**Introduction**

In addition to their conventional antimicrobial activity, the macrolide group of antibiotics is known to have a number of anti-inflammatory/immunomodulatory activities, which may be of benefit to patients with chronic obstructive pulmonary disease (COPD), both in the stable state and during acute exacerbations. These activities involve interactions of the macrolides with the various components of inflammation in patients with COPD, namely the ciliated airway epithelium, the immune and phagocytic cells of the host and their proinflammatory mediators, as well as with the microbes themselves. There are a number of factors that have been shown to cause injury to the bronchial epithelium and its mucociliary mechanism, including bacterial, chemical, mechanical and host-derived factors. The macrolide, azalide, ketolide group of antibiotics has been shown in many studies to be cytoprotective of human ciliated epithelium in vitro, protecting against both chemical mediator- and bacterial-mediated injury. Mucus hypersecretion is an important and characteristic feature of many respiratory diseases, including COPD, and increases the morbidity and mortality of these diseases especially as a consequence of pulmonary infection. Chronic inflammation causes not only airway damage, but also goblet cell hyperplasia, which leads to the mucus hypersecretion. Mediators of this inflammation include cytokines, chemokines and oxygen radicals. Both in vitro and in vivo studies have shown that macrolides reduce mucus and sputum secretion, which may also contribute to their beneficial effects on airway inflammation. Among many other anti-inflammatory actions, clarithromycin (and other macrolides) has been shown to interfere with the generation of neutrophil-selective chemoattractants by bronchial epithelial cells, eosinophils, monocytes, fibroblasts, and airway smooth muscle cells by mechanisms which appear to involve inhibition of intracellular signalling mechanisms. Macrolides also appear to selectively down-regulate exuberant inflammatory responses which result from the interaction of viruses and Gram-negative bacteria with toll-like receptors, TLR3 and TLR4, respectively, while preserving the interaction of Gram-positive bacteria with TLR2. In addition to their standard antimicrobial activity, the macrolide group of antibiotics has been documented to have additional effects against bacteria which are not associated with inhibition of bacterial proliferation, and occur even in microorganisms that are totally resistant to their anti-proliferative actions. This has been most well studied in the case of *Pseudomonas aeruginosa*, a microorganism against which macrolides have no anti-proliferative activity. Macrolides have the ability to interfere with a number of virulence factors produced by *P. aeruginosa*. We have recently reported that the production of pneumolysin, a major protein virulence factor of Streptococcus pneumoniae, which promotes extra-pulmonary dissemination of this microorganism, is attenuated by exposure of the bacteria to sub-inhibitory concentrations of clarithromycin. Interestingly, clarithromycin-mediated inhibition of the production of pneumolysin was observed not only with macrolide-susceptible strains of the pneumococcus, but also with macrolide-resistant strains which harboured either the ribosomal methylase (ermB gene)- or efflux pump (mef gene)-based mechanisms of macrolide resistance. Taken together, the evidence presented in this review, supports the contention that macrolides possess a seemingly unique profile of complementary therapeutic activities.

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**Non-antimicrobial activity of macrolides: therapeutic potential in chronic inflammatory airway disorders**

C Feldman, R Anderson

In addition to their conventional antimicrobial activity, the macrolide group of antibiotics are known to have a number of anti-inflammatory/immunomodulatory activities, which may be of benefit to patients with chronic obstructive pulmonary disease (COPD), both in the stable state and during acute exacerbations. These activities involve interactions of the macrolides with the various components of inflammation in patients with COPD, namely the ciliated airway epithelium, the immune and phagocytic cells of the host and their proinflammatory mediators, as well as with the microbes themselves. There are a number of factors that have been shown to cause injury to the bronchial epithelium and its mucociliary mechanism, including bacterial, chemical, mechanical and host-derived factors. The macrolide, azalide, ketolide group of antibiotics has been shown in many studies to be cytoprotective of human ciliated epithelium in vitro, protecting against both chemical mediator- and bacterial-mediated injury. Mucus hypersecretion is an important and characteristic feature of many respiratory diseases, including COPD, and increases the morbidity and mortality of these diseases especially as a consequence of pulmonary infection. Chronic inflammation causes not only airway damage, but also goblet cell hyperplasia, which leads to the mucus hypersecretion. Mediators of this inflammation include cytokines, chemokines and oxygen radicals. Both in vitro and in vivo studies have shown that macrolides reduce mucus and sputum secretion, which may also contribute to their beneficial effects on airway inflammation. Among many other anti-inflammatory actions, clarithromycin (and other macrolides) has been shown to interfere with the generation of neutrophil-selective chemoattractants by bronchial epithelial cells, eosinophils, monocytes, fibroblasts, and airway smooth muscle cells by mechanisms which appear to involve inhibition of intracellular signalling mechanisms. Macrolides also appear to selectively down-regulate exuberant inflammatory responses which result from the interaction of viruses and Gram-negative bacteria with toll-like receptors, TLR3 and TLR4, respectively, while preserving the interaction of Gram-positive bacteria with TLR2. In addition to their standard antimicrobial activity, the macrolide group of antibiotics has been documented to have additional effects against bacteria which are not associated with inhibition of bacterial proliferation, and occur even in microorganisms that are totally resistant to their anti-proliferative actions. This has been most well studied in the case of *Pseudomonas aeruginosa*, a microorganism against which macrolides have no anti-proliferative activity. Macrolides have the ability to interfere with a number of virulence factors produced by *P. aeruginosa*. We have recently reported that the production of pneumolysin, a major protein virulence factor of Streptococcus pneumoniae, which promotes extra-pulmonary dissemination of this microorganism, is attenuated by exposure of the bacteria to sub-inhibitory concentrations of clarithromycin. Interestingly, clarithromycin-mediated inhibition of the production of pneumolysin was observed not only with macrolide-susceptible strains of the pneumococcus, but also with macrolide-resistant strains which harboured either the ribosomal methylase (ermB gene)- or efflux pump (mef gene)-based mechanisms of macrolide resistance. Taken together, the evidence presented in this review, supports the contention that macrolides possess a seemingly unique profile of complementary therapeutic activities.
Table 1: Conditions which may respond to the anti-inflammatory effects of macrolides.

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Agents</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cystic fibrosis</td>
<td>Azithromycin, clarithromycin</td>
<td>9, 10</td>
</tr>
<tr>
<td>Diffuse panbronchiolitis</td>
<td>Clarithromycin, erythromycin</td>
<td>11, 12</td>
</tr>
<tr>
<td>Bronchiectasis</td>
<td>Erythromycin</td>
<td>13</td>
</tr>
<tr>
<td>Chronic obstructive pulmonary disease</td>
<td>Clarithromycin</td>
<td>5</td>
</tr>
<tr>
<td>Acute and chronic sinusitis</td>
<td>Macrolides generally</td>
<td>4, 14, 18</td>
</tr>
<tr>
<td>Chronic bronchitis</td>
<td>Macrolides generally</td>
<td>4, 14</td>
</tr>
<tr>
<td>Bronchial asthma</td>
<td>Macrolides generally</td>
<td>14, 15, 17</td>
</tr>
<tr>
<td>Bronchiolitis obliterans syndrome after lung transplantation</td>
<td>Azithromycin</td>
<td>16</td>
</tr>
<tr>
<td>Undifferentiated connective</td>
<td>Clarithromycin</td>
<td>19</td>
</tr>
</tbody>
</table>

The effects of the macrolide group of antibiotics on microorganisms which is most likely to confer optimum anti-inflammatory activity on the macrolide/azalide/ketolide group of antimicrobial agents.

In the case of macrolide-susceptible microbial pathogens, the anti-infectious properties of macrolides are attributable to two distinct, albeit complementary, mechanisms. These are: i) interference with the growth of microbial pathogens by conventional ribosomal-targeted antimicrobial mechanisms, which in turn effectively eliminates the stimulus for protracted, ineffective inflammatory responses and ii) suppression of the activities of various types of inflammatory cells, including granulocytes, monocytes/macrophages, and dendritic cells, as well as epithelial cells. More recently, however, it has been speculated that macrolides, in addition to possessing antimicrobial activity, also have immunomodulatory activity, which may explain their therapeutic activity against ostensibly macrolide-resistant microorganisms such as *Pseudomonas aeruginosa* and some strains of *Streptococcus pneumoniae*. In this latter setting, macrolides appear to target microbial virulence factors and quorum sensing mechanisms.

The contribution of the secondary immunomodulatory properties of macrolides to therapeutic efficacy remains to be conclusively established, and is complicated by their activity against atypical microbial pathogens. Interestingly, in a retrospective study designed to address these issues by excluding patients with primary atypical infection, Metesky et al have recently reported that initial antibiotic treatment strategies which included a macrolide, as opposed to a fluoroquinolone or tetracycline, were associated with improved outcome in patients hospitalised with bacteremic pneumonia. As stated by the authors, these results have implications in respect of the mechanisms by which the use of a macrolide for treatment of pneumonia is associated with improved outcome, and appear to support the involvement of the beneficial, secondary immunomodulatory/anti-inflammatory properties in complementing the antimicrobial activity of the macrolides.

**Effects on human ciliated respiratory epithelium**

Both in the upper and the lower respiratory tract, the human airway is lined by a specialised epithelium which consists of a number of different cell types, of which the specialised ciliated columnar epithelial cells are particularly important. The bronchial epithelium is the primary barrier between the environment and the conducting airways and the cilia acting in concert with the mucus layer secreted above the epithelium, constitute the mucociliary escalator. This is the first-line host defence mechanism of the airway which serves to keep the lower respiratory tract normally sterile. There are a number of factors that have been shown to cause injury to the epithelium and its mucociliary mechanism, including bacterial, chemical, mechanical and host-derived factors. For example, cigarette smoke affects ciliary beating, although the ciliary slowing that may occur is reversible provided exposure is stopped before ciliostasis occurs. The consequences of attenuation of mucociliary function may include chronic inflammation, mucosal injury, and persistent bacterial colonisation, as well as bacterial invasion that occur in a variety of chronic airway disorders, including COPD.

In contrast to this is a number of factors that protect the epithelium from these harmful effects. The macrolide, azalide, ketolide group of antibiotics has been shown in studies to be cytoprotective of human ciliated epithelium in vitro. This may serve to protect the epithelium from both chemical- and bacterial-mediated injury. The effects appear to be mediated both directly and indirectly. In the first instance, it has been noted that macrolides stimulate ciliary beat frequency and improve mucociliary clearance. This is in contrast to the actions of several bacteria, including *Haemophilus influenzae*, one of the most important pathogens associated with infective exacerbations of COPD. This microorganism has been shown to cause ciliary slowing and damage to respiratory epithelium. However, incubation of nasal epithelial tissue with sub-minimum inhibitory concentrations (MICs) of dirithromycin significantly reduced these effects caused by *H. influenzae*, in particular the epithelial damage. The authors of that study suggested these effects may be direct and possibly associated with elevations in cyclic adenosine monophosphate (AMP).

Furthermore, erythromycin in sub-MIC levels has been shown to inhibit the adherence to human respiratory cells of another important respiratory pathogen encountered in patients with COPD exacerbations, namely *S. pneumoniae*. The mechanism may be related to inhibition of the microorganism’s ability to produce one of its most important virulence factors, namely its thiol-activated, cytolytic, protein toxin pneumolysin. Pneumolysin has been shown to slow ciliary beating and to cause damage to human ciliated epithelium. It is thought that these effects may aid in the initial colonisation and the subsequent invasion of the respiratory epithelium by the pneumococcus. Similarly, *P. aeruginosa*, which is a microorganism that may be associated with infective exacerbations in patients with severe COPD, particularly occurring in association with structural airway changes and/or bronchiectasis of the airways, causes ciliary slowing and disruption of the structural integrity of respiratory epithelium. However, macrolides are able to decrease its production of various virulence toxins, thereby protecting the epithelium directly, as well as indirectly by inhibiting neutrophil-associated cytotoxicity.

The effects of the macrolide group of antibiotics on microorganisms are discussed more fully below.

**Effects on mucus secretion**

Mucus hypersecretion is an important and characteristic feature of many respiratory diseases, including COPD, and increases the morbidity and mortality of these diseases especially as a
consequence of pulmonary infection. Chronic inflammation causes not only airway damage, but also goblet cell hyperplasia, which leads to mucus hypersecretion. Mediators of this inflammation include cytokines, chemokines and oxygen radicals. Both in vitro and in vivo studies have shown that macrolides, and in particular clarithromycin, reduce mucus and sputum secretion which may also contribute to their beneficial effects on airway inflammation.

P. aeruginosa has been shown to stimulate NCI-H292 epithelial cells to produce the mucin core protein, muc5ac. The mechanism appears to be related to stimulation of these cells by the pseudomonal virulence factor, N-(3-oxododecanoyl) homoserine lactone (30-C12-HSL), which is a component of the microorganism’s quorum sensing mechanism. In contrast, clarithromycin, inhibits muc5ac gene expression. Azithromycin also inhibited muc5ac production in an ERK 1/2-dependent manner. Other macrolides have also been shown to have effects on additional mucus secreting pathways. Macrolides may also decrease sputum production by decreasing water secretion into the airway by inhibiting chloride secretion. Furthermore, in one study of patients with purulent rhinitis, clarithromycin-treated patients showed a reduction in mucus volume and improvement in the quality of nasal mucus, such as viscoelasticity, cohesion, hydration, and transportability. In addition to decreasing the volume of secretions and increasing the mucus clearance by 30%, the rheology, hydration, cohesion and transportability of mucus in patients were similar to controls.

Some of these effects may be due to inhibition of neutrophil accumulation and function. Erythromycin, clarithromycin and azithromycin inhibited tumour necrosis factor (TNF)-induced release of granulocyte-monocyte colony-stimulating factor (GM-CSF) from A549 human airway epithelial cells, thereby inhibiting the ability of these cells to sustain neutrophil survival in vitro. Furthermore, anti-GM-CSF antibodies reduced epithelial cell-conditioned medium-mediated neutrophil survival. These combined inhibitory effects of macrolides on both inflammatory cells and mucus secretion were confirmed in an experimental rat model in which mucus production and neutrophil infiltration induced by intranasal ovalbumin and lipopolysaccharide were inhibited. These combined inhibitory effects are also thought to underlie the longstanding beneficial clinical effects seen with macrolides in the treatment of diffuse pan-bronchiolitis and other chronic inflammatory disorders of the airways.

**Effects on neutrophil and macrophage function**

As previously described, the inhibitory effects of macrolides on inflammatory cells are achieved by several mechanisms, including interference with the synthesis and expression of the adhesion molecules ICAM-1 and VCAM-1 on vascular endothelium, as well as with the upregulated expression of β2-integrins on activated neutrophils. Most importantly, however, clarithromycin (and other macrolides) interfere with the generation of neutrophil-selective chemoattractants by bronchial epithelial cells, eosinophils, monocytes, fibroblasts, and airway smooth muscle cells by mechanisms which appear to involve inhibition of intracellular signalling mechanisms, specifically the activities of mitogen-activated protein kinases, and extracellular signal-regulated kinases 1/2, as well as nuclear translocation of the transcription factors nuclear factor kappa B, activator protein-1, and specificity protein 1. Interference with the synthesis of IL-8, and possibly TNF, appears to be key events in macrolide-mediated attenuation of neutrophil influx into the airway.

Phagocytic clearance of apoptotic cells is impaired in COPD and aggravates and perpetuates the chronic inflammation found in this condition as a consequence of secondary necrosis. However, clarithromycin and azithromycin have been shown to increase the phagocytosis of apoptotic epithelial cells and neutrophils by alveolar macrophages. More recently Hodge and colleagues have demonstrated a decrease in alveolar macrophage mannose receptor expression in patients with COPD, which increased by 50% following azithromycin therapy, concomitant with both improvement in alveolar macrophage phagocytic ability and reduced inflammatory markers in the peripheral blood. These investigators suggested that the findings implicated the mannose receptor abnormality in the defective phagocytic function, which appeared to be amenable to azithromycin therapy.

**Macrolides and toll-like receptors**

Toll-like receptors (TLRs) are the prototype pattern recognition molecules which function primarily as the sentinels of the innate immune system. Although they perform key roles in host defence against microbial and viral pathogens, TLRs have also been identified as potential targets in the control of infection-associated harmful acute/hyperacute and chronic inflammatory responses. Interestingly, clarithromycin has recently been reported to down-regulate the expression of TLR4 on monocytes infected with *Helicobacter pylori*, while treatment of monocyte-derived dendritic cells with erythromycin caused differential modulation of expression of TLRs, resulting in up-regulation of TLR2, down-regulation of TLR3, and no effect on expression of TLR4. Activation of dendritic cells with peptidoglycan, polyribosinic:polyribocytidylic acid (poly I:C), or lipopolysaccharide (LPS), agonists of TLR2, 3 and 4, respectively, resulted in up-regulation of expression of costimulatory molecules and cytokine production. These pro-inflammatory activities of dendritic cells were effectively attenuated by treatment with erythromycin when the cells were activated with poly I:C or LPS, but not with peptidoglycan. In the case of poly I:C, erythromycin-mediated inhibition of pro-inflammatory activity was associated with decreases in both the activation of NF-κB and levels of TLR3 mRNA transcripts, while the mechanism by which the macrolide attenuated TLR4 signalling was unclear. These findings suggest that macrolides may selectively down-regulate exuberant inflammatory responses which result from the interaction of viruses and Gram-negative bacteria with TLR3 and TLR4, respectively, while preserving the interaction of Gram-positive bacteria with TLR2.

**Effects of macrolides on dendritic cells**

In one recent study, Sugiyama et al reported that cytokine production by LPS-activated murine dendritic cells is differentially modulated by 14-(clarithromycin) and 15-membered (azithromycin) macrolides, but is unaffected by a 16-membered macrolide (midecamycin). Clarithromycin was found to suppress the production of IL-6 by LPS-activated dendritic cells, and to render these cells less efficient inducers of IL-2 production by naïve T lymphocytes, while...
azithromycin augmented the production of the anti-inflammatory cytokine, IL-10.42. Taken together with the inhibitory effects of macrolides on TLR3/TLR4-mediated dendritic cells,41 it is clear that in addition to neutrophils, eosinophils, monocytes/macrophages, and epithelial cells, dendritic cells also represent potentially important targets for the anti-inflammatory actions of macrolides. The direct anti-inflammatory actions of macrolides are summarised in Table 2.

Table 2: Direct anti-inflammatory activities of macrolides

<table>
<thead>
<tr>
<th>Anti-inflammatory activity</th>
<th>Mechanisms</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhibition of neutrophil influx</td>
<td>Interference with:</td>
<td>7*</td>
</tr>
<tr>
<td></td>
<td>i) synthesis and release of IL-8 by various types of cells</td>
<td>7*</td>
</tr>
<tr>
<td></td>
<td>ii) synthesis of ICAM-1 and VCAM-1</td>
<td>7*</td>
</tr>
<tr>
<td></td>
<td>iii) upregulation of β2-integrins</td>
<td>7*</td>
</tr>
<tr>
<td>Inhibition of reactive oxygen species</td>
<td>Interference with:</td>
<td>7*</td>
</tr>
<tr>
<td></td>
<td>i) activity of NAPDH oxidase</td>
<td>7*</td>
</tr>
<tr>
<td></td>
<td>ii) synthesis of type II nitric oxide synthase (macrophages)</td>
<td>7*</td>
</tr>
<tr>
<td>Inhibition of cytokine production</td>
<td>Interference with:</td>
<td>32-35,40,41</td>
</tr>
<tr>
<td></td>
<td>i) activation of TLIs and intracellular signalling mechanisms</td>
<td>41,42</td>
</tr>
<tr>
<td></td>
<td>ii) dendritic cell activation and function</td>
<td>7*</td>
</tr>
<tr>
<td>Pre-apoptotic</td>
<td>i) inhibition of synthesis of IL-8 (anti-apoptotic for neutrophils)</td>
<td>7*</td>
</tr>
<tr>
<td></td>
<td>ii) induction of apoptosis</td>
<td>7*</td>
</tr>
<tr>
<td></td>
<td>iii) accelerated clearance of apoptotic neutrophils by macrophages</td>
<td>7*</td>
</tr>
</tbody>
</table>

*recently reviewed by Feldman et al

Table 2 modified from Feldman C, Anderson R. The cytoprotective interactions of antibiotics with human ciliated epithelium. In: Rubin BK, Tamaki J (eds.). Antibiotics as Anti-inflammatory and Immunomodulatory Agents. Basel: Birkhauser Verlag, 2005 (Table 2 page 57) with permission.

Additional effects of macrolides against bacteria

In addition to their conventional antimicrobial activity, the macrolide group of antibiotics has been documented to have additional effects against bacteria, which are not associated with inhibition of bacterial proliferation, and occur even in microorganisms that are totally resistant to their anti-proliferative actions.43-50 This has been most well studied in the case of *P. aeruginosa*, a microorganism against which macrolides have no antiproliferative activity.42,44 Macrolides have the ability to interfere with a number of virulence factors produced by *P. aeruginosa*.42,44 In addition to inhibiting virulence toxin production by this microorganism, clarithromycin also inhibits their ability to undergo ‘twitching motility’.44 The latter is mediated by the *P. aeruginosa* type IV pilus and mediates cell surface translocation. Type IV pilus act as adhesions for binding to host cells in the initial stages of infection. This, together with alginate production, contributes to the virulence of the organism.44 Alginate is an exopolysaccharide capsule of the organism, which is inhibited by clarithromycin in a dose-dependent manner, which occurs even at very low, sub-MIC concentrations of the antibiotic.44 By inhibiting alginate, macrolides decrease the viscosity of media-containing *P. aeruginosa*, which is conversely markedly increased if alginate is present.

Another important virulence factor is biofilm, which is a self-generated polymer matrix.44-47 Biofilms have the ability to bind cells and organic and inorganic materials to each other and to various other substrates.46 They have a tightly formed structure which reduces antimicrobial activity, promotes bacterial adherence to lung epithelium and prevents bacterial dehydration.46 These are important mechanisms for chronic pseudomonal persistence in the airways of patients with conditions such as diffuse panbronchiolitis, cystic fibrosis, bronchiectasis and even COPD. Quorum sensing is a mechanism of cell-to-cell communication utilizing diffusible signalling molecules which enables bacteria to detect and regulate their population density. Importantly, quorum sensing is necessary for biofilm formation, which in turn enhances microbial virulence by creating a milieu which not only favours persistence, but also protects microbial pathogens against antibiotics. Indeed, conventional *in vitro* antibiotic sensitivity testing procedures do not reflect the responses of bacteria which are encased in biofilm. Clarithromycin does not inhibit initiation of biofilm formation, but rather the quality of biofilm is impaired.44 It has also been shown that incubation of biofilm-grown *P. aeruginosa* with clarithromycin altered the structure and architecture of the biofilm.44 It is thought that the reason macrolides have been shown to be effective in chronic airway disorders, such as diffuse panbronchiolitis and cystic fibrosis, even in cases colonised with *P. aeruginosa*, is due to their ability to inhibit these various pseudomonal virulence factors, which are essential for bacterial survival.

These effects of macrolides have been much less well studied in other microorganisms, such as *S. pneumoniae*. We have recently reported that the production of pneumolysin, a major protein virulence factor of *S. pneumoniae*, which promotes extra-pulmonary dissemination, is attenuated by exposure of the bacteria to sub-inhibitory concentrations of clarithromycin. Interestingly, clarithromycin-mediated inhibition of the production of pneumolysin was observed not only with macrolide-susceptible strains of the pneumococcus, but also with macrolide-resistant strains which harboured either the ribosomal methylase (ermB) gene- or efflux pump (mef gene)-based mechanisms of macrolide resistance.49 These observations are in agreement with those of Lagrou et al.49 as well as those of Fukuda et al.49 who reported that erythromycin, or clarithromycin/azithromycin inhibited the production of pneumolysin by macrolide-resistant strains of *S. pneumoniae in vitro*, as well as in the lungs of experimentally infected mice. In a recent study, we reported that it is only macrolides (clarithromycin, erythromycin),
ketolides (telithromycin), clindamycin, and to a lesser extent azalides (azithromycin), which, at sub-MIC concentrations, attenuate the production of pneumolysin by both macrolide-susceptible and macrolide-resistant strains of the pneumococcus, while other types of antimicrobial agent commonly used in the treatment of infections caused by this microbial pathogen, including several which also target microbial protein synthesis, were largely without effect. The other antibiotics tested were amoxicillin, ceftriaxone, ciprofloxacin, moxifloxacin, and tobramycin, while doxycycline showed modest effects.51

Importantly, these mechanisms of macrolide-mediated antimicrobial activities counteract microbial persistence and perpetuation of harmful, ineffectual inflammatory responses. The various mechanisms by which macrolides suppress and reduce lung inflammation are summarised in Figure 1.

**Experimental studies with macrolides**

In one experimental model in which mice were exposed to cigarette smoke daily for six months, those treated for six months with clarithromycin had decreased airspace enlargement and destruction of the alveolar walls and impaired accumulation of macrophages in the lung (demonstrated on bronchoalveolar lavage) in a dose-dependent manner. The authors concluded that at clinically achievable dosages, clarithromycin was able to prevent cigarette smoke-induced emphysema in this animal model by modulating lung inflammation.52

In another very recent study, administration of azithromycin at a dose of 20 mg/kg 24 hours after experimental infection of cystic fibrosis mice with an alginate-overproducing strain of P. aeruginosa was associated with significantly improved survival, reductions in bacterial load, decreased lung inflammation and increased levels of interferon-gamma.53 The authors concluded that azithromycin enhances bacterial clearance and reduces lung inflammation by improving innate immune defence mechanisms in cystic fibrosis mice.

**Use of macrolides in patients with COPD**

Antibiotics are often used in patients with acute exacerbations of chronic obstructive pulmonary disease, which are now recognised to be due to bacterial infection in many cases. The indications for their use and the choice of agent, which may include a macrolide, are reviewed elsewhere.4,5,31 Many studies investigating the efficacy of macrolides in patients with exacerbations of COPD, as well as comparator studies against other classes of antibiotics, have confirmed them to be very effective.54 However, studies have also been undertaken with macrolides in patients with COPD in which they have been used for their anti-inflammatory activities as well as their effects on mucus secretion.2,15,28 For example, in a double-blind, placebo-controlled parallel group trial of 31 patients with chronic bronchitis, bronchiectasis or diffuse panbronchiolitis, clarithromycin 100 mg twice daily for eight weeks reduced sputum production from 51 g/day to 24 g/day and also increased sputum elasticity.15 Treatment with clarithromycin was associated with improved hypoxia, hypercapnia, pulmonary function and quality of life. In addition, in a study of patients with chronic bronchitis or bronchiectasis, seven days of treatment with clarithromycin (400 mg/day) decreased sputum production.55 The percentage of patients in whom it decreased >30% (so-called responders) was 38%. During treatment with clarithromycin, the sputum solid composition increased, while chloride concentration decreased in responders, but not in non-responders.56

In one double-blind study, 27 patients with COPD were randomised 2:1 to either azithromycin 500 mg once daily for three days or to placebo.58 In patients randomised to azithromycin, there were significant changes in serum cytokine values and indices of degranulation as well as oxidative burst of neutrophils, that lasted for 15 days after the end of treatment. Decreases in IL-8, lactoferrin and beta2-microglobulin levels were documented.

Preliminary data also suggest that improvements in clinical endpoints and/or in lung function may occur in patients with COPD who are treated with a macrolide. In one study, 67 patients with moderate to severe stable COPD were randomised to either three months of clarithromycin 500 mg daily or placebo and numerous markers of airway inflammation, bacterial colonisation, lung function, exercise tolerance and overall health status were evaluated.59 At the end of the time period there were no changes in sputum total cell count, inflammatory cytokine levels, or pathogen count in the clarithromycin group. It has been suggested by others that this may be due to the fact that the investigators only included patients on inhaled corticosteroids, since benefit on inflammatory markers has been shown in placebo-controlled studies in patients not on corticosteroids (see below).60 The clarithromycin group did have a small reduction in the neutrophil count and neutrophil chemotaxis. While total cell count and absolute neutrophil count did not correlate with lung function or walk test, they did correlate with health status score, respiratory symptoms and activity scores in the clarithromycin-treated group (p<0.03). The same authors were not able to show an effect of clarithromycin on health status, sputum bacterial numbers or exacerbation rates.61

Shorter term studies have also noted benefit from clarithromycin therapy. Nixon and colleagues documented improvement in spirometry, as well as clinical benefit, in their study of patients with COPD.62 In that study, 25 patients with COPD experiencing an exacerbation were given clarithromycin 500 mg daily for two weeks. FEV1 increased from 1.12 L to 1.34 L (p=0.003), and the mean total scores for signs and symptoms of COPD decreased. Another group investigated the effects of clarithromycin on inflammatory markers in 30 men with mild to moderate COPD, who were not on corticosteroids, in a double-blind, placebo-controlled study.63 They documented significant decreases in sputum total cell count, IL-8 and TNF levels, with similar decreases in serum inflammatory markers.

More recently, in a randomised, controlled trial, investigators administered erythromycin (250 mg twice daily) or placebo to 109 patients with moderate to severe COPD for one year.7 The primary end-point was frequency of exacerbations requiring antibiotics. The frequency of exacerbations in the erythromycin group was decreased by 35% and there was an increased median time to first exacerbation from 89-271 days (both significantly different from control). There were no significant differences in any of the secondary endpoints including lung function and selected sputum and serum inflammatory markers.
References

27. Inamura Y, Yamagata K, Mizuta Y, et al. Azithromycin inhibits MUC5AC production induced by the Pseudomonas aeruginosa subline N-3 oxidodocetol homoeostatic tissue factors in N2-hexadecyl-2