao M, et al. Minocycline induces autophagy and inhibits cell proliferation in LPS-stimulated THP-1 cells. *Biomed Res Int*. 2020;2020:5459209.
- D'Agostino P, La Rosa M, Barbera C, et al. Doxycycline reduces mortality to lethal endotoxemia by reducing nitric oxide synthesis via an interleukin-10-independent mechanism. *J Infect Dis*. 1998;177:489–92.
- Milano S, Arcoleo F, D'Agostino P, Cillari E. Intraperitoneal injection of tetracyclines protects mice from lethal endotoxemia downregulating inducible nitric oxide synthase in various organs and cytokine and nitrate secretion in blood. *Antimicrob Agents Chemother*. 1997;41:117–21.
- Maitra SR, Bhaduri S, Chen E, Shapiro MJ. Role of chemically modified tetracycline on TNF- $\alpha$  and mitogen-activated protein kinases in sepsis. *Shock*. 2004;22:478–81.
- Maitra SR, Bhaduri S, Valane PD, Tervahartiala T, Sorsa T, Ramamurthy N. Inhibition of matrix metalloproteinases by chemically modified tetracyclines in sepsis. *Shock*. 2003;20:280–5.
- Maitra SR, Shapiro MJ, Bhaduri S, El-Maghrabi MR. Effect of chemically modified tetracycline on transforming growth factor-beta1 and caspase-3 activation in liver of septic rats. *Crit Care Med*. 2005;33:1577–81.

41. Tai K, Iwasaki H, Ikegaya S, Ueda T. Minocycline modulates cytokine and chemokine production in lipopolysaccharide-stimulated THP-1 monocytic cells by inhibiting I $\kappa$ B kinase  $\alpha/\beta$  phosphorylation. *Transl Res*. 2013;161:99–109.
42. Patel R, Attur MG, Dave M, et al. A novel mechanism of action of chemically modified tetracyclines: inhibition of COX-2-mediated prostaglandin E2 production. *J Immunol*. 1999;163:3459–67.
43. Amin AR, Patel RN, Thakker GD, Lowenstein CJ, Attur MG, Abramson SB. Post-transcriptional regulation of inducible nitric oxide synthase mRNA in murine macrophages by doxycycline and chemically modified tetracyclines. *FEBS Lett*. 1997;410:259–64.
44. D'Agostino P, Ferlazzo V, Milano S, et al. Anti-inflammatory effects of chemically modified tetracyclines by the inhibition of nitric oxide and interleukin-12 synthesis in J774 cell line. *Int Immunopharmacol*. 2001;1:1765–76.
45. Bode C, Diedrich B, Muenster S, et al. Antibiotics regulate the immune response in both presence and absence of lipopolysaccharide through modulation of Toll-like receptors, cytokine production and phagocytosis in vitro. *Int Immunopharmacol*. 2014;18:27–34.
46. Castro JEZ, Vado-Solis I, Perez-Osorio C, Fredeking TM. Modulation of cytokine and cytokine receptor/antagonist by treatment with doxycycline and tetracycline in patients with dengue fever. *Clin Dev Immunol*. 2011;2011:370872.
47. Fredeking TM, Zavala-Castro JE, González-Martínez P, et al. Dengue patients treated with doxycycline showed lower mortality associated to a reduction in IL-6 and TNF levels. *Recent Pat Antiinfect Drug Discov*. 2015;10:51–8.
48. Nukarinen E, Tervahartiala T, Valkonen M, et al. Targeting matrix metalloproteinases with intravenous doxycycline in severe sepsis—a randomised placebo-controlled pilot trial. *Pharmacol Res*. 2015;99:44–51.
49. Wilson JG, Calfee CS. ARDS subphenotypes: understanding a heterogeneous syndrome. *Crit Care*. 2020;24:102.

### **Publisher's Note**

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.