Immunomodulation by macrolides: therapeutic potential for critical care

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Critical illness is associated with immune dysregulation, characterised by concurrent hyperinflammation and immune suppression. Hyperinflammation can result in collateral tissue damage and organ failure, whereas immune suppression has been implicated in susceptibility to secondary infections and reactivation of latent viruses. Macrolides are a class of bacteriostatic antibiotics that are used in the intensive care unit to control infections or to alleviate gastrointestinal dysmotility. Yet macrolides also have potent and wide-ranging immunomodulatory properties, which might have the potential to correct immune dysregulation in patients who are critically ill without affecting crucial antimicrobial defences. In this Review, we provide an overview of preclinical and clinical studies that point to the beneficial effects of macrolides in acute diseases relevant to critical care, and we discuss the possible underlying mechanisms of their immunomodulatory effects. Further studies are needed to explore the therapeutic potential of macrolides in critical illness, to identify subgroups of patients who might benefit from treatment, and to develop novel non-antibiotic macrolide derivatives with improved immunomodulatory properties.

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Introduction

Immune dysregulation is ubiquitous in patients who are critically ill.[1–3] Sepsis exemplifies this dysregulation, in which pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) expressed by microbes and released from damaged tissue, respectively, initiate a strong inflammatory response by the binding of pattern recognition receptors, including Toll-like receptors (TLRs) and Nod-like receptors (NLRs), resulting in organ failure.[1] Immune suppression and exhaustion, occurring in parallel with hyperinflammation, predispose individuals to secondary infections and reactivation of latent viruses. Tissue damage in other critical illnesses—such as the acute respiratory distress syndrome (ARDS), polytrauma, and severe acute pancreatitis—induces a similar immune response. Those who survive this initial response often have severe long-term disturbances in immune function that are associated with increased mortality, including persistent inflammation, immunosuppression, and catabolism (known as post-intensive care syndrome).[3] However, despite three decades of trials that have explored the potential of treatments to moderate and control this immune dysregulation, no therapy has reached clinical practice.[2,4]

Macrolides are a class of bacteriostatic antibiotics that inhibit protein synthesis by binding to the bacterial ribosome, with a broad spectrum of activity against many gram-positive and some gram-negative bacteria. Erythromycin is a macrolide that also serves as a motilin receptor agonist and is therefore given in the intensive care unit (ICU) at lower doses to alleviate gastrointestinal dysmotility. Notably, macrolides also have potent and wide-ranging immunomodulatory potential, altering the immune response beyond simple suppression or stimulation.[5] Macrolides appear to expedite the return to immune homoeostasis and preserve or even enhance crucial antimicrobial defences.

Perhaps the most striking example of the immunomodulatory potency of macrolides comes from diffuse panbronchiolitis—an idiopathic, progressively destructive disease of the bronchioles—which can be transformed from a fatal to a treatable disease with daily low-dose erythromycin.[6] In addition to their well-established benefit in chronic airway diseases, including chronic obstructive pulmonary disease (COPD), cystic fibrosis, and bronchiectasis,[5] accumulating evidence suggests a

Key messages

- Excessive release of damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs) in critical illness can lead to severe and long-lasting immune dysregulation, characterised by concurrent hyperinflammation with organ failure and immune suppression associated with secondary infections
- Independent of their antimicrobial effects, macrolides modulate key pathways and mechanisms involved in this immune dysregulation, which improves survival and reduces tissue-destructive inflammation in animal models that are relevant to critical care
- The overall effect of macrolides is to dampen excessive and detrimental inflammation, and simultaneously to protect the host from secondary infections by enhancing specific immune functions and reducing bacterial virulence
- Clinical studies of the immunomodulatory effects of macrolides in critically ill patients are scarce, but these drugs show promise in improving survival and reducing the duration of symptoms in severely ill patients with pneumonia, sepsis, and acute respiratory distress syndrome
- Novel non-antibiotic macrolides with enhanced immunomodulatory capacity are in development and have been shown to alter the immune response and improve survival in preclinical studies

potential role for macrolides in correcting immune dysregulation in patients who are critically ill.

Strategies of immunomodulation in the ICU have received much attention in the past 30 years, but effective therapies remain unknown. In this Review, we discuss immunomodulation by macrolides in the acute setting, particularly relating to the ICU. We provide an overview of evidence from preclinical and clinical studies for the beneficial effects of macrolides in acute diseases relevant to critical care. We discuss the mechanisms underlying the immunomodulatory effects of macrolides, and conclude by exploring the potential of novel non-antibiotic macrolide derivatives.

See Online for appendix

Models of critical illness

The potential of immunomodulation by macrolides in the context of critical illness has been explored in numerous preclinical models pertaining to pneumonia, sepsis, and lung injury (appendix pp 2–3). The overall outcome can be summarised as follows: macrolides—either alone or in addition to an antibiotic that is effective against the pathogen—modulate the immune response

	Disease (participants)	Study design	Intervention	Main effect attributed to macrolide*
Ceccato et al,[17] 2019	Community-acquired pneumonia (n=1715)	Prospective, observational (1996–2016)	β-lactam and macrolide (n=932); β-lactam and fluoroquinolone, or fluoroquinolone alone (n=783)	Reduced mortality in patients with *Streptococcus pneumoniae* infection and a high inflammatory response (C-reactive protein >150 mg/L; OR 0·28, 95% CI 0·09–0·93)
Lorenzo et al,[18] 2015	Non-responding community-acquired pneumonia (n=52)	Prospective, observational	β-lactam and macrolide (n=23) or other regimens	Significantly lower IL-6 and TNF-α, and non-significantly lower IL-8 and IL-10, in bronchoalveolar lavage fluid; lower plasma IL-6, IL-8, and IL-10; and lower median time to clinical stability (8 days *vs* 14 days) in patients with nonresponsive community-acquired pneumonia receiving a macrolide-containing antibiotic regimen
Cilloniz et al,[19] 2015	Pneumococcal community-acquired pneumonia (n=643)	Retrospective analysis of prospective cohort (2000–13)	Macrolide, quinolone, β-lactam, or dual therapy with combinations of the above	Lower percentage of patients admitted to ICU (15 [21%] *vs* 14 [42%]) but no difference in 30-day mortality in patients with macrolide-resistant *S pneumoniae* receiving dual therapy that included a macrolide (n=71) versus other combinations (n=33)
van Delden et al,[20] 2012	Mechanically ventilated patients colonised with *Pseudomonas aeruginosa* (n=95)	Randomised controlled trial (2002–05)	Azithromycin (n=43) or placebo (n=42)	Pseudomonal ventilator-associated pneumonia in two patients in the azithromycin group versus six in the placebo group (p=0·16); in participants with *P aeruginosa* producing high levels of rhamnolipids: pseudomonal ventilator-associated pneumonia in one of five patients in the azithromycin group versus five of five in the placebo group (p=0·048)
Laserna et al,[21] 2014	Pseudomonal community-acquired pneumonia (n=402)	Retrospective, observational (2001–07)	Macrolide-containing regimen (n=171) or other regimen (n=231)	All patients: no benefit of macrolide versus no macrolide on 30-day mortality (31 [18·7%] *vs* 38 [16·5%]); ICU patients: no benefit of macrolide (n=61) versus no macrolide (n=75) on 30-day mortality (20 [32·8%] *vs* 21 [28·0%])
Restrepo et al,[22] 2009	Community-acquired pneumonia with sepsis (n=237)	Retrospective, observational (1999–2002)	Macrolide-containing regimen (n=104) or other regimen (n=133)	Lower 30-day mortality (HR 0·3, 95% CI 0·2–0·7) and lower 90-day mortality (0·3, 0·2–0·6) in patients on macrolide-containing regimen; benefit remained in patients with macrolide-resistant bacteria (HR 0·10, 95% CI 0·02–0·49)
Afshar et al,[23] 2016	Mechanically ventilated patients with sepsis (n=105)	Retrospective observational (2010–12)	Azithromycin-containing regimen (n=29) or other regimen	5·47 more 28-day ICU-free days in unadjusted analysis in azithromycin-treated patients; association significant in multivariable analysis and only in patients without pneumonia (n=74); trend towards reduced in-hospital mortality (one of 29 patients receiving azithromycin *vs* 13 of 76 patients in control group; p=0·07)
Giamarellos-Bourboulis et al,[24] 2008	Ventilator-associated pneumonia with sepsis (n=200)	Randomised controlled trial (2004–05)	Clarithromycin (n=100) or placebo (n=100)	No difference in 28-day mortality (31 [31%] for clarithromycin *vs* 28 [28%] for placebo); lower median time until resolution of ventilator-associated pneumonia (10·0 *vs* 15·5 days); lower median time until weaning from mechanical ventilation (16·0 *vs* 22·5 days); lower OR for mortality due to septic shock and multiple organ failure (clarithromycin: OR 3·78, 95% CI 1·36–10·45; placebo: 19·00, 5·64–64·03; p=0·043)
Giamarellos-Bourboulis et al,[25] 2014	Gram-negative sepsis (n=600)	Randomised controlled trial (2007–11)	Clarithromycin (n=302) or placebo (n=298)	No difference in 28-day mortality (56 [18·5%] for clarithromycin *vs* 51 [17·1%] for placebo); lower 28-day mortality in patients with septic shock and multiple organ failure with clarithromycin (15 of 28, 53·6%) versus placebo (19 of 26, 73·1%; p=0·020); lower 28-day mortality in patients with ARDS on clarithromycin (ten of 35, 28·5%) versus placebo (19 of 34, 55·9%; p=0·020); no difference in time until resolution of infection overall (median of 5 days in both groups); shorter time until resolution of infection in group with severe sepsis or septic shock on clarithromycin versus placebo (6 days *vs* 10 days; p=0·037)
Spyridaki et al,[26] 2012	Ventilator-associated pneumonia with sepsis (n=200)	Randomised controlled trial (analysis of serum and immune cells at multiple timepoints)	Clarithromycin (n=100) or placebo (n=100)	Patients with septic shock and multiple organ failure (n=69) treated with clarithromycin on day 4 after randomisation: lower ratio of serum IL-10 to TNF-α, increased apoptosis of monocytes, increased expression of co-stimulatory molecule CD86 on monocytes, increased production of IL-6, but reduced production of TNF-α in monocytes in response to lipopolysaccharides
Tsaganos et al,[27] 2016	Ventilator-associated pneumonia with sepsis (n=200)	Blinded retrospective analysis after a randomised controlled trial	Clarithromycin (n=100) or placebo (n=100)	Lower 90-day mortality in clarithromycin-treated patients (43 [43%] *vs* 60 [60%]); lower cumulative hospitalisation costs by day 45 of €19 382·32 versus €27 089·71 per patient

(Table continues on next page)

	Disease (participants)	Study design	Intervention	Main effect attributed to macrolide*
(Continued from previous page)				
Walkey et al,[28] 2012	ARDS (n=235)	Retrospective analysis of prospective cohort	Macrolide (n=47) or no macrolide (n=188)	Unadjusted trend towards lower 180-day mortality in macrolide-treated patients (11 [23%] *vs* 67 [36%]); pronounced mortality benefit in multivariable and propensity adjusted models (macrolide group: HR 0·46, 95% CI 0·23–0·92; control group: 0·37, 0·16–0·88); shorter time until successful discontinuation of mechanical ventilation (adjusted HR 1·93, 95% CI 1·18–3·17)
Kawamura et al,[29] 2018	ARDS (n=191)	Retrospective analysis of prospective cohort (2004–17)	Azithromycin (n=62) or no macrolide (n=129)	Unadjusted 90-day mortality of 18 (29·0%) in azithromycin-treated patients versus 57 (44·2%) in controls (HR 0·59, 95% CI 0·35–1·01); lower 90-day mortality after propensity score matching (HR 0·49, 95% CI 0·27–0·87) and inverse probability of treatment weighting (0·35, 0·15–0·40); shorter time until discontinuation of mechanical ventilation after propensity score matching (HR 1·71, 95% CI 1·06–2·78) and inverse probability of treatment weighting (1·89, 1·22–2·93)
Simonis et al,[30] 2018	ARDS (n=873)	Retrospective analysis of prospective cohort (2011–14)	Macrolides for non-antibiotic purposes (97% erythromycin, n=158) or no macrolides (n=715)	Unadjusted 30-day mortality of 36 of 158 (22·8%) in macrolide-treated patients versus 226 of 715 (31·6%) in controls (p=0·03); lower 30-day mortality remained after propensity matching (OR 0·62, 95% CI 0·39–0·96); subgroup analyses of propensity-matched cohort: lower 30-day mortality remained only in ARDS of non-pulmonary origin (OR 0·50, 95% CI 0·26–0·95) and non-hyperinflammatory phenotype 1 (0·20, 0·06–0·65])
Pons et al,[31] 2019	Acute respiratory failure (n=7182)	Retrospective analysis of prospective cohort (1997–2015)	Macrolide (n=1295) or no macrolide (n=5887)	Probability of better outcome with macrolides (using desirability of outcome ranking) after inverse probability of treatment weighting: 51·0% (95% CI 48·9%–53·2%)† for death and secondary infection, and 49·4% (95% CI 46·8%–51·6%)† for death and mechanical ventilation

ARDS=acute respiratory distress syndrome. CD86=cluster of differentiation 86. HR=hazard ratio. ICU=intensive care unit. IL=interleukin. OR=odds ratio. TNF=tumor necrosis factor. *All findings reported as different in the table are statistically significant (p<0·05); p-values are listed only to emphasise trends or barely significant findings. †In a desirability of outcome ranking analysis, if the 95% CI contains a probability of 50% then this suggests that the intervention has no benefit.

Table: **Evidence of non-antibiotic benefits of macrolides from clinical studies involving patients with high severity of disease**

to attenuate inflammation-induced tissue damage and improve survival, independent of bacterial load.[7–15] The use of macrolides in other acute systemic inflammatory conditions is underexplored, although a recent study by Weis and colleagues[16] did not show a benefit of azithromycin in a ceruletide-induced model of acute pancreatitis, possibly because the disease severity was insufficient.

In a cecal ligation and puncture model (sepsis with gut bacteria that are predominantly macrolide resistant), the addition of azithromycin to a subprotective dose of ceftriaxone doubled the survival rate of mice when compared with ceftriaxone alone, despite similar bacterial loads in the blood of both groups of animals.[7] Azithromycin reduced the concentrations of pro-inflammatory cytokines such as interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF-α) in the plasma and lungs of treated mice.[7] In a mouse model of ventilator-associated pneumonia by multidrug-resistant *Acinetobacter baumanii*, nearly all azithromycin-treated mice (n=22) survived, compared with fewer than half of the mice (n=11) in the untreated group.[13] This protective effect was associated with a markedly reduced infiltration of inflammatory cells in the lungs of azithromycin-treated mice.[13] The lung-protective effect of macrolides was further explored in high-pressure, ventilation-induced lung injury, in which clarithromycin ameliorated lung damage and neutrophilic infiltration, compared with levofloxacin or the vehicle control.[15]

Clinical studies of critical illness

Few clinical studies have investigated the immunomodulatory effects of macrolides in acute critical illnesses. Completed studies were predominantly retrospective and observational and, similar to preclinical studies, mostly related to pneumonia, sepsis, and lung injury (table). Considering the substantial overlap in the immune pathways and clinical phenotypes associated with critical illnesses, the reported benefits could be relevant to other diseases characterised by immune dysregulation, such as severe pancreatitis, polytrauma, and burns.

Pneumonia

In bacterial pneumonia, local inflammation is of paramount importance in clearing invading pathogens, but uncontrolled inflammation can lead to lung damage, ARDS, and sepsis. The reduction in mortality associated with macrolide treatment in severely ill patients with a high inflammatory response in observational studies could be explained by immunomodulatory and other non-antibiotic-related effects.[17,32] In patients with community-acquired pneumonia who were unresponsive to treatment after 72 h,[18] those receiving macrolides had lower concentrations of cytokines (IL-6 and TNF-α) in bronchoalveolar lavage fluid, and a shorter time to clinical stability than did those receiving other antibiotic regimens. The effectiveness of adding a macrolide to β-lactam treatment in moderately severe community-acquired pneumonia is more ambiguous[33,34] and falls outside the scope of this Review.

The benefit of macrolide treatment seems most pronounced in pneumococcal pneumonia[17]—the most commonly identified cause of community-acquired pneumonia—regardless of whether the causative strain is macrolide resistant,[19] which could derive from inhibition of pneumolysin, a toxin produced by pneumococci,[35] or macrolide-stimulated elimination of the bacterial reservoir in splenic macrophages.[36]

Macrolides can inhibit quorum sensing, a mechanism used by bacteria to increase their virulence (capacity to infect a host) in response to changes in the density of the bacterial population.[5] One randomised controlled trial (RCT) explored whether azithromycin could prevent ventilator-associated pneumonia by inhibition of quorum sensing in patients colonised by the inherently macrolide-resistant *Pseudomonas aeruginosa*.[20] The authors reported a lower incidence of ventilator-associated pneumonia in the azithromycin-treated group compared with the control group, but the finding was not statistically significant. When van Delden and colleagues[20] limited the analysis to the group of patients with *P aeruginosa* isolates producing rhamnolipids—previously shown to pose a high risk of quorum sensing-dependent virulence—only one of five patients receiving azithromycin developed ventilator-associated pneumonia, compared with five of five patients receiving placebo. However, no mortality benefit was observed in a study of pseudomonal community-acquired pneumonia, which included a subgroup analysis of ICU patients.[21]

Sepsis

In sepsis, systemic infection leads to severe immune dysregulation that persists long after the initial infection has been cleared. In pneumonia-derived sepsis, macrolide treatment improved survival at 30 and 90 days even when the cultured pathogens were macrolide resistant.[22] Mechanically ventilated patients with sepsis had more ICU-free days and a trend towards lower mortality when treated with azithromycin in a retrospective study that was limited by a small sample size (only 29 of 105 patients were treated with azithromycin).[23]

Two RCTs done by Giamarellos-Bourboulis and colleagues[24,25] evaluated the immunomodulatory effects of intravenous clarithromycin in sepsis. To exclude a potential survival benefit that an extra antibiotic might provide, the researchers included only patients with infection in which the causative pathogens would most likely be macrolide resistant. The first trial enrolled 200 patients with ventilator-associated pneumonia;[24] the second trial enrolled 600 patients with suspected or microbiologically confirmed gram-negative sepsis (pyelonephritis, abdominal sepsis, or primary bacteraemia).[25] Although both trials were negative for the primary endpoint of 28-day mortality, clarithromycin reduced the duration of symptoms by 4 to 5·5 days.[24,25] In line with other observations, clarithromycin had the greatest effect on the more severely ill patients. In the first trial[24] of patients with ventilator-associated pneumonia, the odds ratio of dying from septic shock and multiple organ failure was lower in clarithromycin-treated than in placebo-treated patients (table). Similarly, in the second trial,[25] which focused on gram-negative sepsis, of the 54 patients with shock and multiple organ failure, 15 of 28 patients (54%) died in the clarithromycin group, compared with 19 of 26 (73%) in the placebo group.

In a secondary analysis of the first ventilator-associated pneumonia trial,[24] patients with septic shock and multiple organ failure who received clarithromycin had a lower ratio of serum IL-10 to TNF-α, although their monocytes showed higher IL-6 but lower TNF-α production in response to lipopolysaccharide—a component of the gram-negative bacterial cell wall—with increased apoptosis, and increased expression of the co-stimulatory molecule CD86 on day 4 after randomisation.[26] These findings could signify that clarithromycin reverses immune suppression and endotoxin tolerance and accelerates the return to homoeostasis. This hypothesis was strengthened by a subsequent analysis of the same trial, in which the researchers found that—by contrast with 28-day mortality—90-day mortality was significantly reduced in the clarithromycin group (table).[27] Together, these data suggest that macrolides might aid the return to homoeostasis from immune suppression, and maintain innate immune cell function against (new) invading pathogens.

ARDS

Pneumonia, sepsis, or another local or systemic condition could escalate to ARDS, in which massive bilateral inflammation of the lungs results in alveolar flooding and respiratory failure, with a high chance of death.[2] In a secondary analysis of 235 patients with ARDS (enrolled into the RCT with a different aim), macrolide use was associated with reduced 180-day mortality and shorter duration of mechanical ventilation (table).[28] Following this finding, a Japanese centre started using intravenous azithromycin as an adjunctive for ARDS and reported similar outcomes, although the before-and-after study design and the long inclusion period (2004–17) predisposed the study to major confounding.[29] A study of 873 patients with ARDS, of whom 158 received macrolides for non-antibiotic purposes, found a mortality benefit of macrolide treatment.[30] In a propensity-matched subgroup analysis, this effect was—perhaps surprisingly—significant only in patients with ARDS of non-pulmonary origin with a non-hyperinflammatory phenotype.[30] In an RCT of clarithromycin in gram-negative sepsis,[25] 35 patients in the clarithromycin group and 34 patients in the placebo group also had ARDS, with a respective mortality of 28·5% and 55·9%. By contrast, a recent analysis of 7182 mechanically ventilated patients with acute respiratory failure (including ARDS), 1295 of whom were receiving macrolide treatment for mostly antibiotic purposes, found no differences in mortality,

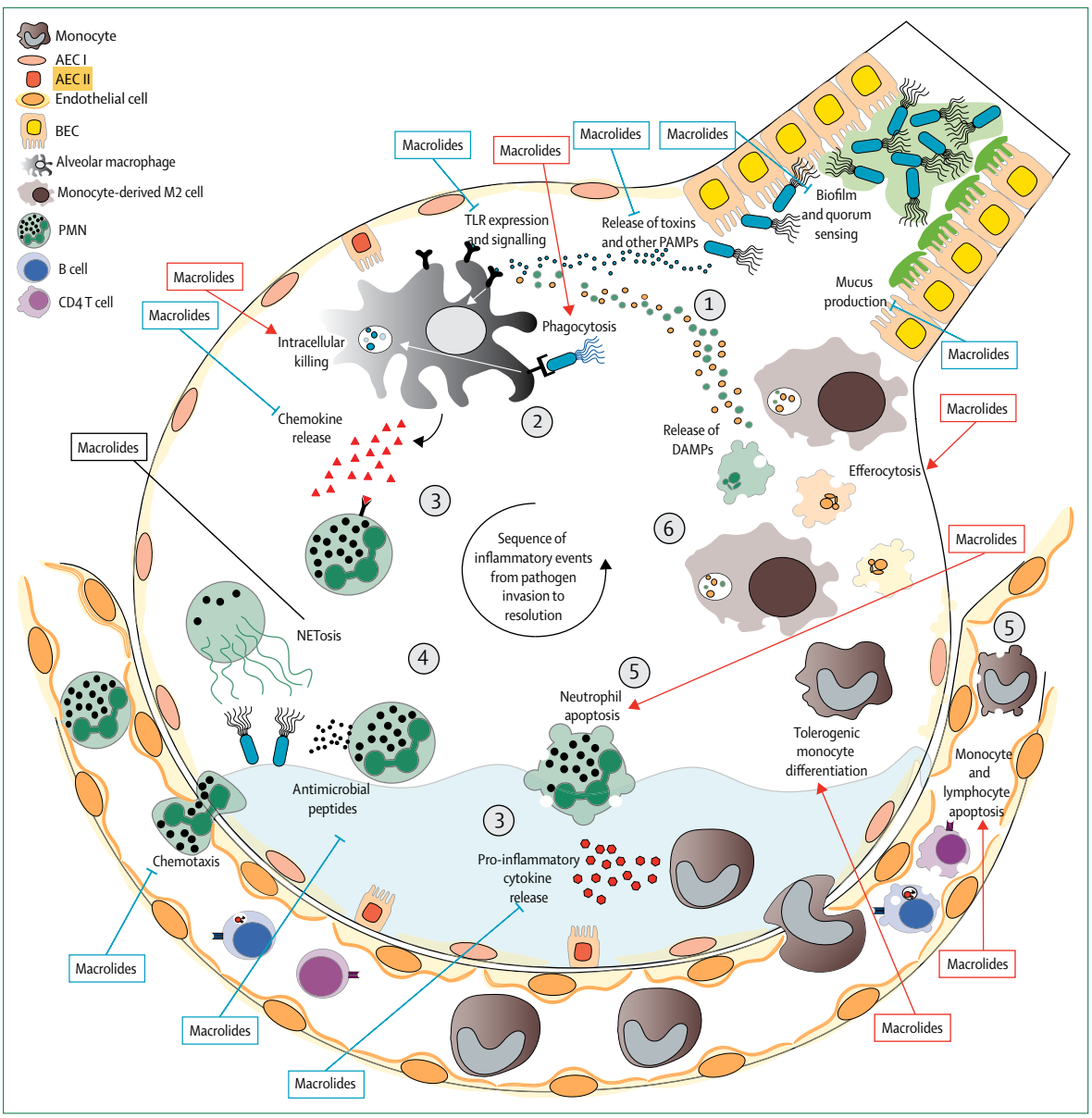

***Figure 1*: Model of the immunomodulatory effects of macrolides during a lung infection**
(1) Macrolides reduce bacterial virulence by inhibiting quorum sensing and biofilm formation. Macrolides also facilitate the initial host defence by reducing the quantity and consistency of sputum. (2) After bacterial invasion, an immune response is initiated when PAMPs are recognised by TLRs expressed on immune cells. Macrolides reduce the release of PAMPs by bacteria and inhibit TLR surface expression and signalling by monocytic immune cells. In parallel, macrolides enhance phagocytosis and subsequent intracellular killing of bacteria by monocytic cells. (3) Macrolides inhibit the release of pro-inflammatory cytokines and chemokines, which impairs recruitment of other immune cells to the lungs. (4) Although macrolides stimulate neutrophil degranulation, the overall number of antimicrobial peptides (such as myeloperoxidase and elastin) in the lungs is lower after macrolide treatment, presumably because of reduced neutrophil influx. Macrolides might also affect NETosis of neutrophils, but results are inconsistent. (5) To resolve immune responses, effector cells undergo apoptosis and specialised cells mediate tissue repair. Macrolides stimulate apoptosis of neutrophils and—at higher concentration—of monocytic cells and lymphocytes. (6) Macrolides also skew the differentiation of monocytic cells towards a tolerogenic (M2-like) phenotype. Timely apoptosis and efferocytosis diminish the release of DAMPs due to immunogenic cell death, and therefore prevent the perpetuation of inflammation. The overall effect of immunomodulation by macrolides is reduced collateral tissue damage caused by excessive inflammation while facilitating efficient immune resolution and tissue repair. Effects of macrolides are shown as inhibition (blue) or stimulation (red). AECI=type I alveolar epithelial cell. AECII=type II alveolar epithelial cell. BEC=bronchial epithelial cell. DAMPs=damage-associated molecular patterns. M2=M2 macrophages. NETosis=neutrophil extracellular trap release. PAMPs=pathogen-associated molecular patterns. PMN=polymorphonuclear neutrophils. TLR=toll-like receptors.

duration of mechanical ventilation, or incidence of secondary infection between the patients who received macrolides and those who did not (table).[31]

Mechanisms of action relevant to critical illness

Although most in-vivo studies of the immunomodulatory activity of macrolides relate to lung inflammation, the

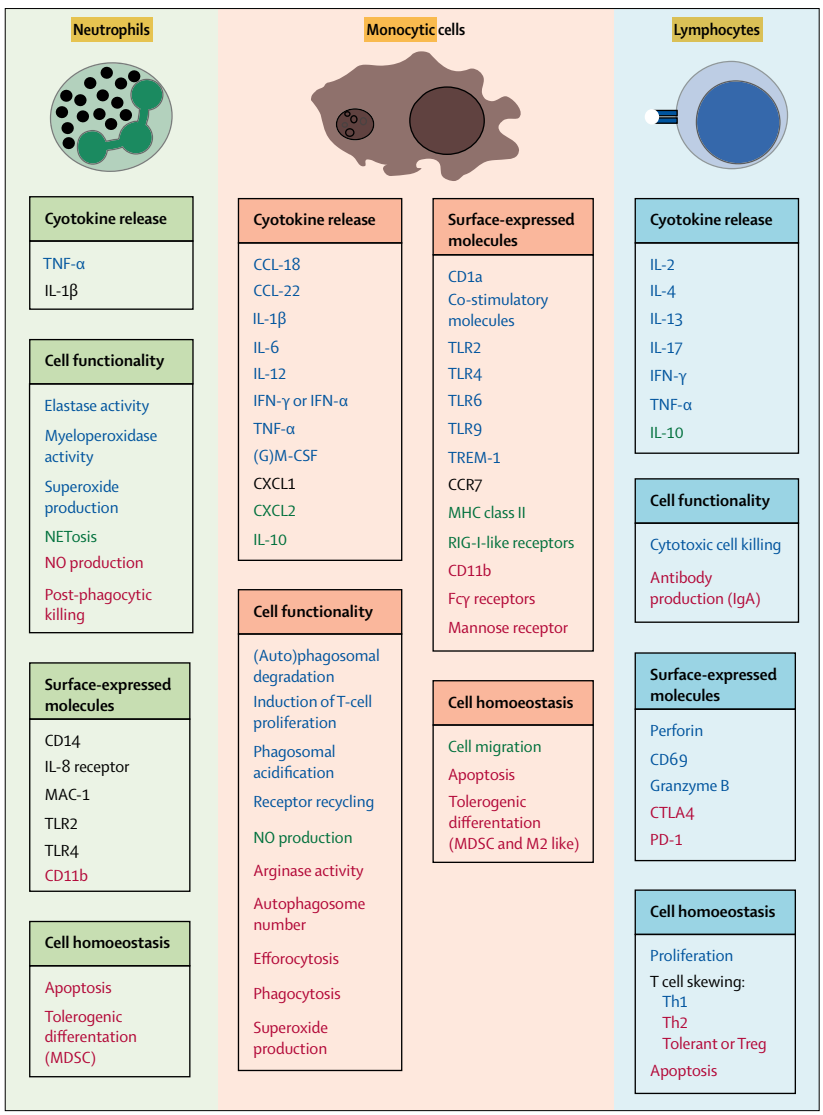

Figure 2: **Immunomodulatory effects of macrolides on neutrophils, monocytic cells, and lymphocytes**
Colours indicate inhibition (blue text), stimulation (red), unaffected (black), and inconclusive results (green). CCL=C-C motif ligand. CCR=C-C chemokine receptor. CD=cluster of differentiation. CTLA=cytotoxic T-lymphocyte-associated protein. CXCL=C-X-C motif ligand. GM-CSF=granulocyte-macrophage colony-stimulating factor. IFN=interferon. IgA=immunoglobulin A. IL=interleukin. M2=anti-inflammatory macrophages. MAC-1=macrophage-1 antigen. MDSC=myeloid derived suppressor cell. MHC=major histocompatibility complex. NETosis=neutrophil extracellular trap release. NO=nitric oxide. PD=programmed cell death protein. RIG-I-like receptors=retinoic acid-inducible gene-I-like receptors. Th=T-helper cell. TLR=toll-like receptor. TNF-α=tumor necrosis factor α. Treg=regulatory T cells. TREM=triggering receptor expressed on myeloid cells.

cellular effects of macrolides suggest that they might also influence extrapulmonary inflammatory disorders. Figure 1 shows the clinically relevant effects of macrolides in the context of lung infection, and figure 2 provides an overview of reported effects for various immune cell types. In this Review, we consider only macrolides with 14-membered and 15-membered lactone rings (eg, azithromycin, clarithromycin, and erythromycin) because immunomodulation has mainly been described for these drugs rather than for 16-membered varieties (eg, josamycin).[37] Some of the effects occur because of direct modifications of the target cell, whereas other effects most likely result from complex systemic interplay—eg, the intestinal microbiome in mice can be altered for an extended period of time after a single macrolide treatment, which in turn could also modulate the immune response.[38] In addition, macrolides also induce non-immunological effects that increase (airway) defence against pathogens, including altered consistency and reduced hypersecretion of sputum (leading to improved pathogen clearance), enhanced airway epithelial integrity,[5] and reduced eicosanoid metabolism.[39]

Effects on initiation of the inflammatory response

In patients who are critically ill, DAMPs and PAMPs induce a strong immune response by interaction with pattern recognition receptors such as TLRs. Macrolides reduce TLR surface expression on dendritic cells[40,41] and macrophages,[42] but not on neutrophils or lymphocytes.[43,44] Importantly, macrolides impair TLR signalling using multiple mechanisms (figure 3), which probably accounts for a substantial part of their immunomodulatory effects.[40,42,45–49]

Effects on cytokines, chemokines, and chemotaxis

TLR ligation activates immune cells to eradicate invading pathogens. However, patients who are critically ill can have an excessive inflammatory response, characterised by an overwhelming release of pro-inflammatory cytokines. Macrolides dampen this response by reducing pro-inflammatory cytokine release and limiting immune cell migration. Macrolides reduce the production of IL-6 and TNF-α in airway epithelial cells, monocytes, macrophages, dendritic cells, and T cells.[9,41,50,51] Likewise, macrolides impair NOD-like receptor protein 3 (NLRP3) and NLRC4 inflammasome activation, which diminishes the production of IL-1β by monocytes and macrophages in response to either lipopolysaccharide stimulation or whole bacteria.[47–49] In gram-negative and gram-positive bacterial infection models, macrolides reduced the amount of IL-1β, IL-6, and TNF-α in serum and bronchoalveolar lavage fluid.[7,8,13,52] Macrolides inhibit the production of IL-12 by dendritic cells, which might explain why macrolide treatment diminishes the induction of T-helper-1 cells[40,41,45] and decreases T-helper-1 cytokine interferon γ.[41,46,53]

The effect of macrolides on anti-inflammatory cytokines is more ambiguous. Macrolides upregulate IL-10 production by monocytes and macrophages,[51,54,55] but seem to suppress the production of IL-10 in T cells and dendritic cells.[41,46,53] In mouse models of infection, macrolide treatment either increased[11] or did not affect the concentration of IL-10 in serum or bronchoalveolar lavage fluid.[56] The increased IL-10 observed in some of these studies might be derived, in part, from macrolide-induced myeloid-derived suppressor cells (MDSCs). A recent study[11] showed that IL-10 was vital for the protective effect of clarithromycin-induced MDSCs in lethal shock

(induced by lipopolysaccharide injection) and post-influenza pneumococcal pneumonia models.

Excessive inflammation in critically ill patients is associated with massive recruitment of leucocytes. This process leads to vascular leakage and tissue damage, which results in organ failure.[1,2] Macrolide administration in mouse models of acute infection and high-pressure ventilation lung injury resulted in reduced recruitment of leucocytes—predominantly neutrophils—to the lung and consequently prevented destruction of the lung parenchyma.[13,15,52,57] Reduced concentrations of growth factors and chemokines might explain these observations. Overall, macrolides reduce the production of granulocyte and granulocyte-macrophage colony-stimulating factors,[57–59] and downregulate chemokines that recruit neutrophils, monocytes, and other leucocytes—such as IL-8 (CXCL8), macrophage inflammatory protein 2 (CXCL2), and monocyte chemoattractant protein 1 (CCL2)—in vivo and in vitro.[50,52,58] Moreover, macrolides reduce the production of IL-17 by CD4⁺ T cells in vitro,[53] which might further reduce the activation and mobilisation of neutrophils.

Effects on cell proliferation and differentiation

In addition to recruitment to the site of infection, immune cells also proliferate to control the infection. However, immune responses in critically ill patients are frequently distorted by hyperinflammation and immune exhaustion.[1] Macrolides can dampen hyperinflammation by inhibiting CD4 T-cell proliferation induced by allogeneic dendritic cells or by α-CD3 combined with α-CD28,[11,40,51,53,60] which could have multiple causes. First, macrolides downregulate expression of co-stimulatory molecules on antigen-presenting cells,[37,40,41,51,56] and azithromycin (but not clarithromycin) reduces expression of major histocompatibility complex class II on dendritic cells in vitro.[40] This downregulation, combined with the diminished production of pro-inflammatory cytokines, might reduce the stimulation of T-cell proliferation. Second, T cells exposed to azithromycin-treated dendritic cells produce less interferon γ and more IL-10,[40] which dampens autocrine activation and subsequent cell proliferation. Reduced cell viability could also explain diminished proliferation, because macrolides have been reported to induce T-cell apoptosis at higher concentrations.[53,60]

Macrolides can further dampen hyperinflammation in patients who are critically ill by skewing cell differentiation towards more tolerogenic phenotypes, which is suggested by the observation that T cells stimulated with macrolide-exposed antigen-presenting cells release more of the anti-inflammatory IL-10,[40] and differentiate towards T-helper-2 cells rather than T-helper-1 cells.[37] Macrolides also push macrophages and monocytes towards a tolerogenic or M2-like phenotype (ie, specialising in efferocytosis and tissue repair) in vitro and in vivo.[51,55,56,61] Clarithromycin treatment was recently shown to increase the number of monocytic and granulocytic MDSCs in the spleen and lungs of mice, caused by upregulation of prokineticin 2 and enhanced phosphorylation of the signal transducer and activator of transcription 3, known as STAT3. This study also showed enhanced circulating MDSCs in humans after clarithromycin treatment.[11] These MDSCs reduced pro-inflammatory cytokine release, lessened the associated inflammatory tissue damage, and improved survival in mouse models of lethal lipopolysaccharide-induced shock and post-influenza pneumococcal pneumonia.[11] Although these effects can be beneficial to patients with hyperinflammation, they could be detrimental in the context of immune exhaustion.

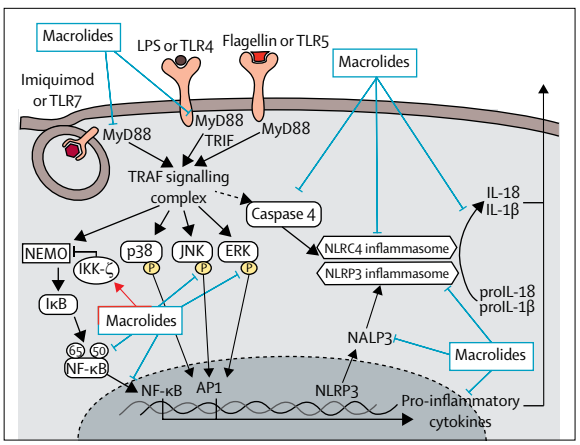

***Figure 3*: Immunomodulatory effects of macrolides mediated by inhibition of TLR signalling molecules**

Ligation of TLRs induces signalling using multiple pathways to ultimately induce gene expression. These signalling pathways include NF-κB, p38, ERK, and JNK. Macrolides inhibit endosome acidification, which impairs TLR-ligand dissociation and thus affects downstream signalling.[42,45] Macrolides enhance expression of IkKζ—an inhibitor of NEMO—leading to reduced induction of p50 and reduced NF-κB nuclear translocation.[42] Macrolides inhibit the phosphorylation of JNK and ERK, which are required for nuclear translocation.[46] These drugs also diminish NLRP3 inflammasome activation, in part by reducing NALP3 protein stability.[47,48] Additionally, macrolides inhibit caspase-4 activation and therefore reduce NLRC4 inflammasome activation.[47,49] These effects, combined, inhibit the expression, activation, and release of pro-inflammatory cytokines.[42,45–49] Effects of macrolides are shown as inhibition (blue) or stimulation (red) and were identified in studies using monocytes,[48,49] macrophages,[42,47] and dendritic cells.[45] AP1=activating protein-1. ERK=extracellular-signal-regulated kinase. IκB=inhibitory κB. IkK-ζ=IκB kinase. IL=interleukin. JNK=c-Jun N-terminal kinase. LPS=lipopolysaccharide. MyD88=myeloid differentiation primary response protein 88. NALP=nucleotide-binding oligomerization domain, leucine-rich repeat, and pyrin domain containing. NEMO=nuclear factor-κB essential modulator. NF-κB=nuclear factor κ-light-chain-enhancer of activated B cells. NLR=NOD-like receptor protein. P=phosphorylated. p38=p38 mitogen-activated protein kinase. TLR=toll-like receptor. TRAF=tumor necrosis factor receptor-associated factor. TRIF= toll/IL-1 receptor-domain-containing adapter-inducing interferon-β.

Effects on cell survival

Patients who are critically ill show excessive apoptosis of T cells, B cells, dendritic cells, and macrophages, which could result in nosocomial infections and is associated with multiple organ failure and mortality.[62,63] By contrast, apoptosis of neutrophils is reduced or unaffected in critical illness,[63] which prolongs excessive inflammation.

Macrolide treatment reduces neutrophil survival by directly stimulating apoptosis and by inhibiting the release of pro-survival molecules such as granulocyte colony-stimulating factors.[5,64] This pro-apoptotic effect does not necessarily impair defence against pathogens, as one study showed that azithromycin did not induce apoptosis when neutrophils were in the presence of *Streptococcus pneumoniae*.[65] Azithromycin at high concentrations induces apoptosis of dendritic cells,[41] natural killer cells,[66] and CD4 T cells[41,53] in vitro. However, apoptosis of these cells has not been observed at clinically relevant concentrations and therefore seems unlikely to exacerbate immune suppression in patients who are critically ill.

Effect on cell functionality: phagocytosis, efferocytosis, and bacterial killing

Internalisation of bacteria by phagocytosis prevents their dissemination, facilitates their killing, and is required for antigen presentation to initiate adaptive immunity. The phagocytic capacity of critically ill patients is lower than that of healthy individuals, and correlates with nosocomial infections and mortality.[67,68] Macrolides enhance the phagocytic capacity of dendritic cells[41,51] and alveolar macrophages.[69] However, erythromycin did not enhance phagocytosis of *Streptococcus pyogenes* by neutrophils.[70] Azithromycin might also aid immune cells that are compromised by disease. Administration of azithromycin completely restored the diminished phagocytic capacity of alveolar and monocyte-derived macrophages from patients with COPD.[71]

To aid tissue repair after inflammation, dead and damaged cells need to be cleared by efferocytosis, a mechanism highly similar to phagocytosis. Efferocytosis is impaired in patients with ARDS.[72] Macrolide treatment enhances efferocytosis by macrophages and dendritic cells in vitro and in vivo.[41,51,69,71]

To facilitate efficient killing after phagocytosis, bacteria need to be targeted to phagolysosomes. Acidification of the phagolysosome and high phagosomal stability are essential to this process. Phagosomes are subjected to high oxidative stress—due to the respiratory bursts used to kill bacteria—which can lead to lysosomal membrane permeabilisation. Azithromycin treatment, in vitro and in vivo, improves phagosomal stability by protecting macrophages from oxidative stress-induced lysosomal leakage and subsequent cell death.[73,74] The enhanced phagosomal stability prevents bacterial escape from phagosomes and thereby aids bacterial clearance.

Autophagy—a process that cells use to eliminate unnecessary or dysfunctional cellular components — can also be used to kill pathogens, regulate immune responses, and prevent apoptosis. Autophagy is initially enhanced in sepsis to aid pathogen clearance, but is suppressed in later stages.[62] Azithromycin treatment promotes autophagosomal stability (and thus blocks bacterial escape)[73] and prevents acidification, which impairs autophagosome maturation and subsequent degradation.[42]

Macrolides have been reported to enhance intra-phagosomal killing of pathogens such as *Staphylococcus aureus*,[74] *Aggregatibacter actinomycetemcomitans*,[75] *S pyogenes*,[70] and *Candida albicans*.[76] Taken together, depending on the pathway necessary to kill the specific pathogen, macrolides will enhance or inhibit pathogen killing. One typical mechanism is the respiratory burst, which is affected by macrolides in a time-dependent and context-dependent manner. Macrolides inhibit spontaneous and N-formyl-methionyl-leucyl-phenylalanine-stimulated reactive oxygen species (ROS) production by primary neutrophils[77,78] and enhance *S aureus*-stimulated ROS production by macrophages.[74] Azithromycin inhibits lipopolysaccharide-induced nitric oxide (NO) production and inducible NO synthetase expression in macrophages both in vitro and in vivo,[61] and enhances spontaneous NO production by MDSCs.[11]

Neutrophils have a specialised strategy to trap extracellular bacteria, in which neutrophil extracellular traps (NETs) are formed from DNA expelled by neutrophils, which can be decorated with antimicrobial peptides—a process known as NETosis. In patients with sepsis, NET formation is increased and associated with organ dysfunction.[1] The effects of macrolides on the extent of NET release are inconsistent. Macrolides have been shown either not to affect or to stimulate spontaneous NET release, although macrolides can also inhibit NET release after stimulation with phorbol 12-myristate 13-acetate or cigarette smoke condensate.[77,79,80] The NETs released by macrolide-treated neutrophils show increased decoration with antimicrobial peptides, which could mean that the NETs are more potent bacterial killers.[79]

To kill invading pathogens, neutrophils can also secrete various antimicrobial peptides that are stored in intracellular granules. Macrolides stimulate neutrophil degranulation in vivo and in vitro.[78,81] Conversely, erythromycin can bind and inhibit elastase—a potent protease released by neutrophils.[82] Clarithromycin has also been reported to reduce elastase activity and enhance myeloperoxidase activity.[11] However, macrolide treatment has been shown to reduce lung myeloperoxidase concentration after lipopolysaccharide challenge and high-pressure mechanical ventilation, which might be secondary to the reduced influx of neutrophils.[15,57] Azithromycin also inhibits perforin expression by natural killer cells, which hampers natural killer-cell activation and killing capacity.[66] Finally, in-vivo azithromycin treatment downregulated granzyme B concentrations in CD4 and CD8 T cells.[83]

Effects on bacteria beyond antibiotics

In addition to their direct bacteriostatic effects by inhibition of bacterial protein synthesis, macrolides can reduce bacterial virulence using several mechanisms. They inhibit the release of LytA—an enzyme that aids bacterial immune escape—by *S pneumoniae*, even if the

strain is macrolide resistant.[84] LytA in turn downregulates pneumolysin,[35] which leads to enhanced complement deposition and thus facilitates bacterial killing.[84] Macrolides also inhibit the production of virulence factors by other bacteria, including *Escherichia coli*,[85] *P aeruginosa*,[5] and fungi.[86]

Under favourable growth conditions, bacteria can give rise to biofilms that minimise their susceptibility and exposure to antibiotics and immune cells. Biofilms are a crucial mechanism in the colonisation of indwelling catheters and medical devices in the ICU, and thus represent an important virulence factor in hospital-acquired infections. Macrolides inhibit biofilms produced by bacteria including *P aeruginosa*, *Haemophilus influenzae*, and *Staphylococcus epidermidis*,[64] in part through diminished quorum sensing. Finally, macrolides inhibit bacterial adhesion to the airway epithelium.[5]

Novel non-antibiotic macrolide derivatives

Concerns about unintentional exacerbation of antimicrobial resistance might hinder the widespread use of macrolides for non-antibiotic indications. Fortunately, macrolides can be modified to eliminate their antibacterial effects while maintaining or enhancing their immunomodulatory capacity. EM703, a non-antibiotic erythromycin derivative, improved survival in mice following an airway infection caused by P aeruginosa, probably by reducing the release of pro-inflammatory cytokines.[87] Erythromycin-derived EM900 inhibits infection-induced pro-inflammatory cytokine production, NFκB (nuclear factor κ-light-chain-enhancer of activated B cells) activation, and mucus production by epithelial cells to a similar degree as erythromycin.[88] EM900 reduced viral loads after rhinovirus infection[88] and improved survival after *S pneumoniae* infection by a mechanism that involved enhanced CCL2 secretion and increased counts of F4/80$^+$ macrophages in the lungs.[89] The non-antibiotic azithromycin derivative CSY0073 diminished the concentrations of lipopolysaccharide-induced pro-inflammatory cytokines and chemokines in the lungs of mice.[90]

Macrolides can also be coupled with other molecules, such as steroids, antimicrobial peptides, or small signalling molecules to enhance their functionality or potency. Given that macrolides are relatively stable in vivo and accumulate in phagocytes, they are excellent carrier molecules to deliver drugs or signals specifically to phagosomes or sites of inflammation. DP7 for example, a potent antimicrobial peptide that cannot be administered systemically because of its adverse effects,[60] can be loaded into liposomes with azithromycin. Administration of these liposomes during *S aureus* infection alleviated the adverse effects of DP7 and reduced bacterial load and systemic inflammation.[60] Although promising, to our knowledge such hybrid macrolides are not currently in use in clinical practice and have not been tested clinically.

Conclusions and future directions

Macrolides profoundly modulate the immune response, inducing a multitude of pro-inflammatory and anti-inflammatory effects that have the potential to correct a distorted immune balance in patients who are critically ill. The immunomodulatory effects of macrolides are complex and appear to be dependent on time, dose, and the broader context (eg, severity of disease). Defining and delineating the extent and impact of these effects and separating cause from correlation remains challenging. Matters are complicated further by the observation that some of the potentially detrimental effects reported in vitro seem to contradict clinical observations. For example, despite multiple reports that macrolides induce apoptosis of lymphocytes and expansion of MDSCs—two hallmarks of immune suppression and risk of secondary infections in patients who are critically ill—their net effect seems to be to prevent most opportunistic infections by stimulating immune cells to kill pathogens and to suppress virulence mechanisms. Nevertheless, the anti-inflammatory and pro-repair properties of macrolides

Panel: Future directions for research

Although macrolides hold promise for correcting the immune dysregulation observed in critically ill patients, further research is needed before routine use in clinical practice can be recommended. Non-antibiotic macrolides should be used for immunomodulatory macrolide therapy to minimise the development of antimicrobial resistance. Macrolides can be further optimised—eg, by attaching specific signalling molecules to boost specific immune functions such as bacterial killing—and such macrolides are currently under development, although still in preclinical stages of development.

Most of the data discussed in this Review was obtained from human cell lines, murine animal models, or retrospective clinical studies. This strongly underlines the need for prospective studies and randomised controlled trials (RCTs). The complex and highly heterogeneous host response to critical illness probably warrants a personalised medicine approach for any future therapy. It might be necessary to identify clinical or immunological phenotypes that are most likely to benefit from immunomodulatory treatment with macrolides. Several phenotypes have been delineated in sepsis and acute respiratory distress syndrome (ARDS) that are relevant to the immune response and clinical outcomes. Differential responses to treatment based on phenotype have been described after retrospective stratification in secondary analyses of RCTs.[91,92] As concerns regarding the cardiovascular safety of macrolide treatment persist,[93] researchers should consider restricting the inclusion criteria in future trials to patients without cardiovascular comorbidities.[94]

A new trial of macrolides in patients with sepsis, using (post-hoc) phenotype-based stratification, and a first trial in patients with ARDS should now be done. A third RCT (NCT03345992) of clarithromycin in patients with sepsis is currently underway. This trial includes only patients with multiple organ failure and respiratory dysfunction, who showed a survival benefit in response to treatment with clarithromycin in the previous two trials.[91,92]

Any clinical benefits of macrolides in pancreatitis, burns, and polytrauma should be tested in preclinical models before clinical studies are initiated. Mouse models of persistent inflammation, immunosuppression, and catabolism syndrome can help to explore the effects of macrolides on chronic immune disturbances of the type observed in patients who are critically ill. Given that macrolides inhibit biofilm formation and quorum sensing, it would be of interest to establish whether they could be used prophylactically to prevent catheter-related bloodstream infections or ventilator-associated pneumonia.

Search strategy and selection criteria

We searched PubMed (MEDLINE) for all relevant studies published between Jan 1, 2008, and Jan 26, 2020, and identified older landmark studies by searching the reference lists of original research and review articles. The medical subject headings used were "macrolides" combined with "immunomodulation", "immunity", or "immune system". In addition, we did a more detailed search comprising all antibiotic macrolides used in clinical practice and novel non-antibiotic macrolides combined with relevant immunological processes (eg, "TLRs" and their ligands, "endotoxin tolerance", "phagocytosis", "phagosomal acidification", "immune cells" and "subsets", "complement system", "coagulation system", and "bacterial factors" associated with virulence), relevant disease processes (eg, "sepsis", "pneumonia", "ARDS", "lung injury", "pancreatitis", "trauma", "burns"), and synonyms of critical care. All relevant abstracts were screened independently by two researchers. The final reference list was generated based on relevance to the topics covered in this Review; only papers published in English were included.

mitigate tissue damage from excessive inflammation and cell death, which might translate to reduced duration of symptoms and mortality, as observed in studies of critically ill patients. However, although harnessing the beneficial immunomodulatory effects of macrolides is an appealing prospect, clinical evidence is scarce at present, and caution is required in terms of safety and the exacerbation of antimicrobial resistance. Further studies, as proposed in the panel, are needed to ascertain whether or not immunomodulation by macrolides could be a valid therapeutic avenue for critically ill patients, and to establish which clinical or immunological subsets of patients might benefit the most from such treatments. In parallel, further improvement of novel non-antibiotic macrolides should be pursued to enhance and expand their immunomodulatory potential.

Contributors

TDYR, AS, and TvdP conceived of and planned the Review. TDYR and AS did the literature search, screened all the abstracts independently, read and summarised all selected articles, and prepared the manuscript. TvdP and MJS critically revised the manuscript.

Declaration of interests

We declare no competing interests.

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