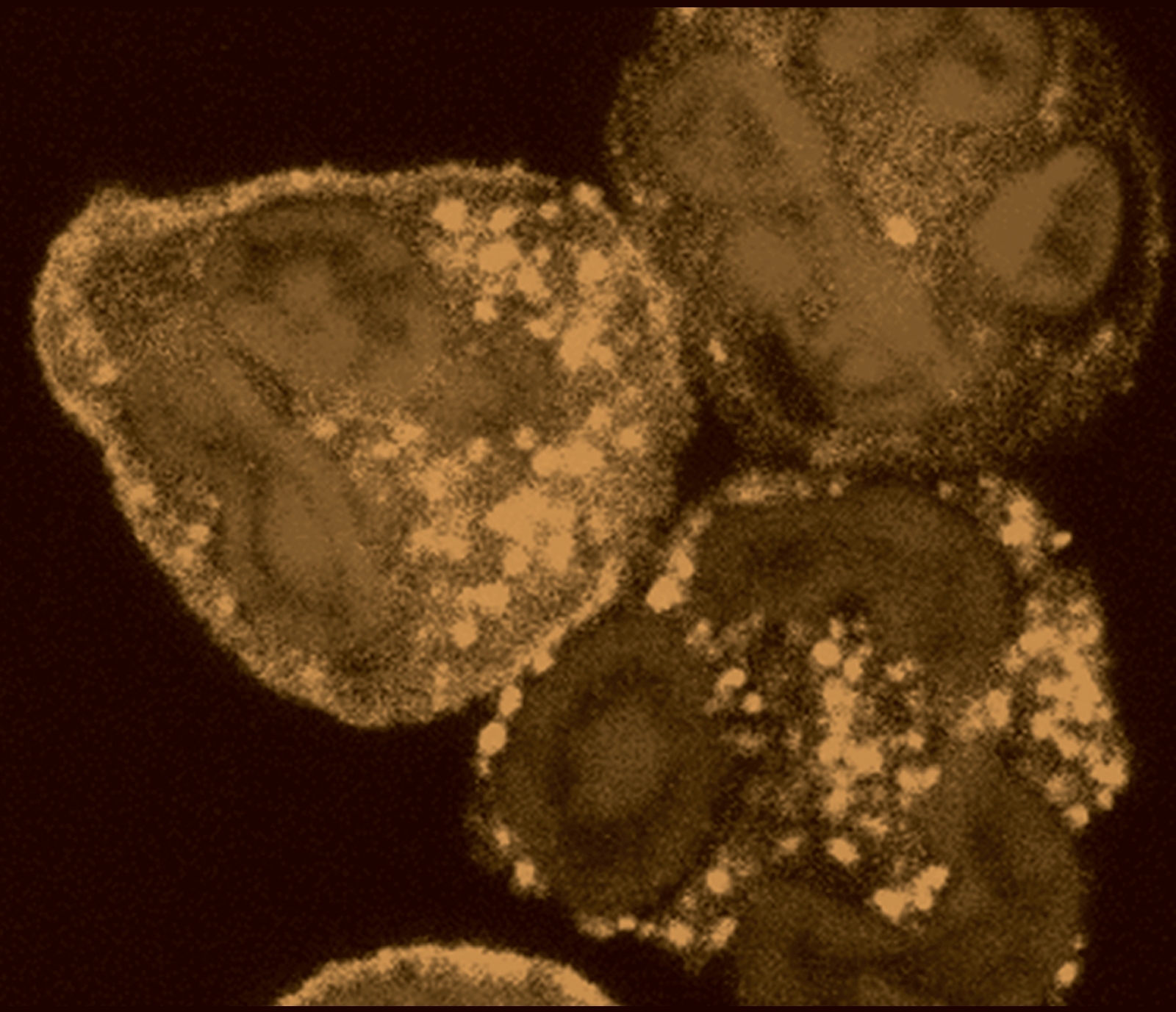
Mediators of Inflammation

Macrolide Therapy in Chronic Inflammatory Diseases

Guest Editors: Kazuhito Asano, Elżbieta Tryka, Joong Saeng Cho, and Naoto Keicho



Macrolide Therapy in Chronic Inflammatory Diseases

Mediators of Inflammation

Macrolide Therapy in Chronic Inflammatory Diseases

Guest Editors: Kazuhito Asano, Elżbieta Tryka,
Joong Saeng Cho, and Naoto Keicho

Editorial Board

Contents

Hindawi Publishing Corporation
Mediators of Inflammation
Volume 2012, Article ID 692352, 2 pages
doi:10.1155/2012/692352

Editorial

Macrolide Therapy in Chronic Inflammatory Diseases

Kazuhito Asano,[1] Elżbieta Tryka,[2] Joong Saeng Cho,[3] and Naoto Keicho[4]

[1] *Division of Physiology, School of Nursing and Rehabilitation Sciences, Showa University, Yokohama 226-8555, Japan*
[2] *Department of Otolaryngology and Laryngological Oncology, Medical University of Lublin, Lublin 20-090, Poland*
[3] *Department of Otolaryngology, School of Medicine, Kyung Hee University, Seoul 100-101, Republic of Korea*
[4] *Department of Respiratory Diseases, Research Institute, National Center for Global Health and Medicine, Tokyo 162-0052, Japan*

Correspondence should be addressed to Kazuhito Asano, asanok@med.showa-u.ac.jp

Received 12 December 2012; Accepted 12 December 2012

Sinobronchial syndrome is well accepted to involve the coexistence of chronic rhinosinusitis (CS) and chronic lower airway inflammation such as chronic bronchitis and diffuse panbronchiolitis (DPB). Although these diseases are resistant to several types of treatment, after discovery of the effectiveness of erythromycin on DPB, low-dose and long-term administration of macrolide antibiotics such as erythromycin, roxithromycin, and clarithromycin, are used frequently in the treatment of these diseases with remarkable success [1]. It has also reported that long-term use of azithromycin, a 16-membered macrolide antibiotic, can improve the lung functions in patients with cystic fibrosis (CF) [2]. These reports clearly indicate that the prognosis of these life-threatening airway diseases, especially DPB and CF, may improve dramatically, but the mode of action of this macrolide therapy is not fully understood. Furthermore, there is little information about the kind of diseases, which can be treated with the macrolide therapy.

This special issue focuses mainly on 9 distinct papers to deal with the therapeutic mechanisms of macrolide on inflammatory diseases, the influence of macrolide antibiotics on respiratory viral infection, and the usefulness of macrolide therapy on inflammatory skin disease.

Therapeutic Mechanisms of Macrolide Antibiotics. Macrolides are a group of antibiotics with a macrocyclic lactone ring, which are classified into 14, 15, and 16 members, combined with sugar. These compounds are also accepted to be active against many species of Gram-positive and some Gram-negative bacteria and used frequently for the treatment of infectious diseases in respiratory tract. Besides their bacteriostatic and bactericidal effects, macrolides are used for the treatment of chronic airway inflammatory disease with remarkable success. However, the precise mechanisms by which macrolides could favorably modify the clinical status of chronic inflammatory diseases are not fully understood. B. Kwiatkowska and M. Maślińska and H. C. Steel review the therapeutic mode of action of macrolides on chronic inflammatory diseases. T. Shimizu and S. Shimizu examine the influence of azithromycin (AZ) on mucus hypersecretion in vitro and in vivo. They reveal the suppressive effects of AZ, but not josamycin and ampicillin on mucus secretion induced by inflammatory stimulation and propose that AZ will be a useful agent for the treatment of inflammatory diseases characterized by mucus hypersecretion. J. Bai et al. examine the influence of macrolide antibiotics on regulatory T-cell (Treg) functions through the choice of erythromycin and a rat model of smoke-induced lung inflammation (emphysema) and revel that oral administration of the agent into rat enhances Treg functions along with inhibition of lung inflammation. This novel data are very worthy to understand the therapeutic mode of action of macrolide antibiotics on airway inflammatory diseases.

Respiratory Viral Infections. The respiratory viral infections such as rhinovirus, respiratory syncytial virus, and influenza virus, among others, cause the high mortality rate through an overactive inflammatory response. Severity of airway viral infection is also accepted to be closely related with virus-induced hyperproduction of both inflammatory cytokines and chemokines, which are responsible for the development of fatal clinical symptoms such as massive pulmonary edema,

acute bronchopneumonia, and acute respiratory distress syndrome. Since there is much evidence showing the suppressive effects of macrolide antibiotics on hyperproduction of inflammatory cytokines, macrolide antibiotics may be considered as promising treatment option in the treatment of airway viral infections. In this regard, J.-Y. Min and Y. J. Jang review the usefulness of macrolides in the treatment of airway viral infections. Furthermore, S. Yokota et al. also show the efficacy of macrolide antibiotics, especially clarithromycin, in the prevention of immunological disorders and secondary bacterial infections during airway viral infections.

Skin Disorders and Bronchiectasis. Long-term therapy with macrolide antibiotics is shown to be effective in the treatment of chronic airway inflammatory diseases such as CF, CS, and DPB. A. A. Alzolibani and K. Zedan and C. Rodrigues-Cerdeira et al. show the potential benefits of macrolide antibiotics in the treatment of cutaneous disorders such as atopic dermatitis, neutrophilic dermatitis, rosacea, and alopecia areata, among others. R. Masekala and R. J. Green also show the efficacy of macrolide antibiotics in the treatment of noncystic fibrosis-related bronchiectasis, an orphan lung disease, which results in impaired quality of life and mortality in paediatrics if it left untreated.

Acknowledgments

We would like to thank all contributors and reviewers and K. Asano personally grateful for support to this special issue as there can be no growth and improvement without their participation.

Kazuhito Asano
Elżbieta Tryka
Joong Saeng Cho
Naoto Keicho

References

[1] N. Keicho and S. Kudoh, "Diffuse panbronchiolitis: role of macrolides in therapy," *American Journal of Respiratory Medicine*, vol. 1, no. 2, pp. 119–131, 2002.

[2] A. Jaffé, J. Francis, M. Rosenthal, and A. Bush, "Long-term azithromycin may improve lung function in children with cystic fibrosis," *The Lancet*, vol. 351, no. 9100, p. 420, 1998.

Hindawi Publishing Corporation
Mediators of Inflammation
Volume 2012, Article ID 563709, 16 pages
doi:10.1155/2012/563709

Review Article

Clinical Application of Development of Nonantibiotic Macrolides That Correct Inflammation-Driven Immune Dysfunction in Inflammatory Skin Diseases

Carmen Rodriguez-Cerdeira,[1] Elena Sanchez-Blanco,[2] and Alberto Molares-Vila[3]

[1] *Dermatology Department, CHUVI and University of Vigo, Vigo, Spain*
[2] *University of Vigo, Vigo, Spain*
[3] *Analytical Chemistry Department, University of Vigo, Vigo, Spain*

Correspondence should be addressed to Carmen Rodriguez-Cerdeira, crodcer@uvigo.es

Received 18 February 2012; Revised 20 July 2012; Accepted 22 July 2012

Academic Editor: Kazuhito Asano

Background. Inflammation-driven immune dysfunction supports the development of several chronic human disorders including skin diseases. Nonantibiotic macrolides have anti-inflammatory and/or immunomodulatory activity that suggests the exploitation of these in the treatment of skin diseases characterized by inflammatory disorders. *Materials and Methods*. We performed an extensive review of the nonantibiotic macrolide literature published between 2005 and 2012, including cross-references of any retrieved articles. We also included some data from our own experience. *Results*. Calcineurin antagonists such as tacrolimus and ascomycins (e.g., pimecrolimus) act by inhibiting the activation of the nuclear factor for activated T cells (NFAT). There are new applications for these macrolides that have been available for several years and have been applied to skin and hair disorders such as atopic dermatitis, oral lichen planus, vitiligo, chronic autoimmune urticaria, rosacea, alopecia areata, pyoderma gangrenosum, Behcet's disease, neutrophilic dermatosis, and lupus erythematosus. We also reviewed new macrolides, like rapamycin, everolimus, and temsirolimus. In addition to the literature review, we report a novel class of nonantibiotic 14-member macrocycle with anti-inflammatory and immunomodulatory effects. *Conclusions*. This paper summarizes the most important clinical studies and case reports dealing with the potential benefits of nonantibiotic macrolides which have opened new avenues in the development of anti-inflammatory strategies in the treatment of cutaneous disorders.

1. Introduction

The term "macrolide" encompasses a diverse family of unrelated compounds with large macrolactam rings. The activity of these compounds stems from the presence of a macrolide ring. Macrolide rings are comprised of a large macrocycle lactone ring to which one or more deoxy sugars, usually cladinose and desosamine, may be attached. In addition to their antibacterial activity, macrolides have diverse biological effects, including modulation of inflammatory and immune responses without affecting homeostatic immunity [1, 2].

Macrolides are effective antibiotics that have immunomodulatory effects and inhibit the production of many proinflammatory cytokines such as interleukin 6 (IL-6), IL-8, and tumor necrosis factor alpha (TNFα). Macrolides are used in inflammatory skin and hair disorders. Many studies have been performed to assess their effectiveness in the treatment of rosacea, psoriasis, pityriasis rosea, alopecia areata, bullous pemphigoid, and pityriasis lichenoides [3].

However, new strategies for the treatment of cutaneous pathologies are directed towards the development of new nonantibiotic macrolides with anti-inflammatory, antiproliferative, and antiangiogenesis properties. The most known and used are inhibitors of the phosphatase calcineurin (pimecrolimus and tacrolimus), which under normal circumstances induce the transcription of IL-2. In addition, these drugs inhibit lymphokine production and interleukin release, which lead to a reduced function of effector T-cells [4].

Nowadays, novel chemical structures with improved therapeutic anticancer and anti-inflammatory properties by affecting skin disease targets have arose from mammalian rapamycin inhibitors. These agents inhibit the response to IL-2 and thus block the activation of T and B lymphocytes [5, 6].

Recently, new synthetic derivatives of the macrolide azithromycin, namely, CSY0073, (8R,9S)-8,9-dihydro-6,9-anhydropseudoerythromycin A (EM900), and (8R,9S)-4″,13-O-diacetyl-8,9-dihydro-6,9-epoxy-8,9-anhydropseudoerythromycin A (EM911) having potent anti-inflammatory properties have been developed [6]. Currently, ridaforolimus has been developed but has only been used *in vitro* thus far. More studies are required to uncover the possible applications of these promising molecules although one of the first possible applications of these compounds is as an antitumor agent [7].

In this paper, we review the clinical use of nonantibiotic macrolides that have become available clinically for chronic inflammatory skin diseases with immune dysfunction.

2. Methodology

We searched the Cochrane Central Register of Controlled Trials (Central), Med-Line (PubMed), and Embase (2005 to January 2012). We also examined references from selected articles. We included case series with 5 or more patients, cohort trials, and randomised controlled trials. Search terms used were: "tacrolimus", "pimecrolimus", "calcineurin inhibitors", "new macrolides", "rapamycin", and so forth and "atopic dermatitis", "psoriasis", and other common dermatitises that have been treated using macrolides. We also include some data from our own experience.

3. Results and Discussion

We have divided the paper into 2 sections.

3.1. Innovative Use of Calcineurin Inhibitors

3.1.1. Pimecrolimus. Pimecrolimus (SDZ ASM 981, Novartis) is one of the new classes of novel ascomycin immunomodulating macrolactams and was developed for the treatment of inflammatory skin diseases (Figure 1) [8]. Ascomycin, first isolated as a fermentation product of *Streptomyces hygroscopicus* var. ascomycetes, in the early 1960s, was initially researched for its antifungal properties. However, more than 20 years later, ascomycin was investigated for its structural and immunomodulatory properties. Pimecrolimus is a colourless, solid compound with a molecular weight of 810.48 Daltons. Interest in pimecrolimus has been intense because it has significant anti-inflammatory and immunomodulatory activity and because it has low potential for systemic immunosuppression [4]. The mechanism of action of pimecrolimus involves the blockage of T cell activation. Ascomycin macrolactams are immunophilin ligands that bind to a specific cytosolic receptor. Pimecrolimus binds to FKBP-12 and immunophilin macrophilin-12, also known as FK506 binding protein. Like tacrolimus and

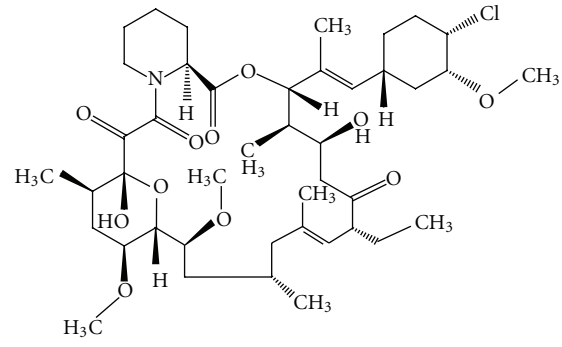


Figure 1: Molecular structure of pimecrolimus extracted from Kyoto Encyclopedia of Genes and Genomes (KEGG) database [8].

cyclosporin A, pimecrolimus acts by binding to macrophilin-12. The pimecrolimus-macrophilin complex then binds to the cytosolic enzyme calcineurin phosphatase. Calcineurin is a Ca^{2+}/calmodulin-dependent protein phosphatase that regulates the translocation of the cytosolic components of NFATs. NFATs, in turn, regulate the promoter activities of several mediators during mRNA transcription. By inhibiting the action of calcineurin, the pimecrolimus-macrophilin complex prevents the dephosphorylation of the cytoplasmic component of NFATs. NFATs regulate the mRNA transcription of a number of inflammatory cytokines. Therefore, pimecrolimus blocks the transcription of these cytokines, especially T-helper Th1 (IL-2-, IFN-γ-) and Th2 (IL-4-, IL-10-) type cytokines (Figure 2) [8]. Pimecrolimus decreases the production of other cytokines, including interleukins IL-5, IL-10, and TNFα, in a dose-dependent manner [4]. Pimecrolimus also targets mast cells, which play an important role in anti-inflammatory activities. Pimecrolimus inhibits not only the transcription and synthesis of cytokines from mast cells, but also inhibits the release of the preformed mediators serotonin and β-hexosaminidase. Additionally, pimecrolimus inhibits Fc Epsilon RI-mediated degranulation and secretion (Figure 3) [9]. It is important to note that all of these inhibitory processes occur only when pimecrolimus is bound to macrophilin-12. In a study of murine mast cell line CPII, pimecrolimus did not inhibit the transcription of a reporter gene that was under the control of human TNFα promoter in the murine dendritic cell line and had no effect on IL-8 release from keratinocytes, fibroblasts, and endothelial cells. This is an indication of the specificity of the pharmacologic activity of pimecrolimus.

Atopic dermatitis (AD) is a pruritic disease of unknown origin that usually develops in early infancy (an adult-onset variant is recognized); it is characterized by pruritus, eczematous lesions, xerosis (dry skin), and lichenification (thickening of the skin and an increase in skin markings). AD may be associated with other atopic (immunoglobulin-E-(IgE-) associated) diseases (e.g., acute allergic reaction to foods, asthma, urticaria, and allergic rhinitis) [10]. Treatment of AD is one of the best known applications for pimecrolimus. Pimecrolimus inhibited cytokines, IL-2 and interferon gamma IFNγ, and Th2-type cytokines, IL-4

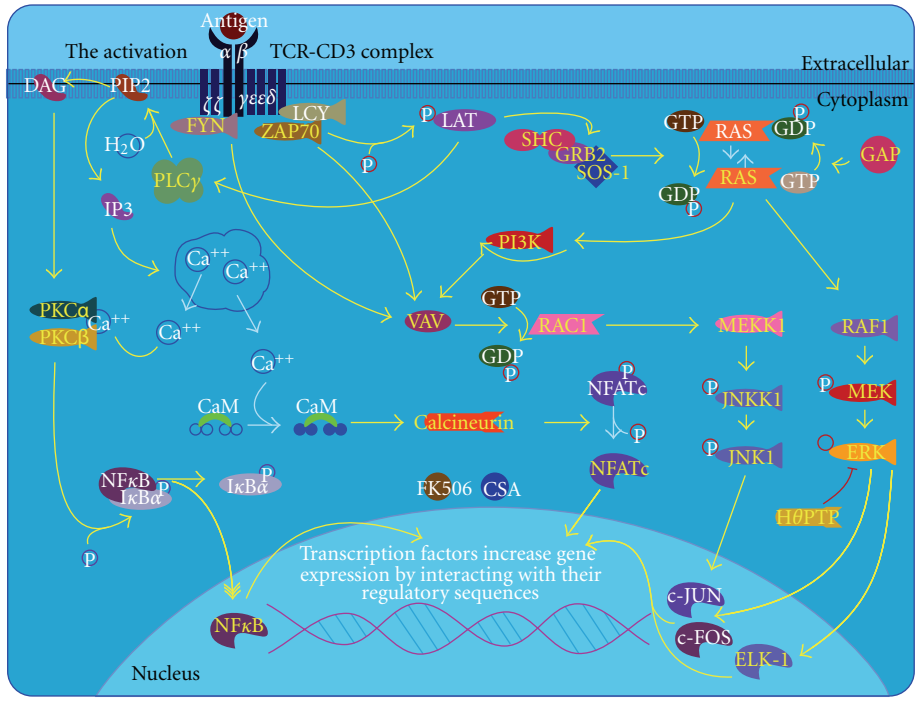


Figure 2: T cell receptor signaling pathway extracted from BioCarta database [9].

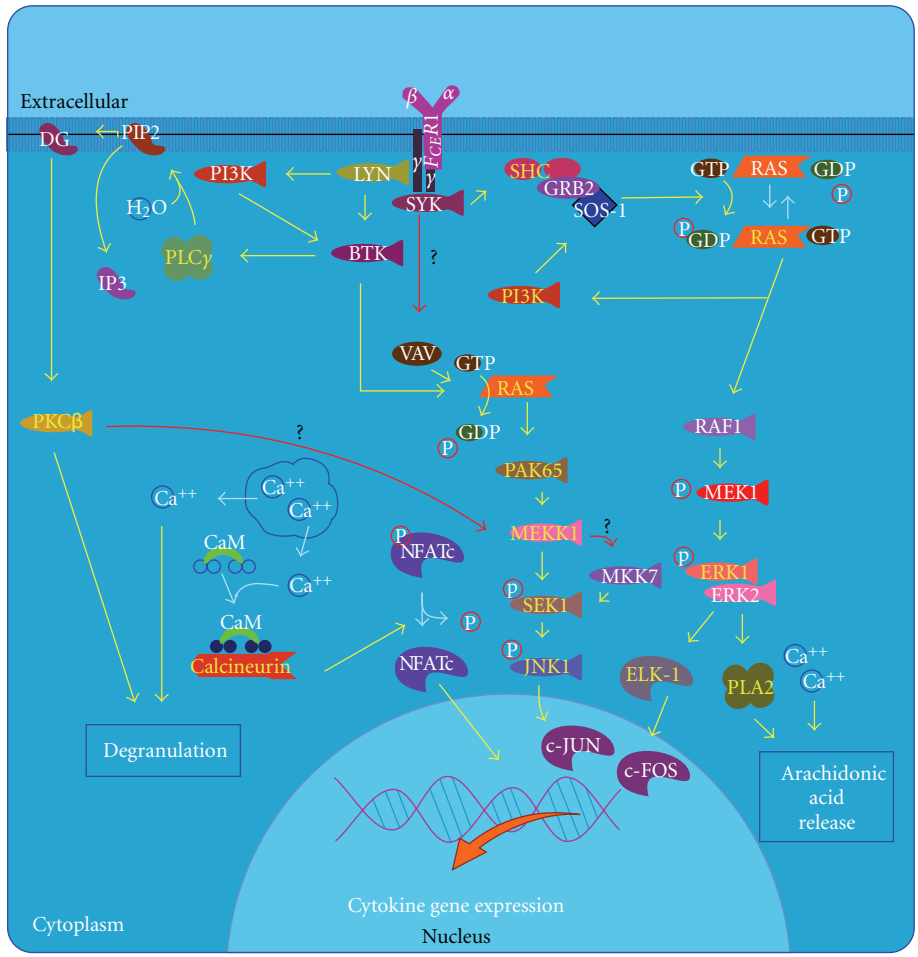


Figure 3: Fc epsilon receptor I signaling in mast cell pathway extracted from BioCarta database [9].

and IL-10. In addition, pimecrolimus prevents the release of inflammatory cytokines and mediators from mast cells *in vitro* after stimulation by antigen/IgE. References are still emerging in the literature for AD treatment with these drugs. Pimecrolimus cream 1% is a good option for treatment of mild to moderate AD in adults and children aged ≥2 years [4, 10]. No novel systemic applications have appeared since 2005. In 2005, however, there was a study of oral pimecrolimus for use in the treatment of moderate to severe AD. This study demonstrated the efficacy and short-term safety of oral pimecrolimus in adults in a double-blind study with a 12-week treatment and 12-week post-treatment phase. Longer-term studies in larger cohorts are now required [11].

Psoriasis is considered a chronic skin condition. However, its exact cause remains unknown. Psoriasis may develop because of a combination of factors, including genetic predisposition and environmental factors. Psoriasis may be commonly observed among members of the same family. The immune system is thought to play a major role in the development of this condition. Psoriasis has a variable course, which periodically improves and worsens. Many people note a worsening of their symptoms in the colder winter months. Psoriasis produces red, dry plaques of thickened skin. The dry flakes and skin scales are thought to result from the rapid proliferation of skin cells that is triggered by abnormal lymphocytes in the blood. Psoriasis commonly affects the skin of the elbows, knees, and scalp [12]. Another important application for pimecrolimus is psoriasis treatment, where it acts through blockage of T-cell activation and signal transduction pathways in T cells and through inhibition of the synthesis of inflammatory cytokines, which play a key role in the pathogenesis of psoriasis [13]. Oral pimecrolimus was tested in healthy adult outpatients with moderate to severe chronic plaque-type psoriasis ($n = 143$) who received either an oral placebo or pimecrolimus for 12 weeks. Oral pimecrolimus was well tolerated and produced a dose-dependent reduction in psoriasis severity. Doses of 20 mg and 30 mg b.d. were the most effective [14, 15].

Oral lichen planus (OLP) is an inflammatory condition that affects the mucous membranes of the mouth. OLP may appear as white lacy patches, red swollen tissues, or open sores. These lesions may cause burning, pain, or other discomfort. OLP is a T-cell-mediated chronic inflammatory oral mucosal disease of unknown cause, and lesions contain few B cells or plasma cells and minimal deposits of immunoglobulin or complement. Therefore, OLP is ideal for studying human T-cell-mediated inflammation and autoimmunity. Antigen-specific mechanisms in OLP include antigen presentation by basal keratinocytes and antigen-specific lysis of keratinocytes by CD8+ cytotoxic T cells. Nonspecific mechanisms include mast cell degranulation and matrix metalloproteinase activation in OLP lesions. A combination of these mechanisms may cause T cell accumulation in the superficial lamina propria, basement membrane disruption, intraepithelial T cell migration, and apoptosis of keratinocytes in OLP (Figure 4) [9, 16]. Pimecrolimus, as described above, inhibits dephosphorylation of nuclear factor of activated T cells by calcineurin, thus, reducing T-cell cytokine production and inhibiting T-cell activation. Pimecrolimus significantly reduces the symptoms of OLP [17, 18].

Vitiligo is a common depigmenting disorder affecting about 1-2% of the world population. Approximately half of the affected individuals develop the disease before adulthood. Etiologic hypotheses for vitiligo include biochemical, neural, and autoimmune mechanisms. The most compelling of these suggests a combination of genetic and immunologic factors that results in autoimmune melanocyte destruction. Pimecrolimus have comparable efficacy and a better safety profile compared with topical corticosteroids. It was effective in their treatment better than topic corticoids [19, 20].

Patients in whom the cause of urticaria is unknown are said to have chronic idiopathic urticaria; however, findings suggest that in 25–45% of patients, chronic idiopathic urticaria is not idiopathic but is an autoimmune disease termed as chronic autoimmune urticaria [21]. Chronic autoimmune urticaria is dependent not only on the cross-linking of IgE receptors (by anti-Fc Epsilon RIa or anti-IgE), but also on the activation of complement. Cross-linking of IgE receptors leads to histamine release via a calcineurin-dependent signal transduction pathway, whereas complement C5a receptors act through G-proteins. Histamine release by patient sera or isolated IgG can be inhibited by ascomycin but not the C5a. The failure of pimecrolimus to satisfactorily treat chronic autoimmune urticaria may at least in partly result from this [22].

Rosacea is a common cutaneous disorder, which occurs most frequently in light-skinned middle-aged individuals. Cutaneous signs are flushing, erythema, telangiectasia, and papules and pustules. An important reference we found to the use of pimecrolimus for the treatment of rosacea was a study "by Kim" in 26 patients with mild to moderate inflammatory rosacea [23].

Alopecia areata (AA) is an autoimmune disease of the hair follicle caused by a T-lymphocytic infiltrate, although its pathogenesis is not yet completely clear. AA results in hair loss and baldness, and may frequently remit and relapse. Histologically, the peribulbar infiltration consists mainly of activated CD4+ and CD8+ T-cells. Type 1 cytokines, including IL-2, IFN-c, and TNFα, mediate initiation of the immune response in AA. Pimecrolimus prevents calcineurin-mediated dephosphorylation of the NFATs, which inhibits the synthesis of Th1 and Th2 cytokines in T lymphocytes. Topical pimecrolimus treatment is as effective as topical corticosteroids for the treatment of AA and frontal fibrosing alopecia, and has fewer side effects than topical corticosteroids [24, 25].

Pyoderma gangrenosum (PG) is an uncommon ulcerative cutaneous condition of uncertain cause. PG is associated with systemic diseases in at least 50% of the patients. This condition is diagnosed by excluding other causes of similar-appearing cutaneous ulcerations, including infection, malignancy, vasculitis, collagen vascular diseases, diabetes, and trauma. Pathergy involves development of new ulcerations after trauma or injury to the skin in 30% of patients with existing PG. The pathogenesis of PG is not entirely understood, but defects in cell-mediated immunity, humeral immunity, neutrophil chemotaxis, and monocyte

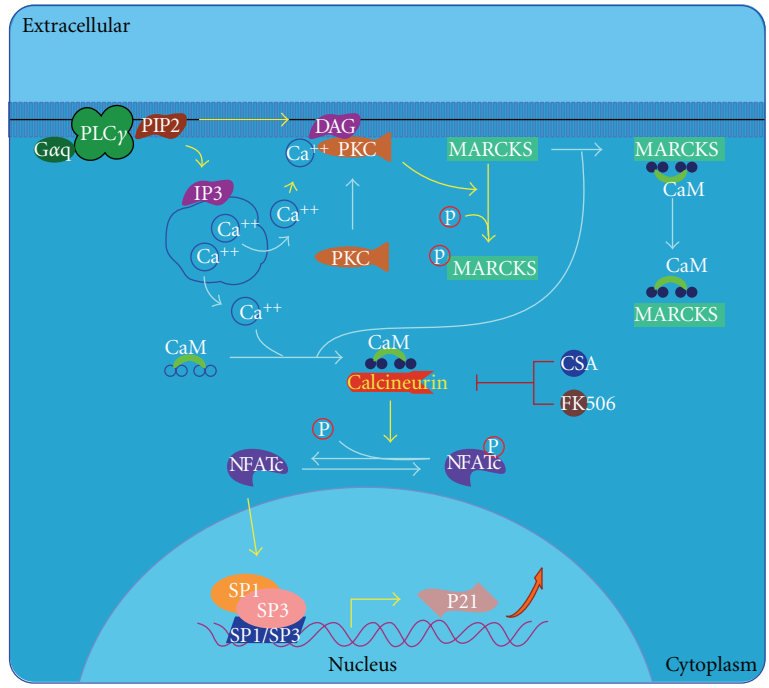


Figure 4: Effects of calcineurin in keratinocyte differentiation pathway extracted from BioCarta database [9].

phagocytosis along with diminished lymphokine production have been observed in patients with PG [26]. Positive clinical results from treatment of PG with pimecrolimus and tacrolimus are probably due, in part, to a decrease in the release of TFNα. TFNα release is considered to be very important in the development of the neutrophilic dermatoses. Pimecrolimus does not affect the differentiation, maturation, and functions of Langerhans cells and does not induce their apoptosis [27].

Discoid lupus erythematosus (DLE) is a chronic skin condition of sores with inflammation and scarring on the face, ears, and scalp, and at times, on other areas of the body. These lesions develop as a red inflamed patch with a scaling and crusty appearance. Localized DLE typically manifests as skin lesions localized above the neck and mainly involves sites such as the scalp, bridge of nose, cheeks, lower lip, and ears [28]. Lesions have elevated levels of IL-2, IFNγ, and TNFα mRNA, as compared to normal skin. Elevated type I IFN (IFN-α/β) has also been found in these skin lesions. Type 1 IFN is correlated with Th1-associated inflammation. In addition, unlike cyclosporine and tacrolimus, the action of pimecrolimus is more selective for T-cells and mast cells, thus reducing the likelihood of systemic immunosuppression [29].

Behçet's disease (BD) was named in 1937 after the Turkish dermatologist Hulusi Behçet who first described the triple-symptom complex of recurrent oral aphthous ulcers, genital ulcers, and uveitis. Painful genital ulcerations usually develop around the anus, vulva, or scrotum and cause scarring in 75% of the patients. The cause is not well defined, but it is primarily characterized by autoinflammation of the blood vessels. The primary mechanism of the damage is an overactive immune system that seems to target the patient's own body. The primary cause is not well known. In fact, as of now, no one knows why the immune system starts to behave this way in Behçet's disease. There does however seem to be a genetic component involved, as first degree relatives of the affected patients are often affected in more than expected proportion for the general population [30]. Pimecrolimus is safe and effective for the treatment of BD genital ulcers and accelerates the healing process [31].

Graft-versus-host disease (GVHD) is a common complication of an allogeneic tissue transplant. GVHD is commonly associated with stem cell or bone marrow transplant, but the term also applies to other forms of tissue graft. Immune cells (white blood cells) in the tissue (the graft) recognize the recipient (the host) as "foreign." Subsequently, the transplanted immune cells attack the cells of the host's body. GVHD can also occur after a blood transfusion if irradiated blood products are not used. In the classical sense, acute GVHD is characterized by selective damage to the liver, skin (rash), mucosa, and the gastrointestinal tract. New research indicates that target organs of GVHD other than those mentioned above include the immune system (the hematopoietic system, e.g., the bone marrow and the thymus) itself, and the lungs in the form of idiopathic pneumonitis. Further, chronic GVHD involves the above organs but can also cause damage to the connective tissue and exocrine glands over a long term. T cells present in the graft, either as contaminants or intentionally introduced into the host, attack the tissues of the transplant recipient after perceiving host tissues as antigenically foreign. The T cells produce an excess of cytokines, including TNF-α and interferon-gamma (IFNγ). A wide range of host antigens

can initiate GVHD, such as the human leukocyte antigens (HLAs). The only study in which the treatment of GVHD was reported was that by Schmook. Further research is required to address this issue [32].

3.1.2. Tacrolimus. Tacrolimus (Figure 5) [9] was first isolated in 1984 from a Japanese soil fungus. Tacrolimus is structurally dissimilar to cyclosporine, but has similar immunosuppressive properties. The macrolide antibiotic tacrolimus (FK 506) was discovered as a naturally occurring metabolite of the fungus *Streptomyces tsukubaensis*. Tacrolimus is a "prodrug" that becomes active after forming complexes with intracytoplasmic proteins called immunophilins. Once activated, tacrolimus binds to FKBP. At least 4 FKBP are described: 12, 13, 25, and 59. The main effect of tacrolimus appears to result from the inhibition of T-cell function. Following the binding of an antigen-presenting cell to a T cell via the T cell receptor, intracytoplasmic levels of calcium rise, leading to calmodulin activation of the phosphorylase enzyme, calcineurin phosphatase. Calcineurin phosphatase is the main target of this drug. The activation of calcineurin phosphatase leads to the dephosphorylation of a cytoplasmic protein-NFAT. Once dephosphorylated, NFAT translocates into the nucleus where it combines with a nuclear subunit (NFATn). The resulting nuclear complex binds to the promoter units of several genes. The binding of NFATn enables transcription of proinflammatory cytokines, including IL-2, IL-4, IFNγ, and TGF-β and upregulation of receptors, such as IL-2R (CD25). Transcription of these cytokines initiates T-cell activation (Figure 2) [9, 33]. Activated tacrolimus inhibits the action of calcineurin, thus preventing the dephosphorylation of nuclear factors and blocking this path to gene transcription. In stimulated T cells, tacrolimus inhibits activation principally by suppressing IL-2 production and IL-2R expression. Inhibition of IL-2 production blocks the activation of T-helper cells, T-regulatory cells (autocrine loop), natural killer cells, and monocytes. In addition to inhibiting IL-2 transcription, other calcium-dependent events, including nitric oxide synthase activation (Figure 6) [9], cell degranulation, and apoptosis (Figure 7) [9] are also inhibited. In stimulated mast cells, tacrolimus decreases histamine release, impairs Langerhans' cell function, and downregulates high-affinity IgE receptors. It also decreases the production of chemotactic protein-1 and IL-8 in monocytes and affects other cell types, including neutrophils, eosinophils, and endothelial cells. Inhibition of calcineurin interferes with superantigen stimulation of T cells and may decrease the production of vascular endothelial growth factor. Tacrolimus also inhibits the function of B cells and the production of other cytokines such as IL-3, IL-4, IL-5, IFNγ, TNFα, and granulocyte-macrophage colony stimulating factor (GM-CSF) [34].

When used to treat AD, tacrolimus inhibits the T lymphocytes, which release the cytokines that trigger the inflammation underlying AD. Tacrolimus also affects other cells including Langerhans and mast cells. By downregulating T cells, the symptoms of AD begin to fade within a few days of applying a topical ointment that contains tacrolimus. Such ointments penetrate the skin sufficiently to allow local immunomodulation. However, the skin does not act as a reservoir for this drug, as discussed by Kim and Kono [35]. Oral tacrolimus is an additional therapeutic option for management of severe and extensive AD [36].

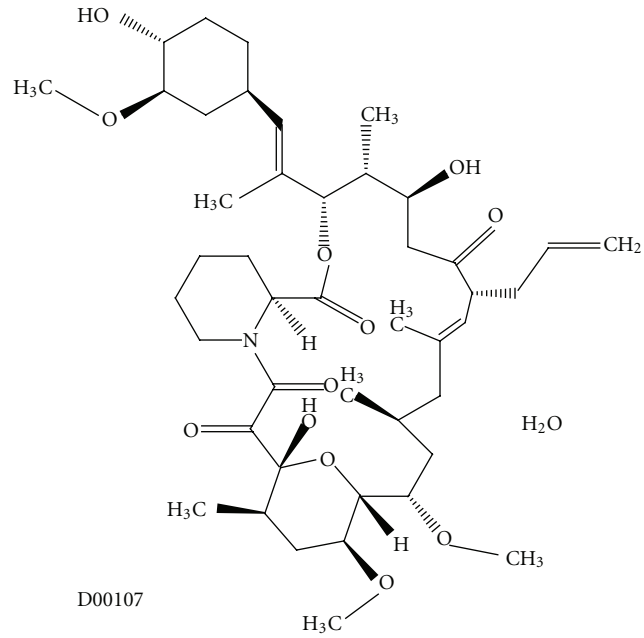


Figure 5: Molecular structure of tacrolimus extracted from KEGG database [9].

Tacrolimus inhibits the production of many proinflammatory cytokines, such as IL-6, IL-8, and TNFα, perhaps by suppressing the transcription factors NF-κB or activator protein-1. It also reduces neutrophil activity. Studies of topical tacrolimus as a treatment for psoriasis have yielded disappointing results. However, topical tacrolimus that was applied under occlusion to descaled psoriatic plaques is an effective treatment. There is good evidence that topical tacrolimus is a highly effective treatment for psoriasis of the face and flexures [37–39].

In our clinical practice, treatment with 0.15 mg/kg b.d. oral tacrolimus for 1 week resulted in a marked reduction in the erythema and scaling of severe psoriasis patients. Complete remission occurred after 4 weeks of treatment. Administration of tacrolimus at a dose of 0.3 mg/kg per day to 7 patients with recalcitrant psoriasis resulted in remission with minor metabolic effects, including minimal elevation of urea, creatinine, and glucose in the blood [40].

More recently, tacrolimus has been used to treat genital lichen sclerosus, a condition in which patches of the skin become thin and wrinkled. Thus, the skin tears easily, and bright red or purple bruises are common. Sometimes, the skin becomes scarred. Tacrolimus blocks the proliferation of T lymphocytes and the release of inflammatory cytokines from these cells. The skin on the patches becomes thin and crinkled. Then the skin tears easily, and bright red or purple bruises are common [41]. Sometimes, the skin becomes scarred. If the disease is a mild case, there may be no symptoms. Tacrolimus ointment 0.1% may also be effective and well tolerated for the treatment of anogenital lichen

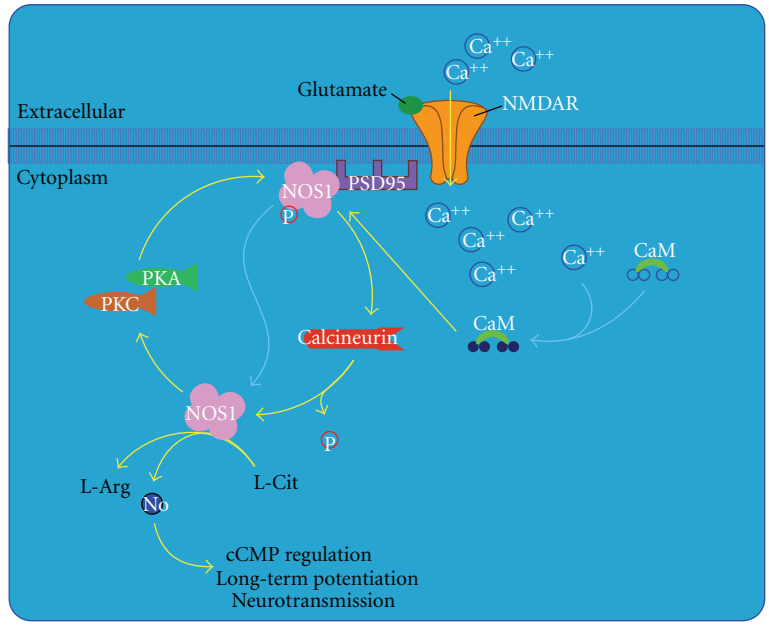


Figure 6: Nitric oxide signaling pathway extracted from BioCarta database [9].

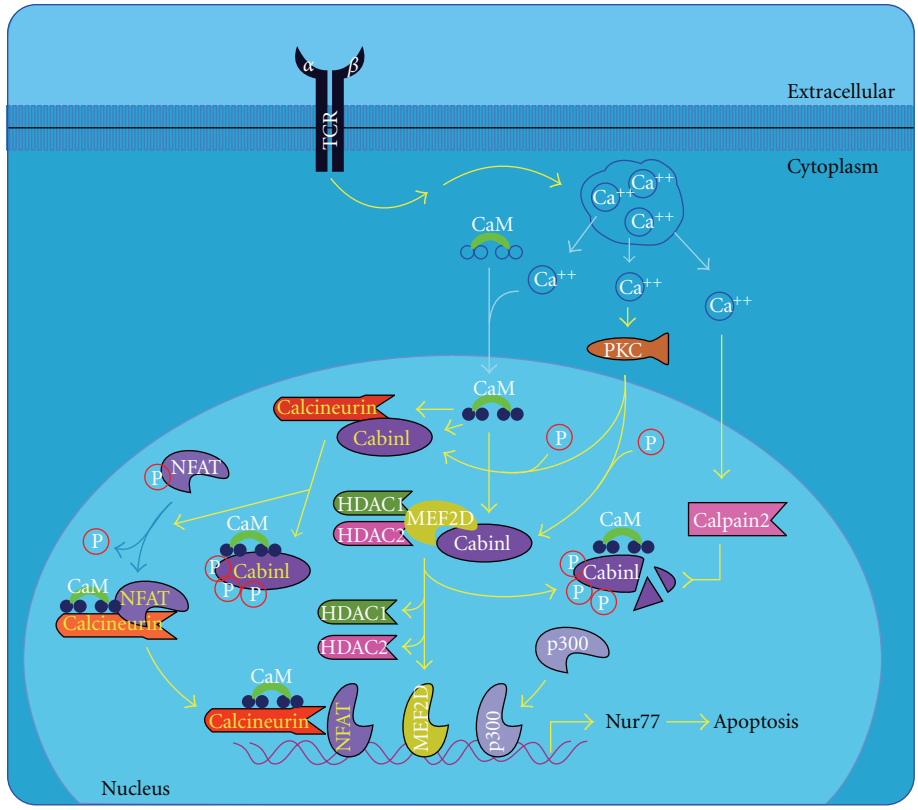


Figure 7: T cell apoptosis pathway extracted from BioCarta database [9].

sclerosus, both in adults and in prepubertal girls. Active lesions cleared in 43% of patients after 24 weeks of treatment. Partial resolution was reached in 34% of patients [42]. Recent reports describe the use of 0.1% tacrolimus in a topical formulation for the management of OLP [43]. Therefore, there is a need for more effective and safer therapies for symptomatic OLP. The activation of IL-2 production occurs after antigen, with a major histocompatibility complex type II antigen, is presented to the T-cell receptor-CD3 complex. Antigen presentation results in the release of calmodulin,

which binds and activates the protein calcineurin that is involved in the dephosphorylation of NFAT. The activated NFAT induces the transcription of the IL-2 gene. Tacrolimus and the intracellular immunophilin protein known as the FK-binding protein form a complex that binds to and inactivates the protein calcineurin. As a result, the T-cell receptor-mediated induction of IL-2 production is inhibited, resulting in suppression of T-cell-dependent immune functions [43, 44].

Contact dermatitis is a condition in which the skin becomes red, sore, or inflamed after direct contact with a substance. There are 2 types of contact dermatitis: irritant and allergic. Treatment of contact dermatitis is often palliative and directed against cutaneous inflammation itself. Tacrolimus has good anti-inflammatory effects and penetrates well through inflamed skin. In a human study, topical tacrolimus (at concentrations of 0%, 0.01%, 0.1%, and 1%) in ethanol were applied to the skin of 5 volunteers and left for 48 hours. 1-Chloro-2,4-dinitrobenzene (DNCB) was then applied to the skin. Biopsies of the test patches showed no inflammation on the DNCB-challenged skin sites that were pretreated with FK 506, while there was intense dermatitis ethanol-only. The ability to suppress reactions in previously sensitized patients is important because contact dermatitis patients do not present until after primary sensitization. The ability to treat such sensitized individuals is crucial because many antigens, such as nickel, are ubiquitous and complete avoidance is often impossible. Topical tacrolimus also suppresses irritant reactions in animal models, suggesting that topical tacrolimus may also be useful for primary irritant contact dermatitis. This may be applicable to the treatment of chronic hand dermatitis and occupational irritant dermatitis (in which allergic contact often coexists) [45].

The efficacy and safety of 0.1% tacrolimus ointment in vitiligo were compared favourably to that of 0.05% fluticasone propionate cream for the treatment of segmental vitiligo in a randomized controlled trial [46].

Even diseases that are not considered to be classic T-cell-mediated inflammatory processes have been considered as targets for tacrolimus therapy. Goldman noted that the anti-inflammatory properties of topical tacrolimus that unlike steroids, tacrolimus may not have intrinsic rosacea-promoting properties. He treated the patients who had steroid-induced rosacea and were previously unable to taper off and discontinue the use of steroid therapy. The eruptions were controlled in all 3 patients, and they were able to successfully taper off tacrolimus therapy and switch to a long-term regimen of topical antibiotics [47, 48].

While AA is another candidate disease for tacrolimus therapy, some authors have expressed reservations regarding its use for this purpose, as AA generally responds poorly to treatment. Thiers published a report of the failure of 0.3% tacrolimus ointment to treat AA in a 9-year-old [49]. We found no other descriptions of AA tacrolimus therapy published after 2000. Steroid intralesionals in combination with topically applied tacrolimus yield better results than topical tacrolimus alone [50, 51]. In 50–75% of patients, PG is associated with inflammatory bowel disease, rheumatoid arthritis, chronic autoimmune hepatitis, or haematological solid tumours. Some reports have indicated that topical tacrolimus is an effective treatment for PG. Immunosuppressive agents have also been used for the management of PG [50–53].

Tacrolimus ointment (0.1%) was applied to DLE lesions twice daily and the erythematous plaques readily diminished after 4–8 weeks. Adverse effects, such as burning sensation or irritations, were not observed. Cutaneous LE is a broad term, which includes a variety of lesions that may appear in the absence of the systemic manifestations of systemic lupus erythematosus [52, 53]. In an open-label study of tacrolimus (0.1 mg/kg) administered for 1 year with dosage adjustment showed that serum C3 level, and anti-ds DNA antibody titre improved with tacrolimus treatment. The mean titre of anti-ds DNA antibodies provides a representative indicator of immunological parameters reflecting disease activity. Therefore, a T cell blockade is considered a reasonable therapeutic target for cutaneous and systemic LE [54, 55]. Dosages differ between reports (1.5–6 mg/day). Tacrolimus can therefore be considered both effective and safe for treating mild manifestations of LE, including skin dermatosis, in systemic LE patients. However, for severe active conditions, its efficacy is limited at current dose settings and usage [56].

Crohn's disease (CD), also known as regional enteritis, is a type of inflammatory bowel disease that may affect any part of the gastrointestinal tract from the mouth to anus and causes a wide variety of symptoms. CD is caused by interactions between environmental, immunological, and bacterial factors in genetically susceptible individuals. This results in a chronic inflammatory disorder, in which the immune system of the body attacks the gastrointestinal tract possibly directed at microbial antigens. In addition, CD may involve the skin, blood, and endocrine system. One type of skin manifestation, erythema nodosum, presents as red nodules usually appearing on the shins. Erythema nodosum is due to inflammation of the underlying subcutaneous tissue and is characterized by septal panniculitis. Another skin lesion, pyoderma gangrenosum, is typically a painful ulcerating nodule. A new view is that CD results from an impaired innate immunity, in that impaired cytokine secretion by macrophages contributes to impaired innate immunity and leads to a sustained microbial-induced inflammatory response in the colon, where the bacterial load is high [57]. Despite the poor quality of the majority of trials examining the role of tacrolimus in CD, there is some evidence suggesting that tacrolimus may be of some benefit in this disease. Although systemic immunosuppressants are generally believed to increase the rate of cancer development, one study has shown that in female CD-1 mice there was a dose-related inhibition of 7,12-dimethylbenz[a]anthracene-(DMBA-) initiated and 12-tetradecanoylphorbol-13-acetate-(TPA-) promoted skin papillomas when 0.1 μmol tacrolimus was applied topically. The application of this formulation to mouse skin almost completely inhibited tumour formation. This antineoplastic effect may be unrelated to the suppression of T-cell functions and might occur after endogenous protein phosphorylation by TPA. This study was contradicted by a later study of the occurrence of de novo

neoplasms in organ transplant recipients. This later study indicated that tacrolimus is as an inducer of skin cancer [58].

Cutaneous T cell lymphoma (CTCL) is a class of non-Hodgkin's lymphoma, which is a type of cancer of the immune system. The malignant T cells in the body initially migrate to the skin, which result in the development of various lesions. These lesions change shape as the disease progresses, typically beginning as what appears to be a rash, which can be very itchy, and eventually forming plaques and tumors before metastasizing to other parts of the body. CTCLs are a heterogeneous group of lymphoproliferative disorders caused by clonally derived skin-invasive T cells. Few studies have reported the efficacy of topical tacrolimus for the treatment of CTCLs [59–61].

Topical application of 0.3% tacrolimus in isotonic solution or cream is a promising treatment modality for pathology ocular in BD [62]. Pulmonary and intestinal lesions evanesced and skin lesions improved after the oral administration of FK506 at a dose of 0.1-0.2 mg/kg for 8 weeks [63, 64].

In a case of refractory GVHD, the patient responded to a combination of oral tacrolimus, psoralen, and UV-A therapy. This suggests that systemic tacrolimus may benefit recipients of solid organ or bone marrow transplants with GVHD that is refractory to cyclosporine, high-dose systemic steroids, and antithymocyte globulin [65]. Tacrolimus is effective in the prevention of acute GVHD. The initial intravenous FK506 dose of 0.04 mg/kg per day and should be maintained for 7 days post-transplant. After day 7, intravenous FK506 doses should be decreased if serum creatinine is elevated to approximately 0.03 mg/kg per day [66]. Sabry et al. suggested that tacrolimus and mycophenolate mofetil is a good option for prophylaxis in HLA-matched nonmyeloablative transplants [67].

Sarcoidosis is a systemic inflammatory disease that can affect any organ. Sarcoidosis involves the skin in about 25% of patients. The most common lesions are erythema nodosum, plaques, maculopapular eruptions, and subcutaneous nodules. The exact cause of sarcoidosis is unknown. The current working hypothesis is that in genetically susceptible individuals, sarcoidosis is caused through an alteration in immune response after exposure to environmental, occupational, or infectious agents [68]. Granulomatous inflammation is characterized primarily by accumulation of monocytes, macrophages, and activated T lymphocytes with increased production of key inflammatory mediators, TNF-α, IFNγ, and IL-12, characteristic of a Th1-polarized response (T-helper lymphocyte-1 response). Sarcoidosis has contrasting effects on inflammatory processes; it is characterized by increased macrophage and CD4 helper T-cell activation, which results in accelerated inflammation; however, immune response to antigen challenges such as tuberculin is suppressed. Regulatory T lymphocytes in the periphery of sarcoid granulomas appear to suppress IL-2 secretion, which is hypothesized to cause a state of anergy, by preventing antigen-specific memory responses. Topical tacrolimus has proved effective for the treatment of cutaneous sarcoidosis [69].

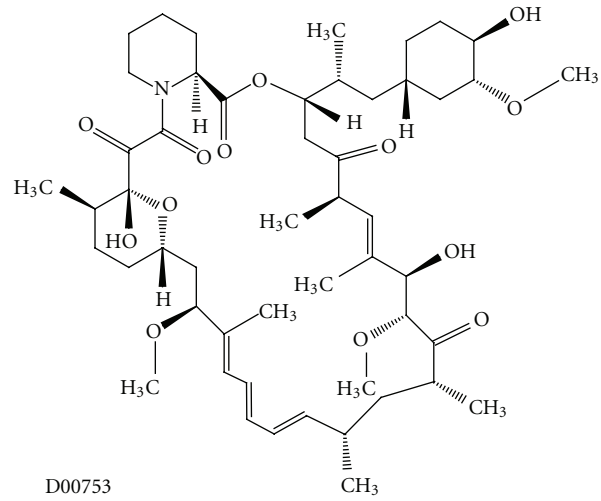


Figure 8: Molecular structure of sirolimus extracted from KEGG database [9].

In the recent years, tacrolimus has been used to suppress the inflammation associated with diverse autoimmune or granulomatous diseases. "As described by Alijotas-Reig," 7 patients with severe and refractory late-onset inflammatory reactions, including large panniculitis, which complicate silicone gel injections were evaluated. After an average of 18 months after tacrolimus administration (in increasing doses, up to 0.08 to 0.1 mg/kg of body weight, 2 times per day), 5 patients experienced mild, sparse bouts of inflammatory processes, including nodules, plaques, and panniculitis. The symptoms were rapidly reversed, and 2 patients showed remission. No side effects related to tacrolimus were observed. The ability of silicone to initiate immunologic processes remains to be clarified. An exhaustive federally sponsored review failed to find evidence to support immunological effects [70].

Long-lasting implants of any type that interact with commensal or infectious microorganisms, trauma, or localized or generalized inflammatory processes could theoretically induce autoimmune disorders or granulomata. These events may occur because of epigenetic alterations in DNA expression in genetically susceptible hosts. An excellent candidate for pathologic mischief on the face is *Propionibacterium acnes* that under certain circumstances can act as an opportunistic pathogen, which stimulates the production of TNF-α and polysaccharides [71, 72].

3.2. Rapamycin and Future Directions in the Development of Mammalian Rapamycin Inhibitor Development

3.2.1. Rapamycin. Another widely used macrolide is rapamycin, also known as sirolimus (Figure 8) [9]. Rapamycin acts through the inhibition of mammalian target of rapamycin (mTOR), a molecule that is activated via phosphoinositide 3-kinase (PI3K) and controls downstream proteins involved in the cell cycle. After binding with tacrolimus binding protein (FK-BP) immunophilin, the rapamycin complex inhibits the stimulatory effect of mTOR on cell cycle

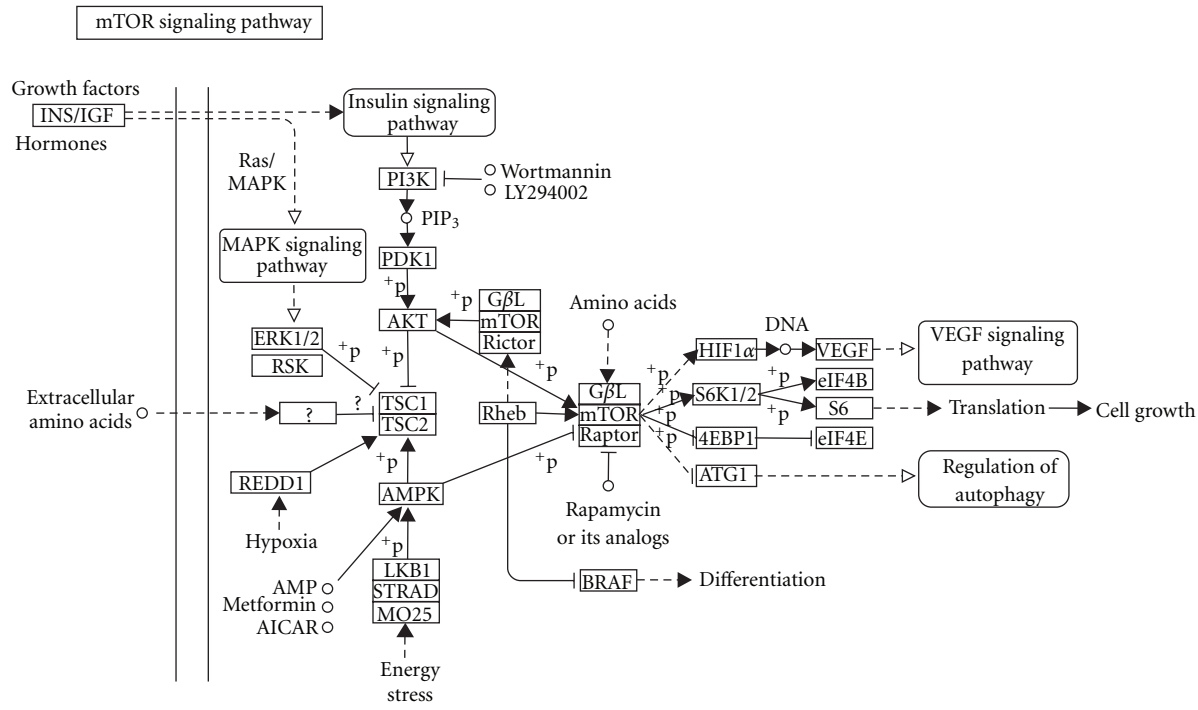


Figure 9: mTOR signaling pathway extracted from KEGG database [8].

protein translation, arresting the G_1 to S transition (Figure 9) [8, 73]. This inhibitory effect can be partly explained because of a reduction of the phosphorylation of eIF-4e binding protein 1 (4E-BP1), a repressor of cap-mediated translation in mammalian cells [74]. Since 1999, rapamycin has been broadly used in human skin transplantation because it carries a low risk of renal dysfunction and reduces the risk of allograft rejection in comparison with other [75–77].

Rapamycin may potentially be used as an antiangiogenic agent to inhibit, for example, growth of pathological blood vessels in combination with laser treatment [78]. The application of a laser to an area of skin provokes the shutdown of major capillary vessels and results in the induction of a severely hypoxic microenvironment. This can cause overexpression of hypoxia-inducible factor-1 alpha (HIF1α) and promote the secretion of angiogenesis-stimulating factors like platelet-derived growth factor (PDGF) [79] and vascular endothelial growth factor (VEGF) [80]. Rapamycin may prevent vascular reperfusion by acting as an inhibitor of this mTOR-HIF1α-VEGF pathway and through the inhibition of the PI3K-p70S6 kinase pathway in endothelial cells stimulated by VEGF [81, 82].

There are some side effects to take into account, such as mild cholangitis [75] and delays in wound closure [75, 83]. These side effects may result from the multiple effects rapamycin may exert upon mTOR inhibition in the epithelial and stromal tissues of the wound area. This includes the important role of mTOR in the wound healing process downstream from phosphatidylinositol 3 kinase (PI3K) and phosphatase and tensin homolog (PTEN) [84].

A randomized, double blind, left-right comparative, dose-ranging clinical trial was carried out to determine the efficacy and safety of rapamycin applied to skin for the treatment of psoriasis [85]. The trial showed that rapamycin was able to penetrate human skin and exerted beneficial effects. A few subjects, however, developed contact sensitization to rapamycin [85].

3.2.2. Everolimus. Everolimus (RAD001) is a rapamycin derivative (Figure 10) [9] with potent immunosuppressive effects, antiproliferative properties, and anticancer effects in many preclinical and clinical studies [86]. In addition, everolimus has shown *in vivo* antitumor activity with a significant cytostatic activity in a variety of preclinical models of haematological and solid tumours.

It has been reported that everolimus, while and effective treatment for psoriasis [87], became ineffective in 2 cases of severe atopic dermatitis when it was combined with prednisone or cyclosporine A [88]. More studies are needed to confirm this result.

3.2.3. Temsirolimus. Temsirolimus (CCI-779, Torisel, Wyeth) is another rapamycin derivative (Figure 11) [9] and has properties that are similar to everolimus [86]. It has been used for the treatment of metastatic renal cell carcinoma and mantle cell lymphoma.

Rapamycin, everolimus, and temsirolimus all prevent tumour cell proliferation and angiogenesis through inhibition of the HIF1α/VEGF pathway [89–91].

3.2.4. New Macrolides and Their Applications. A new synthetic azythromycin-derivative-macrolide, called CSY0073 [92], has anti-inflammatory and immune-modulatory effects, but no antibiotic effects. CSY0073 exerts

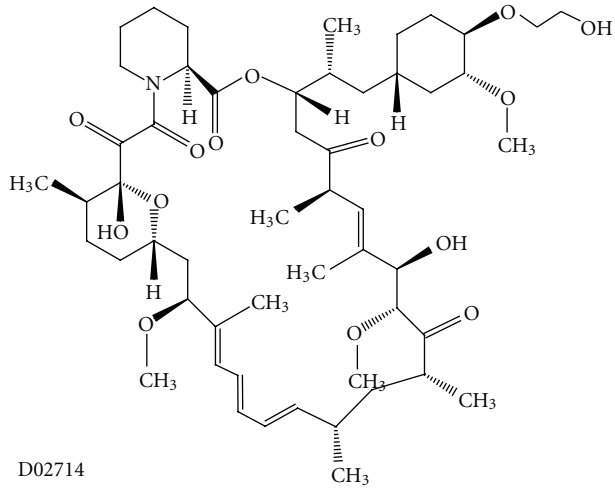


Figure 10: Molecular structure of everolimus extracted from KEGG database [9].

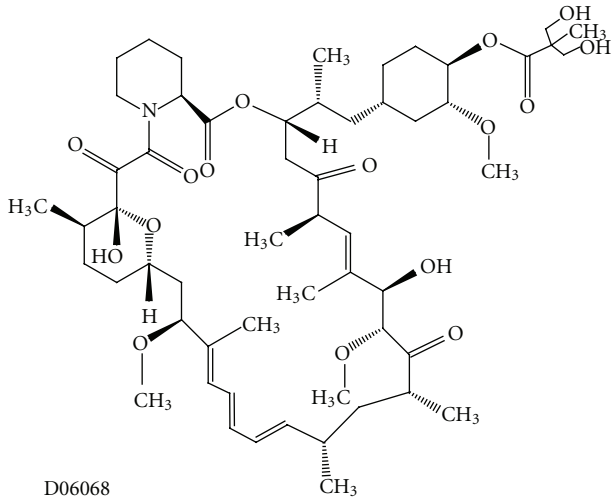


Figure 11: Molecular structure of temsirolimus extracted from KEGG database [9].

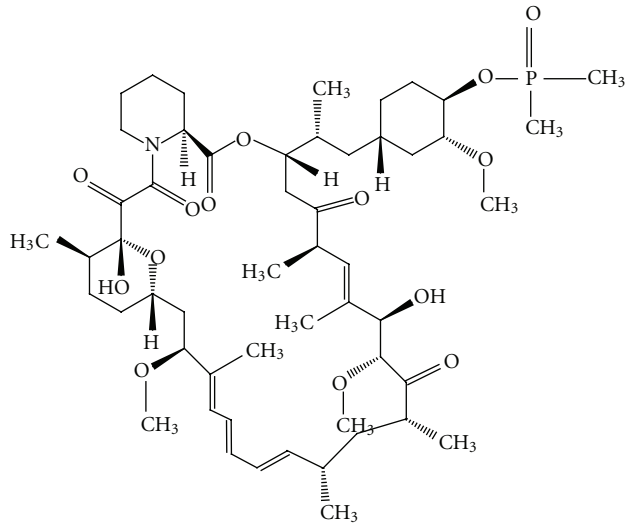


Figure 12: Molecular structure of ridaforolimus extracted from KEGG database [9].

counterregulatory activity on nuclear factor kappa B (NF-κB), activator protein-1 (AP-1) and extracellular signal-regulated kinase 1/2 (ERK1/2) signalling. The anti-inflammatory activity of CSY0073 was demonstrated in rodent models of intestinal inflammation and hold potential as a treatment of inflammation-driven immune dysfunction. CSY0073 may reduce the colonic expression of cytokines involved in the development and maintenance of colon inflammation, such as tumour necrosis factor α (TNFα), interleukin 2 (IL-2), and interferon γ (IFNγ) [93]. In addition, CSY0073 effectively attenuated the immune response of mucosal macrophages. This is consistent with studies of other macrolides that indicate that these compounds penetrate the cell membrane of macrophages and accumulate in subcellular compartments [94]. CSY0073 is also being developed as a therapeutic drug for rheumatoid arthritis. Initial results indicate that treatment with CSY0073 attenuates the development of several signs of arthritis [92].

Recently, 2 new potential macrolides with anti-inflammatory and immunomodulatory characteristics were discovered. These compounds, (8R,9S)-8,9-dihydro-6,9-anhydropseudoerythromycin A (EM900) and (8R,9S)-4″,13-O-diacetyl-8,9-dihydro-6,9-epoxy-8,9-anhydropseudoerythromycin A (EM911), are derivatives of erythromycin A [95]. EM900 and EM911 have so far only been used *in vitro*. More studies are needed to uncover the possible applications of these promising molecules.

Another newly developed molecule is ridaforolimus (also known as deforolimus, AP23473, MK-8669, Merck), a rapamycin analogue (Figure 12) [9], which has broad inhibitory effects on the cell growth, proliferation, division, metabolism, and angiogenesis of a broad panel of cell lines [96]. *In vitro* and *in vivo* studies show that ridaforolimus inhibits mTOR function in a selective and potent manner.

Table 1: Main advantages and drawbacks of different nonantibiotic macrolides in skin diseases.

Macrolide	Advantages	Drawbacks
Pimecrolimus	(i) Plays an important role in the anti-inflammatory activities. (ii) Applied for the treatment of atopic dermatitis (AD) [4, 9, 10]. (iii) Inhibits the synthesis of inflammatory cytokines in psoriasis [13, 14]. (iv) Produces a dose-dependent reduction in the severity of psoriasis [15]. (v) Significantly reduces the symptoms in oral lichen planus (OLP) [16, 17]. (vi) Applied for the treatment of rosacea [23]. (vii) Shows positive clinical results in pyoderma gangrenosum (PG) [27]. (viii) Acts more selectively on T cells and mast cells in lupus dermatosis and thus has a lower possibility of systemic immunosuppression [28, 29]. (ix) Is safe and efficient for the treatment of Behçet's disease genital ulcers, by accelerating the healing process [31].	(i) Tacrolimus is used more often for vitiligo [19, 20, 46]. (ii) Failure in treatment of chronic autoimmune urticaria [22]. (iii) Topical 1% is not a therapeutic option in alopecia areata (AA), especially for patients unresponsive to other treatments [24]. (iv) In PG, the pharmacologic activity is more selective than tacrolimus, and the rate of cutaneous permeation is 9 times lower than that of tacrolimus and, therefore, has a lower risk of systemic immune suppression [27].
Tacrolimus	(i) Oral formulation offers an additional therapeutic option for management of severe and extensive AD [36]. (ii) Topical formulation is a highly effective treatment for psoriasis of the face and flexures [39] and is proposed as an alternative treatment for inflammatory skin diseases in thin skin areas, as well as, pruritus ani [42]. In addition, it is effective in PG [51–53] and in cutaneous T cell lymphomas [60]. (iii) Topical formulation (0.1%) has been used for the management of OLP and may be effective and well tolerated in the treatment of anogenital lichen sclerosus [41]. (iv) Treatment of contact dermatitis is often palliative and directed against the cutaneous inflammation itself. (v) It has been shown to reduce the incidence of lupus dermatosis in the autoimmune-prone MRL/Mp-lpr/lpr (MRL/lpr) mouse. (vi) Better results in AA treatment are achieved in combination with intralesional steroids [50]. (vii) It can be considered both effective and safe for treating skin dermatosis in systemic lupus erythematosus (LE) patients [56].	(i) For severe active LE, its efficacy is considered limited at current dose settings and usage [55, 56]. (ii) There are contradictory results of tacrolimus as an inducer of skin cancer.
Sirolimus (rapamycin)	(i) A clinical trial has shown that macrolides, in a suitable formulation, can penetrate the human skin and exert beneficial effects for the treatment of psoriasis [75, 85].	(i) Contact sensitization to rapamycin could be developed [85].
Everolimus	(i) Potent immunosuppressive effects, antiproliferative properties, and anticancer effects have been observed. (ii) Effective in psoriasis treatment [87].	(i) It was ineffective in combination with prednisone or cyclosporine A in 2 patients with severe AD [88].
Temsirolimus	(i) Potent immunosuppressive effects, antiproliferative properties, and anticancer effects.	(i) Applications for different types of dermatitis are not yet known.
CSY0073	(i) Anti-inflammatory and immunomodulatory effects have been observed [93].	(i) Applications for different types of dermatitis are not yet known.
EM900 EM911	(i) Anti-inflammatory and immunomodulatory characteristics observed.	(i) Applications for different types of dermatitis are not yet known.
Ridaforolimus	(i) One of the first possible applications as an antitumor agent.	(i) Applications for different types of dermatitis are not yet known.

Inhibitory effects on VEGF, endothelial cell growth (EGF), HIF-1α, and glucose metabolism were also observed. In particular, ridaforolimus was found to arrest cell growth without evidence of cell death or apoptosis, accumulating cells in the G_1 phase of the cell cycle. This was due, in part, to a blockade of 4E-BP1/eIF4E signalling [97, 98]. One of the first possible applications for this compound is as an antitumour agent.

4. Conclusions

New uses are being developed for older macrolides, such as pimecrolimus and tacrolimus, due to their interesting anti-inflammatory properties. These drugs work through the inhibition of the calcineurin promotion of several cytokines, such as interleukins, interferons, and TNFα. This approach is opening a broad field of skin disease treatments that have minimal side effects (Table 1).

On the other hand, newer macrolides (rapamycin, everolimus, and temsirolimus) work through the downregulation of the mTOR pathway. The mTOR pathway controls downstream proteins that are involved in the cell cycle. These newer macrolides arrest the G_1 to S transition, an important early event in the control of mammalian cell growth and proliferation. These macrolides also demonstrate antiproliferative, cytostatic, and antiangiogenic properties. There are many examples of successful applications for these compounds in cancer diseases and organ transplantation. These compounds have also been used in the treatment of skin diseases. There were a variety of responses to these compounds, and some of them were not at all positive. Further research in this field is required to determine potential applications for these macrolides.

References

[1] G. Martín Ezquerra, M. Sánchez Regaña, E. Herrera Acosta, and P. Umbert Millet, "Topical tacrolimus for the treatment of psoriasis on the face, genitalia, intertriginous areas and corporal plaques.," *Journal of Drugs in Dermatology*, vol. 5, no. 4, pp. 334–336, 2006.

[2] A. K. Gupta and M. Chow, "Pimecrolimus: a review," *Journal of the European Academy of Dermatology and Venereology*, vol. 17, no. 5, pp. 493–503, 2003.

[3] A. A. Alzolibani and K. Zedan, "Macrolides in chronic inflammatory skin disorders," *Mediators of Inflammation*, vol. 2012, Article ID 159354, 7 pages, 2012.

[4] M. Czarnecka-Operacz and D. Jenerowicz, "Topical calcineurin inhibitors in the treatment of atopic dermatitis-an update on safety issues," *Journal of the German Society of Dermatology*, vol. 10, no. 3, pp. 167–173, 2012.

[5] L. Berk, M. M. Mita, J. Kreisberg et al., "Analysis of the pharmacodynamic activity of the mTOR inhibitor ridaforolimus (AP23573, MK-8669) in a phase 1 clinical trial," *Cancer Chemotherapy and Pharmacology*, vol. 69, no. 5, pp. 1369–1377, 2012.

[6] S. Krengel, I. Satzger, M. Alter, A. Kapp, and R. Gutzmer, "Remission of an iatrogenic Kaposi sarcoma in a patient with myasthenia gravis after switching immunosuppressive therapy to the mTOR inhibitor everolimus," *Der Hautarzt*, vol. 63, no. 7, pp. 573–576, 2012.

[7] A. Sugawara, A. Sueki, T. Hirose et al., "Novel 12-membered non-antibiotic macrolides from erythromycin A; EM900 series as novel leads for anti-inflammatory and/or immunomodulatory agents," *Bioorganic and Medicinal Chemistry Letters*, vol. 21, no. 11, pp. 3373–3376, 2011.

[8] M. Kanehisa and S. Goto, "KEGG: Kyoto encyclopedia of genes and genomes," *Nucleic Acids Research*, vol. 28, no. 1, pp. 27–30, 2000.

[9] BioCarta-Charting Pathways of Life, http://www.biocarta.com/.

[10] M. Czarnecka-Operacz and D. Jenerowicz, "Topical calcineurin inhibitors in the treatment of atopic dermatitis-an update on safety issues," *Journal der Deutschen Dermatologischen Gesellschaft*, vol. 10, no. 3, pp. 167–162, 2012.

[11] K. Wolff, C. Fleming, J. Hanifin et al., "Efficacy and tolerability of three different doses of oral pimecrolimus in the treatment of moderate to severe atopic dermatitis: a randomized controlled trial," *British Journal of Dermatology*, vol. 152, no. 6, pp. 1296–1303, 2005.

[12] L. Naldi and B. Rzany, "Chronic plaque psoriasis," *Clinical Evidence*, no. 13, pp. 2070–2098, 2005.

[13] R. E. Kalb, J. Bagel, N. J. Korman et al., "Treatment of intertriginous psoriasis: from the Medical Board of the National Psoriasis Foundation," *Journal of the American Academy of Dermatology*, vol. 60, no. 1, pp. 120–124, 2009.

[14] C. Fabroni and T. Lotti, "Pimecrolimus in dermatology," *Giornale Italiano di Dermatologia e Venereologia*, vol. 144, no. 3, pp. 321–325, 2009.

[15] A. B. Gottlieb, C. E. M. Griffiths, V. C. Ho et al., "Oral pimecrolimus in the treatment of moderate to severe chronic plaque-type psoriasis: a double-blind, multicentre, randomized, dose-finding trial," *British Journal of Dermatology*, vol. 152, no. 6, pp. 1219–1227, 2005.

[16] M. R. Roopashree, R. V. Gondhalekar, M. C. Shashikanth, J. George, S. H. Thippeswamy, and A. Shukla, "Pathogenesis of oral lichen planus-a review," *Journal of Oral Pathology and Medicine*, vol. 39, no. 10, pp. 729–734, 2010.

[17] P. López-Jornet, F. Camacho-Alonso, and N. Salazar-Sanchez, "Topical tacrolimus and pimecrolimus in the treatment of oral lichen planus: an update," *Journal of Oral Pathology and Medicine*, vol. 39, no. 3, pp. 201–205, 2010.

[18] S. Elad, J. B. Epstein, N. Yarom, S. Drucker, R. Tzach, and I. Von Bltzingslöwen, "Topical immunomodulators for management of oral mucosal conditions, a systematic review; part I: calcineurin inhibitors," *Expert Opinion on Emerging Drugs*, vol. 15, no. 4, pp. 713–726, 2010.

[19] N. van Geel, R. Speeckaert, I. Mollet et al., "In vivo vitiligo induction and therapy model: double-blind, randomized clinical trial," *Pigment Cell and Melanoma Research*, vol. 25, no. 1, pp. 57–65, 2012.

[20] O. Köse, E. Arca, and Z. Kurumlu, "Mometasone cream versus pimecrolimus cream for the treatment of childhood localized vitiligo," *Journal of Dermatological Treatment*, vol. 21, no. 3, pp. 133–139, 2010.

[21] Wardhana and E. A. Datau, "Chronic autoimmune urticaria," *Acta Medica Indonesiana*, vol. 44, no. 2, pp. 165–174, 2012.

[22] A. M. Marsland, S. Soundararajan, K. Joseph, and A. P. Kaplan, "Effects of calcineurin inhibitors on an in vitro assay for chronic urticaria," *Clinical and Experimental Allergy*, vol. 35, no. 5, pp. 554–559, 2005.

[23] M. B. Kim, G. W. Kim, H. J. Park et al., "Pimecrolimus 1% cream for the treatment of rosacea," *The Journal of Dermatology*, vol. 38, no. 12, pp. 1135–1139, 2011.

[24] H. Ucak, B. Kandi, D. Cicek, N. Halisdemir, and S. B. Dertlıoğlu, "The comparison of treatment with clobetasol propionate 0.05% and topical pimecrolimus 1% treatment in the treatment of alopecia areata," *The Journal of Dermatological Treatment*, vol. 25, no. 7, pp. 345–249, 2011.

[25] A. Katoulis, S. Georgala, E. Bozi, E. Papadavid, D. Kalogeromitros, and N. Stavrianeas, "Frontal fibrosing alopecia: treatment with oral dutasteride and topical pimecrolimus," *Journal of the European Academy of Dermatology and Venereology*, vol. 23, no. 5, pp. 580–582, 2009.

[26] S. L. Moschella, "Neutrophilic dermatoses," in *Dermatology*, J. L. Bolognia, J. L. Jorizzo, and R. P. Rapini, Eds., vol. 1, pp. 415–418, 2003.

[27] V. Bellini, S. Simonetti, and P. Lisi, "Successful treatment of severe pyoderma gangrenosum with pimecrolimus cream 1% [10]," *Journal of the European Academy of Dermatology and Venereology*, vol. 22, no. 1, pp. 113–115, 2008.

[28] J. R. M. Carneiro, H. T. Fuzii, C. Kayser et al., "IL-2, IL-5, TNF-α and IFN-γ mRNA expression in epidermal keratinocytes of systemic lupus erythematosus skin lesions," *Clinics*, vol. 66, no. 1, pp. 77–82, 2011.

[29] B. Barikbin, S. Givrad, M. Yousefi, and F. Eskandari, "Pimecrolimus 1% cream versus betamethasone 17-valerate 0.1% cream in the treatment of facial discoid lupus erythematosus: a double-blind, randomized pilot study," *Clinical and Experimental Dermatology*, vol. 34, no. 7, pp. 776–780, 2009.

[30] A. Hamzaoui, R. Klii, O. Harzallah, C. Attig, and S. Mahjoub, "Behçet's disease in women," *La Revue de Medecine Interne*, vol. 33, no. 10, pp. 552–555, 2012.

[31] C. Chams-Davatchi, B. Barikbin, F. Shahram et al., "Pimecrolimus versus placebo in genital aphthous ulcers of Behcet's disease: a randomized double-blind controlled trial," *International Journal of Rheumatic Diseases*, vol. 13, no. 3, pp. 253–258, 2010.

[32] T. Schmook, J. Kraft, B. Benninghoff et al., "Treatment of cutaneous chronic graft-versus-host disease with topical pimecrolimus [4]," *Bone Marrow Transplantation*, vol. 36, no. 1, pp. 87–88, 2005.

[33] S. Sakuma, Y. Higashi, N. Sato et al., "Tacrolimus suppressed the production of cytokines involved in atopic dermatitis by direct stimulation of human PBMC system. (Comparison with steroids)," *International Immunopharmacology*, vol. 1, no. 6, pp. 1219–1226, 2001.

[34] S. Sakuma, Y. Kato, F. Nishigaki et al., "FK506 potently inhibits T cell activation induced TNF-α and IL-1β production in vitro by human peripheral blood mononuclear cells," *British Journal of Pharmacology*, vol. 130, no. 7, pp. 1655–1663, 2000.

[35] K. H. Kim and T. Kono, "Overview of efficacy and safety of tacrolimus ointment in patients with atopic dermatitis in Asia and other areas," *International Journal of Dermatology*, vol. 50, no. 9, pp. 1153–1161, 2011.

[36] T. C. Keaney, T. Bhutani, P. Sivanesan et al., "Open-label, pilot study examining sequential therapy with oral tacrolimus and topical tacrolimus for severe atopic dermatitis," *Journal of the America Academy of Dermatology*, vol. 67, no. 4, pp. 636–641, 2012.

[37] L. Laino and A. DiCarlo, "Palmoplantar pustular psoriasis: clinical and video thermographic evaluation before and after topical tacrolimus treatment," *Archives of Dermatology*, vol. 147, no. 6, p. 760, 2011.

[38] A. N. Lin, "Innovative use of topical calcineurin inhibitors," *Dermatologic Clinics*, vol. 28, no. 3, pp. 535–545, 2010.

[39] S. A. Vogel, B. Yentzer, S. A. Davis, S. R. Feldman, and K. M. Cordoro, "Trends in pediatric psoriasis outpatient health care delivery in the United States," *Archives of Dermatology*, vol. 148, no. 1, pp. 66–71, 2012.

[40] V. Madan and C. E. M. Griffiths, "Systemic ciclosporin and tacrolimus in dermatology," *Dermatologic Therapy*, vol. 20, no. 4, pp. 239–250, 2007.

[41] A. T. Goldstein, D. Thaçi, and T. Luger, "Topical calcineurin inhibitors for the treatment of vulvar dermatoses," *European Journal of Obstetrics Gynecology and Reproductive Biology*, vol. 146, no. 1, pp. 22–29, 2009.

[42] E. Suys, "Randomized study of topical tacrolimus ointment as possible treatment for resistant idiopathic pruritus ani," *Journal of the American Academy of Dermatology*, vol. 66, no. 2, pp. 327–328, 2012.

[43] E. Baubion, M. S. Doutre, and M. Beylot-Barry, "Oral lichen planus and treatment with topical tacrolimus rinse," *Annales de Dermatologie et de Venereologie*, vol. 136, no. 3, pp. 276–278, 2009.

[44] E. Baubion, M. S. Doutre, and M. Beylot-Barry, "Oral lichen planus and treatment with topical tacrolimus rinse," *Annales de Dermatologie et de Venereologie*, vol. 136, no. 3, pp. 276–278, 2009.

[45] A. Katsarou, M. Makris, K. Papagiannaki, E. Lagogianni, A. Tagka, and D. Kalogeromitros, "Tacrolimus 0.1% vs mometasone furoate topical treatment in allergic contact hand eczema: a prospective randomized clinical study," *European Journal of Dermatology*, vol. 22, no. 2, pp. 192–196, 2012.

[46] S. Kathuria, B. K. Khaitan, M. Ramam, and V. K. Sharma, "Segmental vitiligo: a randomized controlled trial to evaluate efficacy and safety of 0.1% tacrolimus ointment vs 0.05% fluticasone propionate cream," *Indian Journal of Dermatology, Venereology and Leprology*, vol. 78, no. 1, pp. 68–73, 2012.

[47] D. Goldman, "Tacrolimus ointment for the treatment of steroid-induced rosacea: a preliminary report," *Journal of the American Academy of Dermatology*, vol. 44, no. 6, pp. 995–998, 2001.

[48] G. Garg and G. P. Thami, "Clinical efficacy of tacrolimus in rosacea," *Journal of the European Academy of Dermatology and Venereology*, vol. 23, no. 2, pp. 239–240, 2009.

[49] B. H. Thiers, "Topical tacrolimus: treatment failure in a patient with alopecia areata," *Archives of Dermatology*, vol. 136, no. 1, p. 124, 2000.

[50] C. Kuldeep, H. Singhal, A. K. Khare, A. Mittal, L. K. Gupta, and A. Garg, "Randomized comparison of topical betamethasone valerate foam, intralesional triamcinolone acetonide and tacrolimus ointment in management of localized alopecia areata," *International Journal of Trichology*, vol. 3, no. 1, pp. 20–24, 2011.

[51] C. G. Larsen and J. P. Thyssen, "Pustular penile pyoderma gangrenosum successfully treated with topical tacrolimus ointment," *Acta Dermato-Venereologica*, vol. 92, no. 1, pp. 104–105, 2012.

[52] A. V. Marzano, V. Trevisan, R. Lazzari, and C. Crosti, "Topical tacrolimus for the treatment of localized, idiopathic, newly diagnosed pyoderma gangrenosum," *Journal of Dermatological Treatment*, vol. 21, no. 3, pp. 140–143, 2010.

[53] M. Altieri, K. Vaziri, and B. A. Orkin, "Topical tacrolimus for parastomal pyoderma gangrenosum: a report of two cases," *Ostomy Wound Management*, vol. 56, no. 9, pp. 56–59, 2010.

[54] M. Sugano, Y. Shintani, K. Kobayashi, N. Sakakibara, I. Isomura, and A. Morita, "Successful treatment with topical tacrolimus in four cases of discoid lupus erythematosus," *Journal of Dermatology*, vol. 33, no. 12, pp. 887–891, 2006.

[55] C. E. Lampropoulos and D. P. D'Cruz, "Topical calcineurin inhibitors in systemic lupus erythematosus," *Therapeutics and Clinical Risk Management*, vol. 15, no. 6, pp. 95–101, 2010.

[56] K. Suzuki, H. Kameda, K. Amano et al., "Single center prospective study of tacrolimus efficacy and safety in the treatment of various manifestations in systemic lupus erythematosus," *Rheumatology International*, vol. 31, no. 6, pp. 757–763, 2011.

[57] D. J. B. Marks and A. W. Segal, "Innate immunity in inflammatory bowel disease: a disease hypothesis," *Journal of Pathology*, vol. 214, no. 2, pp. 260–266, 2008.

[58] K. McSharry, A. M. Dalzell, K. Leiper, and W. El-Matary, "Systematic review: the role of tacrolimus in the management of Crohn's disease," *Alimentary Pharmacology and Therapeutics*, vol. 34, no. 11-12, pp. 1282–1294, 2011.

[59] J. Woo, T. M. Wright, B. Lemster, D. Borochovitz, M. A. Nalesnik, and A. W. Thomson, "Combined effects of FK506 (tacrolimus) and cyclophosphamide on atypical B220+ T cells, cytokine gene expression and disease activity in MRL/MpJ-lpr/lpr mice," *Clinical and Experimental Immunology*, vol. 100, no. 1, pp. 118–125, 1995.

[60] E. Rallis, A. Economidi, C. Verros, and P. Papadakis, "Successful treatment of patch type mycosis fungoides with tacrolimus ointment 0.1%.," *Journal of Drugs in Dermatology*, vol. 5, no. 9, pp. 906–907, 2006.

[61] Q. H. Dé Tran, E. Guay, S. Chartier, and J. Tousignant, "Tacrolimus in dermatology," *Journal of Cutaneous Medicine and Surgery*, vol. 5, no. 4, pp. 329–335, 2001.

[62] F. N. Yalçindağ, F. Batioğlu, and O. Ozdemir, "Penetration of topically applied tacrolimus into the aqueous humor in Behçet's disease," *Annals of Ophthalmology*, vol. 39, no. 1, pp. 15–17, 2007.

[63] C. Evereklioglu, "Managing the symptoms of Behçet's disease," *Expert Opinion on Pharmacotherapy*, vol. 5, no. 2, pp. 317–328, 2004.

[64] K. Matsumura, H. Nakase, and T. Chiba, "Efficacy of oral tacrolimus on intestinal Behcet's disease," *Inflammatory Bowel Diseases*, vol. 16, no. 2, pp. 188–189, 2010.

[65] R. F. Jubran and P. A. Dinndorf, "Successful therapy of refractory graft versus host disease with tacrolimus and psoralen plus ultraviolet light," *Therapeutic Drug Monitoring*, vol. 20, no. 2, pp. 236–239, 1998.

[66] K. Fortune and D. Couriel, "Tacrolimus in hematopoietic stem cell transplantation," *Expert Opinion on Drug Metabolism and Toxicology*, vol. 5, no. 7, pp. 835–841, 2009.

[67] W. Sabry, R. Le Blanc, A. C. Labbé et al., "Graft-versus-host disease prophylaxis with tacrolimus and mycophenolate mofetil in HLA-matched nonmyeloablative transplant recipients is associated with very low incidence of GVHD and nonrelapse mortality," *Biology of Blood and Marrow Transplantation*, vol. 15, no. 8, pp. 919–929, 2009.

[68] R. Kettritz, U. Goebel, A. Fiebeler, W. Schneider, and F. Luft, "The protean face of sarcoidosis revisited," *Nephrology Dialysis Transplantation*, vol. 21, no. 10, pp. 2690–2694, 2006.

[69] N. Katoh, H. Mihara, and H. Yasuno, "Cutaneous sarcoidosis successfully treated with topical tacrolimus," *British Journal of Dermatology*, vol. 147, no. 1, pp. 154–156, 2002.

[70] J. Alijotas-Reig, V. Garcia-Gimenez, and M. Vilardell-Tarre's, "Tacrolimus in the treatment of chronic and refractory late-onset immune-mediated adverse effects related to silicone injections," *Dermatologic Surgery*, vol. 38, no. 1, pp. 38–47, 2012.

[71] T. Rosen, "The Propionibacterium acnes genome: from the laboratory to the clinic.," *Journal of Drugs in Dermatology*, vol. 6, no. 6, pp. 582–586, 2007.

[72] J. Raloff, "Triggering autoimmune assaults," *Science News*, vol. 173, p. 10, 2008.

[73] A. M. Marsland and C. E. M. Griffiths, "The macrolide immunosuppressants in dermatology: mechanisms of action," *European Journal of Dermatology*, vol. 12, no. 6, pp. 618–621, 2002.

[74] L. Beretta, A. C. Gingras, Y. V. Svitkin, M. N. Hall, and N. Sonenberg, "Rapamycin blocks the phosphorylation of 4E-BP1 and inhibits cap-dependent initiation of translation," *EMBO Journal*, vol. 15, no. 3, pp. 658–664, 1996.

[75] P. Petruzzo, S. Testelin, J. Kanitakis et al., "First human face transplantation: 5 years outcomes," *Transplantation*, vol. 93, no. 2, pp. 236–240, 2012.

[76] B. D. Kahan, "Fifteen years of clinical studies and clinical practice in renal transplantation: reviewing outcomes with De Novo use of sirolimus in combination with cyclosporine," *Transplantation Proceedings*, vol. 40, no. 10, pp. S17–S20, 2008.

[77] T. H. Mathew, C. Van Buren, B. D. Kahan, K. Butt, S. Hariharan, and J. J. Zimmerman, "A comparative study of sirolimus tablet versus oral solution for prophylaxis of acute renal allograft rejection," *Journal of Clinical Pharmacology*, vol. 46, no. 1, pp. 76–87, 2006.

[78] W. Jia, V. Sun, N. Tran et al., "Long-term blood vessel removal with combined laser and topical rapamycin antiangiogenic therapy: implications for effective port wine stain treatment," *Lasers in Surgery and Medicine*, vol. 42, no. 2, pp. 105–112, 2010.

[79] S. Kourembanas, R. L. Hannan, and D. V. Faller, "Oxygen tension regulates the expression of the platelet-derived growth factor-B chain gene in human endothelial cells," *Journal of Clinical Investigation*, vol. 86, no. 2, pp. 670–674, 1990.

[80] D. Shweiki, A. Itin, D. Soffer, and E. Keshet, "Vascular endothelial growth factor induced by hypoxia may mediate hypoxia-initiated angiogenesis," *Nature*, vol. 359, no. 6398, pp. 843–845, 1992.

[81] M. Guba, P. Von Breitenbuch, M. Steinbauer et al., "Rapamycin inhibits primary and metastatic tumor growth by antiangiogenesis: involvement of vascular endothelial growth factor," *Nature Medicine*, vol. 8, no. 2, pp. 128–135, 2002.

[82] T. Seufferlein and E. Rozengurt, "Rapamycin inhibits constitutive p70(s6k) phosphorylation, cell proliferation, and colony formation in small cell lung cancer cells," *Cancer Research*, vol. 56, no. 17, pp. 3895–3897, 1996.

[83] R. J. Knight, M. Villa, R. Laskey et al., "Risk factors for impaired wound healing in sirolimus-treated renal transplant recipients," *Clinical Transplantation*, vol. 21, no. 4, pp. 460–465, 2007.

[84] C. H. Squarize, R. M. Castilho, T. H. Bugge, and J. S. Gutkind, "Accelerated wound healing by mTOR activation in genetically defined mouse models," *PLoS ONE*, vol. 5, no. 5, Article ID e10643, 2010.

[85] A. D. Ormerod, S. A. A. Shah, P. Copeland, G. Omar, and A. Winfield, "Treatment of psoriasis with topical sirolimus: preclinical development and a randomized, double-blind trial," *British Journal of Dermatology*, vol. 152, no. 4, pp. 758–764, 2005.

[86] A. Fasolo and C. Sessa, "Current and future directions in mammalian target of rapamycin inhibitors development," *Expert Opinion on Investigational Drugs*, vol. 20, no. 3, pp. 381–394, 2011.

[87] E. Frigerio, M. D. Colombo, C. Franchi, A. Altomare, C. Garutti, and G. F. Altomare, "Severe psoriasis treated with a new macrolide: everolimus," *British Journal of Dermatology*, vol. 156, no. 2, pp. 372–374, 2007.

[88] S. G. A. Van Velsen, I. M. Haeck, and C. A. F. M. Bruijnzeel-Koomen, "Severe atopic dermatitis treated with everolimus," *Journal of Dermatological Treatment*, vol. 20, no. 6, pp. 365–367, 2009.

[89] M. Mayerhofer, K. J. Aichberger, S. Florian et al., "Identification of mTOR as a novel bifunctional target in chronic myeloid leukemia: dissection of growth-inhibitory and VEGF-suppressive effects of rapamycin in leukemic cells," *FASEB Journal*, vol. 19, no. 8, pp. 960–962, 2005.

[90] X. Wan, N. Shen, A. Mendoza, C. Khanna, and L. J. Helman, "CCI-779 inhibits rhabdomyosarcoma xenograft growth by an antiangiogenic mechanism linked to the targeting of mTOR/Hif-1α/VEGF signaling," *Neoplasia*, vol. 8, no. 5, pp. 394–401, 2006.

[91] K. A. Furge, J. P. MacKeigan, and B. T. Teh, "Kinase targets in renal-cell carcinomas: reassessing the old and discovering the new," *The Lancet Oncology*, vol. 11, no. 6, pp. 571–578, 2010.

[92] A. Mencarelli, E. Distrutti, B. Renga et al., "Development of non-antibiotic macrolide that corrects inflammation-driven immune dysfunction in models of inflammatory bowel diseases and arthritis," *European Journal of Pharmacology*, vol. 665, no. 1–3, pp. 29–39, 2011.

[93] R. J. Xavier and D. K. Podolsky, "Unravelling the pathogenesis of inflammatory bowel disease," *Nature*, vol. 448, no. 7152, pp. 427–434, 2007.

[94] C. Seral, S. Carryn, P. M. Tulkens, and F. Van Bambeke, "Influence of P-glycoprotein and MRP effux pump inhibitors on the intracellular activity of azithromycin and ciprofloxacin in macrophages infected by Listeria monocytogenes or Staphylococcus aureus," *Journal of Antimicrobial Chemotherapy*, vol. 51, no. 5, pp. 1167–1173, 2003.

[95] A. Sugawara, A. Sueki, T. Hirose et al., "Novel 12-membered non-antibiotic macrolides from erythromycin A; EM900 series as novel leads for anti-inflammatory and/or immunomodulatory agents," *Bioorganic and Medicinal Chemistry Letters*, vol. 21, no. 11, pp. 3373–3376, 2011.

[96] V. M. Rivera, R. M. Squillace, D. Miller et al., "Ridaforolimus (AP23573; MK-8669), a potent mtor inhibitor, has broad antitumor activity and can be optimally administered using intermittent dosing regimens," *Molecular Cancer Therapeutics*, vol. 10, no. 6, pp. 1059–1071, 2011.

[97] S. Hashemolhosseini, Y. Nagamine, S. J. Morley, S. Desrivières, L. Mercep, and S. Ferrari, "Rapamycin inhibition of the G1 to S transition is mediated by effects on cyclin D1 mRNA and protein stability," *Journal of Biological Chemistry*, vol. 273, no. 23, pp. 14424–14429, 1998.

[98] I. B. Rosenwald, R. Kaspar, D. Rousseau et al., "Eukaryotic translation initiation factor 4E regulates expression of cyclin D1 at transcriptional and post-transcriptional levels," *Journal of Biological Chemistry*, vol. 270, no. 36, pp. 21176–21180, 1995.

Hindawi Publishing Corporation
Mediators of Inflammation
Volume 2012, Article ID 636157, 7 pages
doi:10.1155/2012/636157

Review Article

Macrolide Therapy in Chronic Inflammatory Diseases

Brygida Kwiatkowska and Maria Maślińska

Department of Early Diagnosis of Arthritis, Institute of Rheumatology, Spartanska 1, 02-627 Warsaw, Poland

Correspondence should be addressed to Maria Maślińska, maslinskam@gmail.com

Received 20 January 2012; Revised 13 July 2012; Accepted 16 July 2012

Academic Editor: Kazuhito Asano

Macrolides are a group of antibiotics with a distinctive macrocyclic lactone ring combined with sugars (cladinose, desosamine). The action of macrolides is to block protein synthesis by binding to the subunit of 50S ribosome of bacteria. Prototype macrolide was erythromycin, which came into clinical practice in the 50s of the 20th century. Its antimicrobial spectrum covers the scope of the penicillins but is extended to the impact of atypical bacteria. In the 90s more drugs of this group were synthesized—they have less severe side effects than erythromycin, extended spectrum of Gram-negative bacteria. Macrolides are effective in treating mycobacterial infections especially in patients infected with HIV. It is now known that in addition to antibacterial abilities, macrolides have immunomodulatory effects—they inhibit the production of proinflammatory cytokines (TNF, IL1, 6, and 8) affect transcription factors (NF-κB) as well as costimulaton (CD 80) and adhesion molecules (ICAM). This review article focused not only on the their antimicrobial abilities but also on efficacy in the treatment of several inflammatory disorders independent of the infectious agent. Their wider use as immunomodulators requires further study, which can lead to an extension of indications for their administration.

1. Introduction

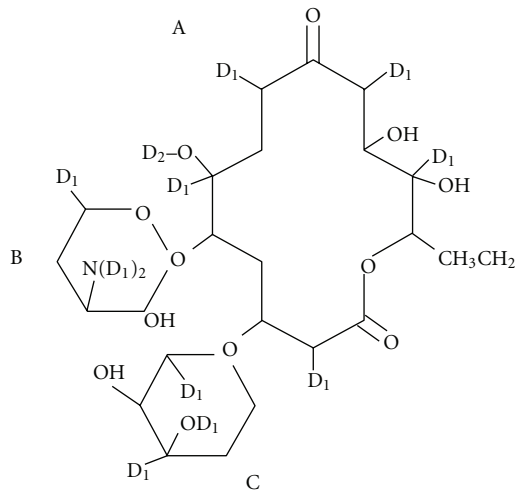
The name "macrolide" covers a family of different antibiotics produced by fungi of the genus *Streptomyces* and some bacteria such as *Arthrobacter* spp. Construction of macrolides is based on the large macrocyclic lacton ring, the activity of which is due to the presence of macrolide ring containing one or more deoxy sugar (usually cladinose-neutral sugar and desosamine-amino sugar). Lactone rings usually consist of 14, 15 or 16 members.

Erythromycin is a macrolide prototype—it contains 14-membered lactone rings, (Figure 1). Its first clinical use in the upper respiratory tract infections occurred in the 50s of the 20th century. Other macrolides with 14-membered ring include clarithromycin, dirithromycin, oleandomycin, roxithromycin, and 16-membered ring: josamycin, midecamycin, mikamycin, and spiramycin. Also stands out azalide—15-membered ring macrolide—azithromycin, and, we can also distinguish ketolides with 14-membered ring such as telithromycin and cethromycin. Tacrolimus isolated from *Streptomyces tsukubaensis* and sirolimus isolated from *Streptomyces hygroscopicus* also belong to this group of antibiotics (Figure 3).

2. The Mechanism of Antibacterial Action of Macrolides

Macrolide antibiotics have been used for many years to treat infectious diseases. Macrolides antibacterial mechanism of action involves binding to the 50S ribosomal subunit, which causes inhibition of the biosynthesis on ribosomal protein level [1, 2]. Both macrolides and ketolides bind domain V of 23S ribosomal RNA (rRNA), contained in the 50S subunit of bacterial ribosomes. However, ketolides have from 10 to 100 greater affinity for the ribosome than erythromycin. Ketolides also, unlike the macrolides, have a greater affinity for binding to the 23S rRNA domain II, which allows them to maintain activity against bacterial strains that are resistant to macrolides due to changes in domain V of 23S [3].

The Spectrum of Antibacterial Activity. Macrolides have become an alternative for people allergic to penicillin.



A: Macrocyclic lactone ring
B: Desosamine
C: Cladinose
D_1: CH_3
D_2: H

Figure 1: 14 member lactone rings of erythromycin.

The first macrolide erythromycin included in its scope spectrum like penicillins, but also demonstrated the effectiveness of intracellular microorganisms such as *Legionella pneumophila, Chlamydia* spp,and *Mycoplasma.* Further discovery and subsequent synthesis of macrolides increased their scope of activity of *Helicobacter pylori* and *Mycobacterium.* The scope of macrolides effect includes also *Bacillus anthracis, Bordetella reccurentis, Corynebacterium diphtheriae*, *Listeria monocytogenes*, *Streptococci* (*S. pneumoniae*), and methicillin-sensitive *Staphilococcus.* They act also on the *Treponema pallidum*, *Toxoplasma gondii*, *Plasmodium* spp, and *Cryptosporidium* [4].

3. Immunoregulation and Anti-Inflammatory Action of Macrolides

In recent years, it has been shown that macrolides beyond the bacteriostatic and bactericidal effect have also anti-inflammatory effect, which was used in chronic inflammatory diseases such as atopic dermatitis, nonspecific inflammatory bowel disease, psoriasis, and arthritis. The effect of macrolides on the inflammatory cell activity by influencing the production and release of proinflammatory cytokines has been demonstrated in many studies. Cytokines and chemokines play a key role in regulating both the proinflammatory immune response—tumour necrosis factor (TNF-), granulocyte—macrophage colony-stimulating factor (GM-CSF), interleukin-L IL-1, IL-6, IL-8, and interferon gamma (IFN-) and anti-inflammatory (e.g., IL-10).

It was shown that macrolides inhibit the production and secretion of IL-1SS and TNF-. in monocytes [5] and IL-1SS, IL-6, TNF-., and GM-CSF in mast cells [6], and IL-8 protein epithelial neutrophil-activating (ENA-78) macrophage inflammatory protein (MIP-1) in macrophages and leukocytes [7]. It was also shown that clarithromycin suppresses the production of IL-6 and IL-1SS by fibroblast-like cells of the synovial membrane [8]. Therapeutic concentrations of erythromycin and clarithromycin reduce the expression of IL-8 mRNA level in bronchial epithelial cells of patients with chronic inflammatory airway disease [9].

Erythromycin also affects the neutrophils migration [10], proliferation of lymphocytes [11], and differentiation of monocytes [12]. Expression of genes involved in immune response and inflammation (e.g., iNOS, COX-2, TNF-alpha, IL-1, and IL-6) at the level of transcription is regulated by nuclear factor-kappa B (NF-κB) [13]. Erythromycin and roxithromycin exhibit antioxidant properties and prevent activation of (NF-κB) [14].

Erythromycin and clarithromycin also show a concentration-dependent inhibition of IL-8 release by eosinophils isolated from people with atopic dermatitis [15]. Macrolides inhibit as well the secretion of eosinophilic chemotactins, cytokines RANTES, and eotaxin in lung fibroblasts [16]. It was also found that macrolides may alter the ratio of IFN-./IL-4 (Th1/Th2) [17]. Macrolides also affect dendritic cells (from mouse bone marrow) by the increase in the expression of CD80, a molecule co-stimulatory T-cell activation [18]. Azithromycin causes increased production of IL-10, while clarithromycin inhibits the production of IL-6 by dendritic cells. All these studies show different effects of macrolides on cytokine production and release of pro- and anti-inflammatory cytokines. Such effects apply only to 14- and 15-membered macrolides [19].

Impact on Other Immunomodulating Mechanisms. Macrolides may influence the metabolism of arachidonic acid by lipoxygenase—modulation cycle of lipoxygenase modulation. Erythromycin and roxitromycin reduce the number and activity of chemotactic neutrophills through the reduction of leukotriene B4 (LBT4) [20].

Several recent studies show the impact of macrolides on the phenomenon of apoptotic epithelial cells and macrophages [21, 22]. In addition, they inhibit angiogenesis by inhibiting the production of vascular endothelial growth factor (VEGF) stimulated by TNF-alpha [23]. The effect of macrolides on the transduction pathways of many different external signals MAPK (mitogen-activated protein kinase) is not limited to the production of cytokines. Erythromycin inhibits IL-1 inducing phosphorylation of p38 MAPK in rheumatoid synovial cells in vitro [24]. Inflammatory cells can produce isoforms of NO using the induced synthesis of nitric oxide (iNOS), which increases the inflammation and causes the destruction of cells. It has been shown in vitro that the macrolides inhibit the production of NO [25, 26].

4. Clinical Practice—Macrolides Use

4.1. Airway Diseases. The most widely from beginning of the introduction into clinical practice, macrolides are used in the treatment of airway diseases. Because of their antibacterial and immunomodulatory abilities, a good tissue penetration

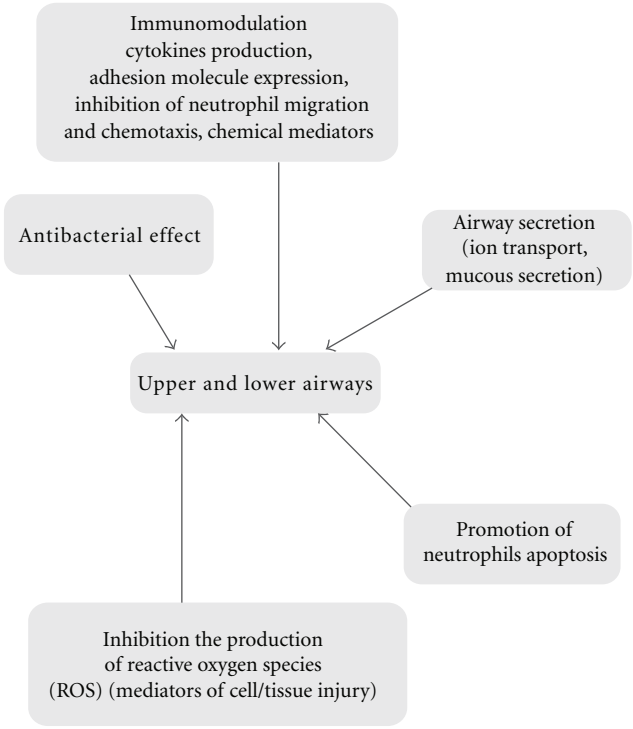


Figure 2: Macrolides influence on inflammatory airway diseases.

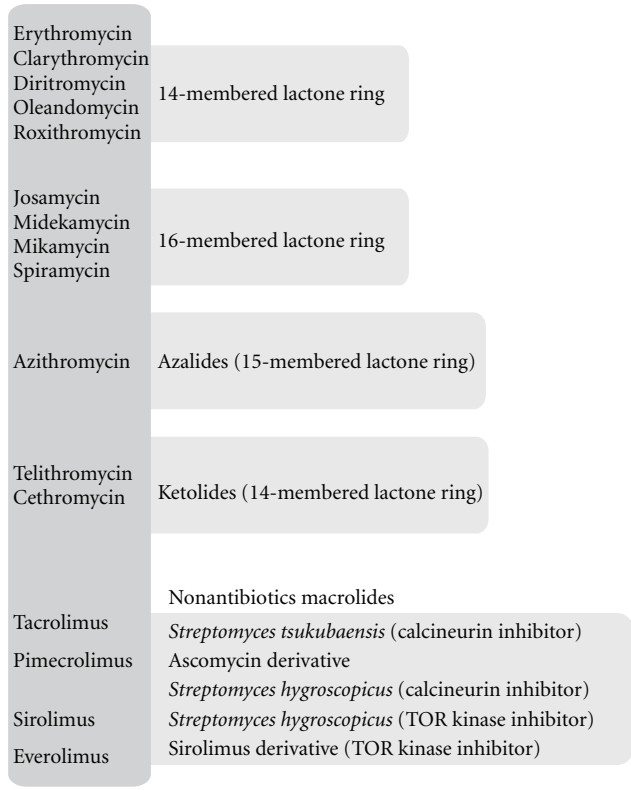


Figure 3: Selected macrolides.

- Rhinosinusitis
- Pharyngitis
- Otitis media
- Exacerbation of chronic bronchitis
- Asthma
- Diffuse panbronchiolitis (DPB)
- Chronic obstructive pulmonary disease (COPD)
- Community acquired pneumonia (CAP)
- Mycobacterium avium complex (HIV-infected patients) (MAC)
- Cystic fibrosis (CF)

Figure 4: Airway diseases in which macrolides are indicated.

and capability for intracellular action are of great importance as well as wide-broad efficacy against many organisms affecting lungs. It was demonstrated that in patients suffering from steroid-dependentasthma the concomitant use of the clarithromycin caused (through theinfluence of cytochrome P450 function) the increase in GKS concentrations, allowing for steroid dose reduction [27, 28]. Until now there is no sufficient evidence and recommendation to treat asthma, by macrolides for long-term therapy, however, it is obvious that atypical bacterial infection in asthma patients is the indication for macrolides therapy [27] (Figures 2 and 4). Several other macrolide properties, such as anti-inflammatory action and production of cytokines (e.g., IL8-a neutrophil chemoattractant), influence on neutrophil migration, antibacterial effect on colonization, and infection by *Pseudomonas aeruginosa*, *Chlamydia pneumonia*, and Mycoplasma pneumonie, may prove beneficial in other various airway diseases. These include diffuse panbronchiolitis (DPB) [29, 30], chronic obstructive lung disease, cystic fibrosis (CF), and bronchiolitis obliterans syndrome (BOS), the latter occurring as a lung transplant complication [31].

4.2. The Use of Macrolides in the Treatment of Skin Diseases. Immunosuppressive macrolides are a new class of anti-inflammatory substances used in the treatment of skin diseases. Tacrolimus (FK506) and pimecrolimus when applied topically penetrate the skin and act locally immunoregulatory [32].

Pimecrolimus and tacrolimus are associated in the cytoplasm of target cells with a specific receptor protein called macrophyllin-12, known as tacrolimus binding protein FKBP (FK506-binding protein). Tacrolimus/pimecrolimus-macrophyllin-12 blocks calcineurin complex. The inhibition of calcineurin results in a lack of gene expression of many mediators of inflammation [33, 34].

Tacrolimus has immunosuppressive activity similar to cyclosporine A, pimecrolimus has a stronger effect. Both drugs were used in the treatment of atopic dermatitis (AD), psoriasis, and contact dermatitis.

Sirolimus (rapamycin) is also a macrolide, but with a different site of action than tacrolimus and pimecrolimus. In the complex with the cytosolic protein FKBP-12, it causes the inhibition of TOR (target of rapamycin) and thereby inhibits intracellular signals pathway conduction. Sirolimus, that acts on T cells, has an effect on angiogenesis by reducing the

production of vascular endothelial growth factor (VEGF). Sirolimus was used in the treatment of psoriasis. The advantage of the use of macrolides for the treatment of skin diseases, both locally and topically, is that they have no effect on collagen synthesis and thus they do not cause skin atrophy in contrast to the glucocorticoids.

Clinical studies have confirmed the effectiveness of oral therapy with macrolide group antibiotics of psoriasis vulgaris [35]. It was shown that 4 weeks of treatment of patients with skin psoriasis with oral macrolides combined with topical treatment with corticosteroids significantly reduced the Psoriasis Area and Severity Index (PASI) and has an impact on the abolition of itching [36].

4.3. Macrolides in Treatment of Nonspecific Inflammatory Bowel Diseases. Due to the immunomodulating effect of macrolides, antibiotics are increasingly used in nonspecific inflammatory bowel diseases, especially Crohn's disease. 2-year observation of patients with Crohn's disease treated with following combination therapy: rifabutin with a macrolide (azithromycin or clarithromycin) for a period of 6 to 35 months showed significant improvement in the assessment of disease activity (Harvey-Bradshaw Crohn's activity index) effect after 6 months of therapy and continuing for the next 24 months [37]. Another study using clarithromycin as immunomodulating drug for 24 weeks and longer showed that 42.9% of patients with active Crohn's disease had remission in the assessment of CDAI (Crohn's Disease Activity Index) after 12 weeks of treatment [38].

Eradication of H. pylori—a Permanent Place for the Use of Macrolides. A lot of studies demonstrate the effectiveness of clarithromycin in the eradication of *H. pylori* infection in combination with another antibiotic and antisecretory agent (proton pump inhibitor-PPI) as standard triple therapy. However, the increasing resistance to the clarithromycin can be observed [39].

5. Prokinetic Effect of Macrolides

It has been demonstrated that 14-membered lacton ring macrolides stimulate gastrointestinal motility, while there is no such effect of the 15- and 16-membered lactone ring macrolides use. It is known that erythromycin acts on the intestinal and gallbladder motility through motilin receptor which causes stimulation of enteric nerves and smooth muscle [40, 41]. Erythromyicin activity, in particular on gastric antral motility, has been also demonstrated to be mediated via cholinergic pathway and activation of a neuromuscular receptor [41]. The attention paid to the prokinetic properties of macrolides is associated with the ongoing search for the effective treatment of gastrointestinal disorders such as gastroparesis in diabetic patients, slow emptying and gastroparesis in intensive care patients undergoing mechanical ventilation, and gastroesophageal reflux and bacterial overgrowth in intensive care patients during enteral nutrition. The prokinetic qualities of macrolides may also be considered in the use of these antibiotics in lung transplant patients, where the risk of graft dysfunction is increased by gastroesophageal reflux (GERD). It is suggested in the literature that erythromycin prokinetic efficacy is dependent on the dose, as it decreases in the days following application. The use of macrolides is associated with risk of inducing and increasing bacterial resistance to macrolides and other side effects, such as arrythmias with prolonged QT interval (ventricular tachycardia—"torsades de pointes"). There is no strong recommendation for macrolide use as a first-line prokinetic treatment. We should consider their use in cases of failure of all other gastrointestinal hyipomotility treatments (e.g., metoclopramide) and of complications of gastrointestinal motility disorders [42, 43].

6. The Use of Macrolides in Rheumatoid Arthritis and Other Rheumatic Diseases

The immunosuppressive effect of tacrolimus is well known in patients with rheumatoid arthritis (RA) for whom methotrexate was ineffective [44] as well as the immunosuppressive effects of sirolimus on the growth of synovial fibroblasts in patients with rheumatoid arthritis [45]. The results of a recent study have demonstrated the effectiveness of roxitromycin as disease-modifying drug in the early forms of rheumatoid arthritis [46]. Clarithromycin showed similar efficacy [47], but it is not a standard therapeutic procedure in the treatment of RA. The application of roxitromycin both in early and late periods of rheumatoid arthritis can be an effective form of therapy that modifies the course of the disease, but requires further studies [48]. In this paper, Matsuoka et al. demonstrated the inhibitory effect of erythromycin costimulating molecule and production of proinflammatory cytokines by synovial fibroblast-like cell. The authors suggested the possibility of further studies in patients with RA [8].

Reactive Arthritis (ReA). In this group of patients particularly chlamydia-induced ReA is an indication for antibiotic therapy. Good effects of treatment Ch– and ReA are described in the application of tetracycline, ciprofloxacin, and doxycycline with rifampicin [49]. Greater efficiency was obtained when using azithromycin and rifampicin [50]—this treatment is particularly effective in the treatment of *Chlamydia pneumoniae* infection [51].

6.1. Conjunctivitis. The studies have shown that the use of azithromycin in the form of eye drops for bacterial conjunctivitis can remove most microorganisms that can cause the inflammation [52].

6.2. Trachoma. Chronic inflammation of the cornea and conjunctiva caused by serotypes A, B, Ba, and C Chlamydia trachomatis, which is the most common cause of blindness in developing countries. In the case of the disease, the drug of choice is azithromycin administered orally (single dose efficacy adults 1 g, children 20 mg per kg) and topical tetracycline [53].

6.3. The Effect of Macrolides on Viral Upper Respiratory Tract. The studies in recent years have shown that macrolides can

inhibit the development of viral infection of upper respiratory tract. Clarithromycin, by inhibiting the production of intracellular adhesion molecule ICAM-1 and secretion of IL-6 and IL-8, significantly influences the pathophysiological changes associated with infection caused by rhinovirus (RV). Clarithromycin inhibits protein and mRNA expression of ICAM induced by infection with the virus and increased levels of proinflammatory cytokines such as IL-1SS, IL-6, and IL-8. This effect is the greatest 3 days after the infection [54] and similar to the effects demonstrated by erythromycin [55]. Similar effects were demonstrated in the case of azithromycin and paramyxovirus infections, particularly respiratory syncytia virus (RSV) [56] and clarithromycin and its effect on infection with influenza virus type A [57]. Macrolides may have future use in the inhibition of chronic inflammation induced by upper respiratory viral infections, such as RV, RSV, or influenza A.

7. New Possibilities of Macrolides

Drugs to build a macrolide such as sirolimus or its derivative everolimus both inhibit the TOR kinases and the proliferation and clonal expansion, therefore, they were applied in transplant rejection reactions as well as in interventional cardiology for coating stents (drug eluting stents), which lowers the risk of restenosis [58]. Further studies are underway on the macrolides, in which no evidence of antibacterial activity was found—only immunomodulating/immunoregulating functions. One of these is a macrolide CSY0073—azithromycin structure showing immumoregulating action in experimental models of inflammatory bowel disease and arthritis [59].

In recent years, it was also revealed that the impact of rapamycin on the inhibition of cell aging which can be important in treating progeria and other age-related diseases [60].

8. Antibacterial Action and Resistance Mechanisms for Macrolides-Clinical Problem

Antibiotic resistance can be the result of adenine methylation associated with the domain V of 23S rRNA, which causes the insensitivity of such a ribosome to macrolides [3]. The resistance to esterase production may also occur. This enzyme, which hydrolyses macrolide, is produced by Enterobacteriaceae. The cause of resistance of bacteria (mainly G) can constitute negative disturbances and abnormal permeability of outer membrane flow hydrophobic molecules.

Cross-resistance to erythromycin and other macrolides can occur as well as cross-resistance to macrolides and clindamycin and streptogramin B—which bind to the same place on the ribosome.

9. Interaction of Macrolides with Other Drugs and the Resulting Toxicity of Drugs

Macrolides inhibit the activity of cytochrome P-450 and its isoform as CYP 3A4 [61]. Macrolides can be divided into 3 groups according to the inhibition of CYP 3A4. Erythromycin and troleandomycin are the strongest inhibitors of cytochrome CYP 3A4. Clarithromycin shows weak inhibition of CYP 3A4, whereas in vitro studies of azithromycin and diritromycyna show almost no inhibition of the cytochrome [62]. Inhibition of CYP3A4 changed metabolism of many drugs, increasing their concentration in serum and exceeding therapeutic levels and thus is the cause of their toxic effects. Special attention should be paid to the potential toxic effects of benzodiazepines, oral anticoagulants (warfarin), theophylline, neuroleptics, statins, and class IA antiarrhythmic drugs such as quinidine and digoxin toxicity risk [63, 64]. Macrolide drugs may also prolong the QT interval and cause torsade pointes.

The most common side effects of this drug class are disorders of the gastrointestinal tract (vomiting, diarrhoea, increased peristalsis). Allergic reactions with eosinophilia, pruritic skin, and urticaria are less common but also observed. In the course of their use, vasculitis (after i.v. administration), elevated transaminases, and hepatitis with cholestasis may occur.

10. Conclusion

Since the discovery of erythromycin and its clinical use as an alternative to penicillin for the introduction of new macrolides such as azithromycin, clarithromycin, telithromycin, which are characterized by greater bioavailability, longer half-life, and extended-antibacterial spectrum and less severe adverse reactions, new abilities of macrolides were discovered. A new class of drugs that have no antibacterial abilities and have been applied not only to treat bacterial infections caused by common G+ bacteria and to a lesser extent G− but also demonstrated their effectiveness in treating atypical infections with bacteria, some protozoa (e.g., *T. Gondii*, *Leishzmania donovani*). They are used in mycobacterial infection (*Mycobacterium avium*). It has been shown that their antibacterial effectiveness involves not only the direct effect on the inhibition of bacterial protein biosynthesis but also their effects on the immune system. Thanks to the influence of co-stimulating particles (CD 80), proinflammatory cytokines production (TNFa, IL1, 6, and 8) and anti-inflammatory cytokines (IL-10), adhesion proteins (ICAM 1), the influence on intracellular signalling pathways, and functions of T cells, their wider use is possible in the treatment of inflammatory conditions beyond the control of infection. Further studies aim to find new indications for macrolides already used in clinical practice and to invent new macrolides of the main immunomodulating action.

Conflict of Interests

All authors have nothing to disclose. All authors have no commercial or financial interest in the products or companies described in this paper.

References

[1] M. N. Alekshun, "New advances in antibiotic development and discovery," *Expert Opinion on Investigational Drugs*, vol. 14, no. 2, pp. 117–134, 2005.

[2] D. N. Wilson, "The A-Z of bacterial translation inhibitors," *Critical Reviews in Biochemistry and Molecular Biology*, vol. 44, pp. 393–433, 2009.

[3] S. Douthwaite, L. H. Hansen, and P. Mauvais, "Macrolide-ketolide inhibition of MLS-resistant ribosomes is improved by alternative drug interaction with domain II of 23S rRNA," *Molecular Microbiology*, vol. 36, no. 1, pp. 183–192, 2000.

[4] B.G Macrolides Katzung, *Basic & Clinical Pharmacology*, vol. 44, 9th edition, 2004.

[5] H. Suzaki, K. Asano, S. Ohki, K. Kanai, T. Mizutani, and T. Hisamitsu, "Suppressive activity of a macrolide antibiotic, roxithromycin, on pro-inflammatory cytokine production in vitro and in vivo," *Mediators of Inflammation*, vol. 8, no. 4-5, pp. 199–204, 1999.

[6] T. Shimane, K. Asano, M. Suzuki, T. Hisamitsu, and H. Suzaki, "Influence of a macrolide antibiotic, roxithromycin, on mast cell growth and activation in vitro," *Mediators of Inflammation*, vol. 10, no. 6, pp. 323–332, 2001.

[7] M. J. Schultz, P. Speelman, C. E. Hack, W. A. Buurman, S. J. H. Van Deventer, and T. Van Der Poll, "Intravenous infusion of eryhtromycin inhibits CXC chemokine production, but augments neutrophil degranulation in whole blood stimulated with Streptococcus pneumoniae," *Journal of Antimicrobial Chemotherapy*, vol. 46, no. 2, pp. 235–240, 2000.

[8] N. Matsuoka, K. Eguchi, A. Kawakami et al., "Inhibitory effect of clarithromycin on costimulatory molecule expression and cytokine production by synovial fibroblast-like cells," *Clinical and Experimental Immunology*, vol. 104, no. 3, pp. 501–508, 1996.

[9] H. Takizawa, M. Desaki, T. Ohtoshi et al., "Erythromycin modulates IL-8 expression in normal and inflamed human bronchial epithelial cells," *American Journal of Respiratory and Critical Care Medicine*, vol. 156, no. 1, pp. 266–271, 1997.

[10] S. Nelson, W. R. Summer, P. B. Terry, G. A. Warr, and G. J. Jakab, "Erythromycin-induced suppression of pulmonary antibacterial defenses. A potential mechanism of superinfection in the lung," *American Review of Respiratory Disease*, vol. 136, no. 5, pp. 1207–1212, 1987.

[11] N. Keicho, S. Kudoh, H. Yotsumoto, and K. S. Akagawa, "Antilymphocytic activity of erythromycin distinct from that of FK506 or cyclosporin A," *Journal of Antibiotics*, vol. 46, no. 9, pp. 1406–1413, 1993.

[12] N. Keicho, S. Kudoh, H. Yotsumto, and K. S. Akagawa, "Erythromycin promotes monocyte to macrophage differentiation," *Journal of Antibiotics*, vol. 47, no. 1, pp. 80–89, 1994.

[13] K. Yamamoto, T. Arakawa, N. Ueda, and S. Yamamoto, "Transcriptional roles of nuclear factor κB and nuclear factor-interleukin-6 in the tumor necrosis factor α-dependent induction of cyclooxygenase-2 in MC3T3-E1 cells," *The Journal of Biological Chemistry*, vol. 270, no. 52, pp. 31315–31320, 1995.

[14] A. Ianaro, A. Ialenti, P. Maffia et al., "Anti-inflammatory activity of macrolide antibiotics," *Journal of Pharmacology and Experimental Therapeutics*, vol. 292, no. 1, pp. 156–163, 2000.

[15] T. Kohyama, H. Takizawa, S. Kawasaki, N. Akiyama, M. Sato, and K. Ito, "Fourteen-member macrolides inhibit interleukin-8 release by human eosinophils from atopic donors," *Antimicrobial Agents and Chemotherapy*, vol. 43, no. 4, pp. 907–911, 1999.

[16] E. Sato, D. K. Nelson, S. Koyama, J. C. Hoyt, and R. A. Robbins, "Erythromycin modulates eosinophil chemotactic cytokine production by human lung fibroblasts in vitro," *Antimicrobial Agents and Chemotherapy*, vol. 45, no. 2, pp. 401–406, 2001.

[17] A. C. Williams, H. F. Galley, A. M. Watt, and N. R. Webster, "Differential effects of three antibiotics on T helper cell cytokine expression," *Journal of Antimicrobial Chemotherapy*, vol. 56, no. 3, pp. 502–506, 2005.

[18] K. Sugiyama, R. Shirai, H. Mukae et al., "Differing effects of clarithromycin and azithromycin on cytokine production by murine dendritic cells," *Clinical and Experimental Immunology*, vol. 147, no. 3, pp. 540–546, 2007.

[19] S. Kanoh and B. K. Rubin, "Mechanisms of action and clinical application of macrolides as immunomodulatory medications," *Clinical Microbiology Reviews*, vol. 23, no. 3, pp. 590–615, 2010.

[20] H. Nakamura, S. Fujishima, T. Inoe et al., "Clinical and immunomodulatory effects of roxitromycin therapy for chronic respiratory tract infection," *European Respiratory Society*, vol. 13, pp. 1371–1379, 1999.

[21] K. Aoshiba, A. Nagai, and K. Konno, "Erythromycin shortens neutrophil survival by accelerating apoptosis," *Antimicrobial Agents and Chemotherapy*, vol. 39, no. 4, pp. 872–877, 1995.

[22] K. Inamura, N. Ohta, S. Fukase, N. Kasajima, and M. Aoyagi, "The effects of erythromycin on human peripheral neutrophil apoptosis," *Rhinology*, vol. 38, no. 3, pp. 124–129, 2000.

[23] T. Oyama, T. Sakuta, K. Matsushita, I. Maruyama, S. Nagaoka, and M. Torii, "Effects of roxithromycin on tumor necrosis factor-alpha-induced vascular endothelial growth factor expression in human periodontal ligament cells in culture," *Journal of Periodontology*, vol. 71, no. 10, pp. 1546–1553, 2000.

[24] T. Fumimori, S. Honda, K. Migita et al., "Erythromycin suppresses the expression of cyclooxygenase-2 in rheumatoid synovial cells," *Journal of Rheumatology*, vol. 31, no. 3, pp. 436–441, 2004.

[25] H. Terao, K. Asano, K. I. Kanai et al., "Suppressive activity of macrolide antibiotics on nitric oxide production by lipopolysaccharide stimulation in mice," *Mediators of Inflammation*, vol. 12, no. 4, pp. 195–202, 2003.

[26] K. Kohri, J. Tamaoki, M. Kondo, K. Aoshiba, E. Tagaya, and A. Nagai, "Macrolide antibiotics inhibit nitric oxide generation by rat pulmonary alveolar macrophages," *European Respiratory Journal*, vol. 15, no. 1, pp. 62–67, 2000.

[27] K. W. Garry, I. Rubinstein, M. H. Gotfried, I. J. Khan, S. Varma, and L. H. Danziger, "Long-term clarithromycin decreases prednisone requirements in elderly patients with prednisone-dependent asthma," *Chest*, vol. 118, no. 6, pp. 1826–1827, 2000.

[28] M. Shinkai, M. O. Henke, and B. K. Rubin, "Macrolide antibiotics as immunomodulatory medications: proposed mechanisms of action," *Pharmacology and Therapeutics*, vol. 117, no. 3, pp. 393–405, 2008.

[29] N. Keicho and S. Kudoch, "Diffuse panbronchiolotis:role of macrolides therapy," *American Journal of Respiratory Medicine*, vol. 1, no. 2, pp. 119–131, 2002.

[30] T. Enomoto, A. Azuma, K. Sakakibara, J. Usuki, and S. Kudo, "Azithromycin therapy for patients with intractable diffuse panbronchiolitis," *The Japanese journal of antibiotics*, vol. 56, supplement A, pp. 12–14, 2003.

[31] B. M. Vanaudenaerde, R. Vos, I. Meyts et al., "Macrolide therapy targets a specific phenotype in respiratory medicine: from clinical experience to basic science and back," *Inflammation and Allergy*, vol. 7, no. 4, pp. 279–287, 2008.

[32] L. M. Gianni and M. M. Sulli, "Topical tacrolimus in the treatment of atopic dermatitis," *Annals of Pharmacotherapy*, vol. 35, no. 7-8, pp. 943–946, 2001.

[33] R. F. Standaert, A. Galat, G. L. Verdine, and S. L. Schreiber, "Molecular cloning and overexpression of the human FK506-binding protein FKBP," *Nature*, vol. 346, no. 6285, pp. 671–674, 1990.

[34] P. Nghiem, G. Pearson, and R. G. Langley, "Tacrolimus and pimecrolimus: from clever prokaryotes to inhibiting calcineurin and treating atopic dermatitis," *Journal of the American Academy of Dermatology*, vol. 46, no. 2, pp. 228–241, 2002.

[35] M. Komine and K. Tamaki, "An open trial of oral macrolide treatment for psoriasis vulgaris," *Journal of Dermatology*, vol. 27, no. 8, pp. 508–512, 2000.

[36] M. Polat, N. Lenk, B. Yalcin et al., "Efficacy of erythromycin for psoriasis vulgaris," *Clinical and Experimental Dermatology*, vol. 32, no. 3, pp. 295–297, 2007.

[37] G. P. H. Gui, P. R. S. Thomas, M. L. V. Tizard, J. Lake, J. D. Sanderson, and J. Hermon-Taylor, "Two-year-outcomes analysis of Crohn's disease treated with rifabutin and macrolide antibiotics," *Journal of Antimicrobial Chemotherapy*, vol. 39, no. 3, pp. 393–400, 1997.

[38] S. Inoue, H. Nakase, M. Matsuura et al., "Open label trial of claritromycin therapy in Japanese patients with Crohn's disease," *Journal of Gastroenterology and Hepatology*, vol. 22, p. 984, 2007.

[39] S. K. Chuah, F. W. Tsay, P. I. Hsu, and D. C. Wu, "A new look at anti-Helicobacter pylori therapy," *World Journal of Gastroenterology*, vol. 17, no. 35, pp. 3971–3975, 2011.

[40] Z. Itoh, T. Suzuki, and M. Nakaya, "Gastrointestinal motor-stimulating activity of macrolide antibiotics and analysis of their side effects on the canine gut," *Antimicrobial Agents and Chemotherapy*, vol. 26, no. 6, pp. 863–869, 1984.

[41] S. M. Catnach and P. D. Fairclough, "Erythromycin and the gut," *Gut*, vol. 33, no. 3, pp. 397–401, 1992.

[42] H. Mentec, H. Dupont, M. Bocchetti, P. Cani, F. Ponche, and G. Bleichner, "Upper digestive intolerance during enteral nutrition in critically ill patients: frequency, risk factors, and complications," *Critical Care Medicine*, vol. 29, no. 10, pp. 1955–1961, 2001.

[43] C. V. Hawkyard and R. J. Koerner, "The use of erythromycin as a gastrointestinal prokinetic agent in adult critical care: benefits versus risks," *Journal of Antimicrobial Chemotherapy*, vol. 59, no. 3, pp. 347–358, 2007.

[44] D. E. Furst, K. Saag, M. R. Fleischmann et al., "Efficacy of tacrolimus in rheumatoid arthritis patients who have been treated unsuccessfully with methotrexate: a six-month, double-blind, randomized, dose-ranging study," *Arthritis and Rheumatism*, vol. 46, no. 8, pp. 2020–2028, 2002.

[45] K. Migita, K. Eguchi, T. Aoyagi et al., "The effects of the immunosuppressant rapamycin on the growth of rheumatoid arthritis (RA) synovial fibroblast," *Clinical and Experimental Immunology*, vol. 104, no. 1, pp. 86–91, 1996.

[46] M. Ogrendik, "Efficacy of roxithromycin in adult patients with rheumatoid arthritis who had not received disease-modifying antirheumatic drugs: a 3-month, randomized, double-blind, placebo-controlled trial," *Clinical Therapeutics*, vol. 31, no. 8, pp. 1754–1764, 2009.

[47] M. Ogrednik, "Effects of clarithromycin in patients with active rheumatoid arthritis," *Current Medical Research and Opinion*, vol. 23, pp. 515–522, 2007.

[48] M. Ogrendik and N. Karagoz, "Treatment of rheumatoid arthritis with roxithromycin: a randomized trial," *Postgraduate Medicine*, vol. 123, pp. 220–227, 2011.

[49] J. D. Carter, J. Valeriano, and F. B. Vasey, "Doxycycline versus doxycycline and rifampin in undifferentiated spondyloarthropathy, with special reference to Chlamydia-induced arthritis. A prospective, randomized 9-month comparison," *Journal of Rheumatology*, vol. 31, no. 10, pp. 1973–1980, 2004.

[50] U. Dreses-Werringloer, I. Padubrin, H. Zeidler, and L. Köhler, "Effects of azithromycin and rifampin on Chlamydia trachomatis infection in vitro," *Antimicrobial Agents and Chemotherapy*, vol. 45, no. 11, pp. 3001–3008, 2001.

[51] X. X. Bin, K. Wolf, T. Schaffner, and R. Malinverni, "Effect of azithromycin plus rifampin versus amoxicillin alone on eradication and inflammation in the chronic course of Chlamydia pneumoniae pneumonitis in mice," *Antimicrobial Agents and Chemotherapy*, vol. 44, no. 6, pp. 1761–1764, 2000.

[52] F. Denis, C. Chaumeil, P. Goldschmidt et al., "Microbiological efficacy of 3-day treatment with azithromycin 1.5% eyedrops for purulent bacterial conjunctivitis," *European Journal of Ophthalmology*, vol. 18, no. 6, pp. 858–868, 2008.

[53] S. K. West, B. Munoz, H. Mkocha, C. A. Gaydos, and T. C. Quinn, "Number of years of annual mass treatment with azithromycin needed to control trachoma in hyper-endemic communities in Tanzania," *Journal of Infectious Diseases*, vol. 204, no. 2, pp. 268–273, 2011.

[54] Y. J. Jang, H. J. Kwon, and B. J. Lee, "Effect of clarithromycin on rhinovirus-16 infection in A549 cells," *European Respiratory Journal*, vol. 27, no. 1, pp. 12–19, 2006.

[55] T. Suzuki, M. Yamaya, K. Sekizawa et al., "Erythromycin inhibits rhinovirus infection in cultured human tracheal epithelial cells," *American Journal of Respiratory and Critical Care Medicine*, vol. 165, no. 8, pp. 1113–1118, 2002.

[56] A. Beigelman, C. L. Mikols, S. P. Gunsten, C. L. Cannon, S. L. Brody, and M. J. Walter, "Azithromycin attenuates airway inflammation in a mouse model of viral bronchiolitis," *Respiratory Research*, vol. 11, article 90, 2010.

[57] M. Yamaya, K. Shinya, Y. Hatachi et al., "Clarithromycin inhibits type A seasonal influenza virus infection in human airway epithelial cells," *Journal of Pharmacology and Experimental Therapeutics*, vol. 333, no. 1, pp. 81–90, 2010.

[58] H. J. Eisen, E. M. Tuzcu, R. Dorent et al., "Everolimus for the prevention of allograft rejection and vasculopathy in cardiac-transplant recipients," *The New England Journal of Medicine*, vol. 349, no. 9, pp. 847–858, 2003.

[59] A. Mencarelli, E. Distrutti, B. Renga et al., "Development of non-antibiotic macrolide that corrects inflammation-driven immune dysfunction in models of inflammatory bowel diseases and arthritis," *European Journal of Pharmacology*, vol. 665, no. 1–3, pp. 29–39, 2011.

[60] M.V Progeria Blagosklonny, "Rapamycin and normal aging: recent breakthrough," *AGING*, vol. 3, no. 7, pp. 685–691, 2011.

[61] P. M. Gascon and P. Dayer, "Comparative effects of macrolide antibiotics on liver mono-oxygenases. Abstract," *Clinical Pharmacology & Therapeutics*, vol. 49, p. 158, 1991.

[62] N. A. Von Rosenstiel and D. Adam, "Macrolide antibacterials: drug interactions of clinical significance," *Drug Safety*, vol. 13, no. 2, pp. 105–122, 1995.

[63] J. F. Westphal, "Macrolide-Induced clinically relevant drug interactions with cytochrome P-450A (CYP) 3A4: an update focused on clarithromycin, azithromycin and dirithromycin," *British Journal of Clinical Pharmacology*, vol. 50, no. 4, pp. 285–295, 2000.

[64] T. Gomes, M. M. Mamdani, and D. N. Juurlink, "Macrolide-induced digoxin toxicity: a population-based study," *Clinical Pharmacology and Therapeutics*, vol. 86, no. 4, pp. 383–386, 2009.

Hindawi Publishing Corporation
Mediators of Inflammation
Volume 2012, Article ID 584262, 17 pages
doi:10.1155/2012/584262

Review Article

Pathogen- and Host-Directed Anti-Inflammatory Activities of Macrolide Antibiotics

Helen C. Steel,[1] Annette J. Theron,[1] Riana Cockeran,[1] Ronald Anderson,[1] and Charles Feldman[2]

[1] *Medical Research Council Unit for Inflammation and Immunity, Department of Immunology, Faculty of Health Sciences, University of Pretoria and Tshwane Academic Division of the National Health Laboratory Service, P.O. Box 2034, Pretoria 0001, South Africa*
[2] *Division of Pulmonology, Department of Internal Medicine, Charlotte Maxeke Academic Hospital, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg 2193, South Africa*

Correspondence should be addressed to Helen C. Steel, hsteel@medic.up.ac.za

Received 1 February 2012; Accepted 2 March 2012

Academic Editor: Kazuhito Asano

Macrolide antibiotics possess several, beneficial, secondary properties which complement their primary antimicrobial activity. In addition to high levels of tissue penetration, which may counteract seemingly macrolide-resistant bacterial pathogens, these agents also possess anti-inflammatory properties, unrelated to their primary antimicrobial activity. Macrolides target cells of both the innate and adaptive immune systems, as well as structural cells, and are beneficial in controlling harmful inflammatory responses during acute and chronic bacterial infection. These secondary anti-inflammatory activities of macrolides appear to be particularly effective in attenuating neutrophil-mediated inflammation. This, in turn, may contribute to the usefulness of these agents in the treatment of acute and chronic inflammatory disorders of both microbial and nonmicrobial origin, predominantly of the airways. This paper is focused on the various mechanisms of macrolide-mediated anti-inflammatory activity which target both microbial pathogens and the cells of the innate and adaptive immune systems, with emphasis on their clinical relevance.

1. Introduction

Macrolides, which are primarily antibiotics, belong to the polyketide group of natural products [1]. They derive their name from their characteristic structural features, a macrocyclic lactone ring to which various deoxy sugars, most commonly cladinose and desosamine, are attached [1]. The most important macrolide antibiotics are 14-, 15-, and 16-membered compounds. The molecular structure of the 14-membered erythromycin, the prototype macrolide, is shown in Figure 1. Drug delivery problems resulting from acid instability prompted the design of newer macrolides. These compounds include (i) clarithromycin, roxithromycin, dirithromycin, and the ketolides and fluoroketolides, all of which have a 14-membered ring structure; (ii) the 15-membered azithromycin; and (iii) the 16-membered agents spiramycin, rokitamycin, and josamycin.

Macrolide antibiotics are generally used to treat respiratory and soft tissue infections caused by Gram-positive bacteria. They are also active against rickettsiae, chlamydiae, and *Mycoplasma pneumoniae*, as well as some Gram-negative bacterial pathogens, including *Bacteroides fragilis, Bordetella pertussis, Campylobacter* species, *Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Moxarella catarrhalis,* and *Neisseria* species. The more advanced macrolides, azithromycin, and clarithromycin, as well as the ketolides/fluoroketolides, have several distinct advantages over erythromycin. These include extended spectrum of activity, improved pharmacokinetics, pharmacodynamics and tolerability, and once-daily administration [2]. Azithromycin and to a lesser extent clarithromycin are noted for their high and prolonged concentrations at sites of infection, reaching tissue levels of 10–100-fold and 2–20-fold greater than serum concentrations, respectively [3–5]. Both agents are also concentrated intracellularly by alveolar macrophages, attaining levels of approximately 400-fold (clarithromycin) and 800-fold (azithromycin) above serum concentrations [3]. The ketolide, telithromycin, also has

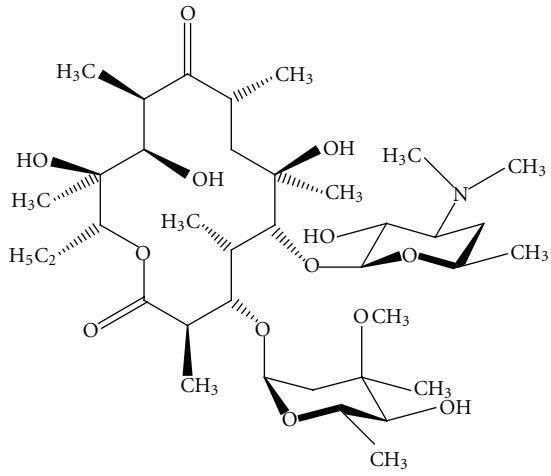


Figure 1: The molecular structure of erythromycin, the 14-membered prototype macrolide [1].

excellent penetration into bronchopulmonary tissues and macrophages, while macrolides and macrolide-like agents are also accumulated by polymorphonuclear leukocytes (PMNL), which, in turn, effect the active delivery of these agents to sites of bacterial infection [3, 6].

With respect to their mechanism of antimicrobial action, macrolides are inhibitors of bacterial protein synthesis. This is achieved by reversible binding of these agents to the P site of the 50S subunit of the bacterial ribosome [1]. The macrolide/ribosome interaction has several apparent consequences, all of which result in inhibition of bacterial protein synthesis. These are (i) interference with peptidyl-transferase, preventing polypeptide chain elongation; (ii) inhibition of ribosomal translocation; and (iii) untimely detachment of peptidyl-tRNA from the ribosome [1, 7, 8]. Macrolides, ketolides, and fluoroketolides possess 1, 2, and 3 ribosomal binding, sites respectively [1]. Although predominantly bacteriostatic, the high tissue and macrophage/PMNL concentrations attained by macrolides and macrolide-like agents may favour bactericidal activity *in vivo*.

Notwithstanding their primary antimicrobial activity, macrolides, unlike most other classes of antibiotic, also possess beneficial anti-inflammatory properties. These latter effects are achieved by two distinct mechanisms. Firstly, as a consequence of their primary ribosomal-targeted mechanism of antimicrobial action, they inhibit the production of proinflammatory microbial toxins and other virulence factors. Surprisingly, this pathogen-directed mechanism of anti-inflammatory activity has also been described for a number of ostensibly macrolide-resistant bacterial pathogens as described hereinafter. Secondly, macrolides have been reported to possess secondary anti-inflammatory activities which target cells of the innate and adaptive immune systems as well as structural cells.

The remainder of this paper is devoted to a consideration of the anti-inflammatory activities of macrolides and their therapeutic relevance.

2. Pathogen-Targeted Anti-Inflammatory Activities of Macrolides

Antibiotics cooperate with host defences to eradicate microbial pathogens. In this setting, the antibiotic-exposed pathogens are weakened, increasing their vulnerability to the cellular and humoral defences of the host. While these antibiotic/host defence interactions are clearly beneficial, some antibiotics may trigger over-exuberant inflammatory responses with potentially harmful consequences for the infected host. These include cell-wall-targeted, bactericidal antibiotics, especially, beta-lactams, as well as fluoroquinolones, which initiate the release of proinflammatory intracellular toxins and cell-wall components from damaged, disintegrating bacteria. Examples of these are the pneumococcal toxin, pneumolysin, as well as cell-wall-derived lipopolysaccharides and lipoteichoic acids. These initiate exaggerated inflammatory responses by several mechanisms, including (i) interactions with Toll-like receptors and nucleotide- oligomerization- (NOD-) like receptors on/in immune and inflammatory cells, as well as epithelial cells; and (ii) activation of complement cascades [9–11]. The harmful, proinflammatory activities of beta-lactams and fluoroquinolones have been demonstrated in a number of studies, either by measuring the release of intracellular toxins following exposure of susceptible bacteria to these antimicrobial agents *in vitro* [12–18], or in animal models of experimental infection in which survival is correlated with the antimicrobial and proinflammatory potencies of antibiotics [19–22].

In contrast to beta-lactams and fluoroquinolones, antibiotics which inhibit bacterial protein synthesis, particularly macrolides and macrolide-like agents, prevent the release of proinflammatory protein toxins from both Gram-positive and Gram-negative bacteria, as well as the production of other virulence factors such as bacterial adhesins and biofilm. Consequently, the pathogen-targeted actions of macrolides have a much lesser propensity to trigger harmful inflammatory reactions than is the case with abruptly bactericidal agents, a contention which is supported by a considerable body of experimental evidence. This includes a number of *in vitro* studies which have demonstrated the inhibitory effects of macrolides and macrolide-like agents, often at subminimal inhibitory concentrations (MICs), on the production of proinflammatory/cytocidal bacterial toxins such as (i) pneumolysin by *Streptococcus pneumoniae* [23, 24], (ii) Panton-Valentine leukocidin and α-haemolysin by *Staphylococcus aureus* [12, 13], and (iii) shiga-like toxins by enterohaemorrhagic strains of *Escherichia coli* [14–18]. In contrast, exaggerated release of these toxins was observed when the bacteria were exposed to beta-lactams or fluoroquinolones [12–18, 25].

These findings have been confirmed in animal models of experimental infection. Spreer et al. in several studies using a rabbit model of experimental meningitis have reported that administration of the macrolide-like agent, clindamycin, as well as rifampicin, but not the beta-lactam, ceftriaxone, significantly reduced concentrations of pneumolysin in cerebrospinal fluid [19–21]. This was associated with an

attenuated inflammatory response and decreased neuronal injury. More recently, others have investigated the effects of treatment with (i) ampicillin only, (ii) azithromycin or clindamycin only, or (iii) ampicillin in combination with either azithromycin or clindamycin on survival using a murine model of secondary, influenza-associated pneumococcal pneumonia [22]. The lowest survival rate in the antibiotic-treated animals was observed in mice treated with ampicillin only, while the highest rates were noted in those treated with azithromycin or clindamycin individually or in combination with ampicillin. Improved survival in the azithromycin/clindamycin-treated groups was associated with an attenuated inflammatory response in the airways characterized by decreases in both the numbers of inflammatory cells and concentrations of proinflammatory cytokines, as well as less severe histopathological changes [22].

In addition to the aforementioned effects of macrolides on dampening potentially harmful responses in the setting of acute bacterial infections caused by macrolide-susceptible pathogens, it is noteworthy that these agents have also been reported to inhibit the production of proinflammatory toxins by ostensibly macrolide-resistant pathogens. Notwithstanding the inhibitory effects of macrolides on the production of shiga toxins by *E. coli* mentioned previously, these agents have also been reported to inhibit the production of pneumolysin by macrolide-resistant strains of the pneumococcus both *in vitro* and *in vivo*. In an earlier study, Lagrou et al. reported that exposure of an *ermAM*-expressing, ribosomal methylase-producing, macrolide-resistant (MIC $\geq$ 256 μg/mL) strain of *Streptococcus pneumoniae* to a sub-MIC concentration of erythromycin prevented the adherence of the bacteria to human nasal respiratory epithelial cells [26]. Although the growth of the bacteria was unaffected, exposure to erythromycin almost completely attenuated the production of pneumolysin, which was the probable cause of interference with bacterial adherence [26]. These findings were confirmed in a later study in which Fukuda et al. reported that both azithromycin and clarithromycin at concentrations of 1–4 μg/mL inhibited the production of pneumolysin by *ermB* and *mefE/A* coexpressing, macrolide-resistant (MIC $\geq$ 256 μg/mL) strains of the pneumococcus *in vitro* [27]. Administration of these agents to mice (40–200 mg/kg) experimentally infected with macrolide-resistant pneumococci was found to result in prolonged survival, which was associated with decreased concentrations of pneumolysin in the airways. Similar findings have been described by Anderson et al., who reported that exposure of an *ermB*-expressing, macrolide-resistant strain of *S. pneumoniae* (MIC $\geq$ 256 μg/mL) to a range of macrolides and macrolide-like agents (0.5 μg $\cdot$ mL) resulted in significant attenuation of the production of pneumolysin, while amoxicillin, ceftriaxone, ciprofloxacin, doxycycline, and tobramycin were ineffective [23, 24].

More recently, Cockeran et al. have attempted to identify the molecular basis of the inhibitory effects of macrolides on the production of pneumolysin by macrolide-resistant strains of the pneumococcus [28]. They observed that exposure of 8 different *ermB*-expressing, macrolide-resistant strains (each with an MIC value of >256 μg/mL) to clarithromycin resulted in significant prolongation of the lag phase of bacterial growth (4.9–12.2 hours in comparison with 1.2–4.9 hours for non-exposed bacteria). Although rapid induction of the *ermB* gene was evident, according to a 4-fold increase in mRNA within 15 minutes of exposure to the antibiotic, synthesis of ribosomal methylase is probably hindered because of binding of clarithromycin to the peptide exit tunnel of the large ribosomal subunit, blocking peptide chain elongation [28]. The consequence is transient susceptibility due to slow acquisition of the full resistance phenotype.

Additional mechanisms which have been reported to underpin the efficacy of macrolides in murine models of experimental infection include high levels of intracellular accumulation of these agents by phagocytes and epithelial cells as well as their beneficial, secondary anti-inflammatory properties described hereinafter [29, 30].

2.1. Macrolides and Pseudomonas aeruginosa. *Pseudomonas aeruginosa* is a persistent opportunistic pathogen which colonizes the airways of immunocompromised individuals causing a chronic, ineffectual inflammatory response. This in turn results in inflammation-mediated tissue damage and pulmonary dysfunction and is particularly serious in patients with cystic fibrosis. Although macrolides do not affect the growth of *P. aeruginosa*, they are nevertheless protective by inhibiting the production of persistence-promoting and proinflammatory virulence factors. These include (i) proadhesive type IV pili, (ii) tissue-damaging pseudomonal elastase, (iii) proinflammatory rhamnolipid, and (iv) alginate and biofilm [31–34]. Alginate is an exopolysaccharide which functions as an antiphagocytic capsule, while biofilm is a self-generated, extracellular polymer matrix in which the pathogen is insulated against both antibiotics and the cellular and humoral defences of the host.

These *P. aeruginosa*-directed anti-infective, anti-inflammatory activities of macrolides, including erythromycin, clarithromycin, and azithromycin, appear to target quorum sensing in *P. aeruginosa*. Quorum sensing is a mechanism of microbial intercellular communication, utilising diffusible signalling molecules known as autoinducers, which enable bacteria to detect and regulate their population density and to upregulate virulence [35]. Gram-negative bacteria most commonly utilize type I family autoinducers known as N-acylated-L-homoserine lactones as their primary mediators of quorum sensing [35]. Both azithromycin and clarithromycin have been reported to inhibit the production of this class of autoinducers by *P. aeruginosa* [31, 36, 37]. Importantly, these effects were evident at sub-MIC concentrations of both macrolides, which in the case of azithromycin was 2 μg/mL [36]. In the case of biofilm formation, the quality of biofilm, as opposed to initiation of synthesis, appeared to be impaired by the macrolides, resulting in altered architecture, structure, and density, favouring the penetration of antibiotics [36, 37]. The pathogen-directed anti-inflammatory activities of macrolides are summarised in Table 1.

As a strategy to counter *P. aeruginosa* in particular, the aforementioned antimicrobial/anti-inflammatory activities

Table 1: Targets of the pathogen-directed anti-inflammatory activities of macrolide antibiotics.

(i) Synthesis and release of proinflammatory toxins and virulence factors
(ii) Quorum sensing
(iii) Biofilm formation

of macrolides are of proven benefit in the long-term therapy of cystic fibrosis [38], as well as the other chronic inflammatory disorders of the airways described hereinafter. However, the benefits of long-term administration of macrolides must be balanced against the potential risks, which include development of macrolide resistance, and, of particular concern, increased susceptibility to infection with nontuberculosus mycobacteria as a consequence of interference with lysosomal acidification [39].

3. Effects of Macrolides on Innate and Adaptive Immune Mechanisms

In addition to pathogen-directed anti-inflammatory activity, macrolides have also been reported to inhibit the proinflammatory activities of cells of both the innate and adaptive immune systems.

3.1. Innate Immunity. In the setting of innate immunity, the predominant anti-inflammatory activity of macrolides appears to be achieved via the modulation of the proinflammatory activities of neutrophils, in particular, inhibition of the production of the potent neutrophil activator and chemoattractant, IL-8 [40, 41]. Increased IL-8 in sputum and bronchoalveolar lavage is associated with severity of chronic inflammatory diseases such as cystic fibrosis (CF) and diffuse panbronchiolitis (DPB) [41–44]. Azithromycin, erythromycin, and clarithromycin have been shown to attenuate the production and secretion of IL-8 by airway smooth muscle cells, alveolar macrophages, and human gingival fibroblasts [40, 45, 46], as well as other cytokines such as (i) IL-1α and IL-2 by murine macrophages and splenocytes, respectively; (ii) IL-1β, GM-CSF, TNF-α, and MCP-1 by macrophages; and (iii) IL-1β, IL-6 and TNF-α from peripheral blood monocytes [47–53]. This is thought to result from the suppression of nuclear translocation of several transcription factors [54] by the macrolides, specifically nuclear factor- (NF-) κB, activator-protein- (AP-) 1, and specificity protein 1 in various types of inflammatory and structural cells [40, 54–60]. Inhibition of intracellular signalling via the extracellular signal-regulated kinase 1 and 2 (ERK 1/2) and p38 mitogen-activated protein kinase (MAPK) pathways are thought to mediate the downregulation of NF-κ-B, AP-1, and specificity protein 1 in response to clarithromycin [56, 57, 61–64]. In addition, azithromycin has been shown to attenuate the LPS/IFN-γ-mediated induction of IL-12p40, probably by the inhibition of the binding of AP-1, nuclear factor of activated T cells (NFAT), and interferon consensus sequence binding protein (ICSBP) to the DNA binding site of the IL-12p40 promoter [65]. This may also prove to be an important mechanism for regulating the anti-inflammatory effects of azithromycin in macrophages.

Interestingly, the ability of macrolide antibiotics to modulate cytokine expression by human neutrophils and their ability to decrease or increase cytokines is thought to depend on the presence or absence of bacteria [66, 67]. Clarithromycin was shown to inhibit the production of IL-6 and TNF-α by neutrophils primed with lipopolysaccharide (LPS), while increasing their expression when bacteria were present [67]. Shinkai et al. reported that clarithromycin initially increased IL-8 secretion by bronchial epithelial cells via ERK signalling but later inhibited ERK signalling leading to reduction (normalisation) in secretion of the chemokine. It is suggested that immunomodulation occurs, in part, by sequential cycles of ERK 1/2 inhibition and activation [60, 63]. This modulation of ERK 1/2 and transcription factors is consistent and unrelated to the antimicrobial properties of macrolides.

Notwithstanding interference with the production of IL-8 by monocytes/macrophages and various types of structural cells, several other mechanisms have been described by which macrolides inhibit neutrophil migration. These include (i) decreased synthesis and expression of the endothelial adhesion molecules ICAM-1 and VCAM-1, possibly as a consequence of decreased synthesis of IL-1β and TNF-α by tissue macrophages and other cell types [68, 69], (ii) interference with the expression of β2-integrins on activated neutrophils [69], (iii) decreased synthesis of leukotriene B_4, a potent neutrophil chemoattractant, possibly as a secondary consequence of inhibitory effects on cytokines/chemokines [70], and (iv) interference with the synthesis and release of the matrix- metalloproteinases- (MMP-), 2, 7, and 9 from nasal polyp fibroblasts, as well as neutrophils, via antagonism of activation of NF-κB and AP-1 [71–73]. MMPs facilitate neutrophil migration.

In addition, macrolides may also interfere with signalling mechanisms initiated by activation of Toll-like receptors (TLRs). TLRs play a key role in innate host defence against viral and microbial pathogens by promoting the release of the neutrophil-mobilizing cytokines, IL-8, and TNF-α, from tissue macrophages and epithelial cells in particular. Treatment of monocyte-derived dendritic cells with erythromycin resulted in up-regulation of TLR2, down-regulation of TLR3, and no effect on expression of TLR4 [74]. However, clarithromycin has been reported to downregulate the expression of TLR4 on monocytes infected with *Helicobacter pylori* [75]. These results indicate that macrolides may selectively downregulate inflammatory responses which result from the interaction of viruses and Gram-negative bacteria with TLR3 and TLR4, respectively, while maintaining the interaction of Gram-positive bacteria with TLR2 [75].

Other anti-inflammatory interactions of macrolides with neutrophils include interference with the generation of reactive oxygen species (ROS) by these cells [76]. Although several mechanisms may exist, membrane-stabilizing activity has been proposed to underpin these effects by neutralizing the sensitizing actions of bioactive phospholipids such as lysophosphatidylcholine, platelet-activating factor (PAF), and lysoPAF on the membrane-associated,

superoxide-generating complex of neutrophils, NADPH oxidase [77]. Macrolides have also been reported to induce phospholipidosis in eukaryotic cells, the magnitude of which appears to correlate with anti-inflammatory activity [78, 79]. Macrolides have also been reported to suppress the production of another type of ROS, nitric oxide, by activated macrophages, presumably by interfering with the induction of inducible nitric oxide synthase via antagonism of NF-κB [80, 81]. The anti-inflammatory interactions of macrolides with the cells of the innate immune system are summarised in Table 2.

In addition to their effects on neutrophils and macrophages, macrolides, as alluded to what is mentioned before, can also downregulate the proinflammatory activities of structural cells, especially epithelial cells. Airway epithelial cells not only provide a mechanical barrier to inhaled microorganisms but are also involved in the direct killing of microbial pathogens, as well as in activating other cells of the innate immune system [63]. The upper and lower respiratory tracts are lined by a highly specialised ciliated columnar epithelium which, together with the mucous layer covering these cells, constitute the mucociliary escalator which functions to keep the lower respiratory tract pathogen-free [82]. Macrolides have been shown to stimulate ciliary beat frequency and improve mucociliary clearance [83, 84]. Moreover, erythromycin, azithromycin, clarithromycin, and roxithromycin have been shown to inhibit chemotaxis and infiltration of neutrophils into the airways and subsequently suppress the synthesis and release of mucus by inhibiting *muc5ac* gene expression [68, 85–87]. Clarithromycin inhibits *muc5ac* gene expression, while azithromycin has been shown to inhibit *muc5ac* production in an ERK 1/2-dependent manner [68, 88]. Macrolides may also decrease sputum production by inhibiting chloride secretion [68]. In addition to these anti-inflammatory effects of macrolides on epithelial cells, these agents have also been reported to protect ciliated respiratory epithelium against the damaging effects of host-derived bioactive phospholipids [89].

3.2. Adaptive Immunity. Although lymphocytes are essential for adaptive immune responses to pathogens, they may also play a harmful role in inflammatory conditions such as autoimmunity and bronchial asthma. Several studies have described the anti-inflammatory effects of macrolides on lymphocytes, particularly T-lymphocytes. These include inhibition of proliferation of (i) Jurkat T cells treated with erythromycin and its non-antibacterial derivatives [90]; (ii) CD4 T cells, when clarithromycin- and roxithromycin-treated and untreated dendritic cells were used as antigen presenting cells [91]; (iii) peripheral blood mononuclear cells treated with azithromycin, clarithromycin, and roxithromycin and activated with concanavalin-A or toxic shock syndrome toxin-1 [92]; and (iv) T cells from house dust mite allergen-sensitive bronchial asthma patients treated with roxithromycin and stimulated with mite antigen [93]. In contrast, cystic fibrosis patients who were treated with clarithromycin (250 mg/day) and followed for a year showed a sustained increase in the *ex vivo* proliferative responses of peripheral blood lymphocytes activated with the T-cell mitogen, phytohemagglutinin [94], possibly reflecting transient inhibitory effects of the macrolides.

The effects of macrolides on cytokine production by T-lymphocytes have also been described in a number of studies. In their study, Pukhalsky et al. reported reversal of the serum IFN-γ/IL-4 ratio in cystic fibrosis patients treated with clarithromycin, compatible with a potentially beneficial elevation in the Th1/Th2 ratio [94]. Others also reported that roxithromycin and clarithromycin increased the Th1/Th2 ratio by decreasing production of IL-4 and IL-5, without affecting IL-2 and IFN-γ levels in several experimental systems, including (i) T cells isolated from the blood of healthy and allergic rhinitis subjects [95], (ii) house dust mite antigen-induced responses of peripheral blood lymphocytes of mite-sensitive bronchial asthma patients [93], and (iii) mononuclear leucocytes, isolated from the blood of healthy donors and stimulated with phorbol 12-myristate 13-acetate (PMA) and ionomycin [96]. In contrast to these findings, Park et al. reported that patients with diffuse panbronchiolitis, receiving long-term treatment with erythromycin, showed decreased levels of IL-2 and IFN-γ, in the setting of increased levels of IL-4, IL-5, and IL-13 in the bronchoalveolar lavage fluid, suggesting a shift from Th1 to Th2 cytokine production following treatment with the macrolide [97]. Inhibition of the production of cytokines by T-lymphocytes by macrolides was also demonstrated in various other studies [91, 92, 98].

T-cell chemotaxis and apoptosis are also affected by treatment with macrolides. Th1, Th2, but not T regulatory cells, treated with roxithromycin, elicited reduced chemotactic responses to the chemokines IP10 (IFN-γ-inducible protein 10) and TARC (thymus- and activation-regulated chemokine) [99]. In addition, erythromycin, clarithromycin, azithromycin, and josamycin have been reported to induce apoptosis in lymphocytes, potentially reducing the number of lymphocytes in the lungs of patients with chronic respiratory tract diseases [90, 100–102].

Apart from effects on T cells, macrolides also appear to affect B-lymphocytes, specifically the expression of co-stimulatory molecules. Asano et al. reported that treatment of B-lymphocytes isolated from BALB/c mice spleens with roxithromycin (5.0 μg/mL) resulted in significant suppression of the expression of the costimulatory molecules, CD40, CD80, and CD86, induced by antigenic stimulation *in vitro* [103]. The anti-inflammatory interactions of macrolides with cells of the adaptive immune system are shown in Table 3.

From a mechanistic perspective, these immunomodulatory activities of macrolides appear to be polymodal. Nonetheless, the weight of evidence favours inhibition of extracellular signal-regulated kinase 1/2 (ERK 1/2) phosphorylation and NF-κB activation as being the predominant mechanisms [104, 105].

4. Immunolides

The clinical efficacy of macrolides in the therapy of apparently nonmicrobial chronic inflammatory diseases of the

Table 2: Anti-inflammatory effects of macrolides on phagocytes and structural cells.

Cellular target	Altered function	Mechanisms
Neutrophils	↓ Migration	Interference with (i) production of IL-8 and TNF-α by macrophages and structural cells, (ii) decreased expression of adhesion molecules on vascular endothelium and neutrophils, and (iii)↓ production/release of MMPs by fibroblasts and neutrophils
	↓ production of ROS	Interference with NADPH oxidase, possibly by antagonizing the sensitizing actions of bioactive phospholipids
Macrophages	↓ cytokine production (IL-1β, IL-6, IL-8, TNF-α)	Interference with intracellular signalling mechanisms and transcription factor activation, resulting in suppression of gene expression
	↓ decreased NO production	As above, resulting in decreased expression of the gene encoding iNOS
Airway epithelial cells, fibroblasts, smooth muscle cells	↓ cytokine production (IL-8, TNF-α)	As above

Table 3: The anti-inflammatory effects of macrolides on T- and B-lymphocytes.

Cellular target	Altered function	Mechanisms
T-lymphocytes	↓ Proliferation	Interference with (i) expression of NFκB,(ii) cellular JNK & ERK activity, and (iii) IFN-γ levels (enhancement may contribute to anti-proliferative activity)
T-lymphocytes	↓ Cytokines of either Th1 (IL-2, TNF-α, IFN-γ), Th2 (IL-4, IL-5, IL-10, IL-13) or both cell types	Interference with cellular JNK and ERK activity
T-lymphocytes	↓ Chemotaxis	Interference with F-actin polymerization and Ca^{2+} influx
T-lymphocytes	↑ Apoptosis	Interference with (i) NF-κB activity,(ii) Bcl-xL expression, and (iii) Fas-Fas ligand pathway
B-lymphocytes	↓ Costimulatory molecules (CD40, CD80, CD86)	—

Abbreviations: NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells; JNK: c-Jun N-terminal kinases; ERK: extracellular-signal-regulated kinases; Bcl-xL: B-cell lymphoma-extra large.

airways has triggered the design and development of a novel class of macrolides, known as immunolides, which are attenuated with respect to antimicrobial activity in the setting of retention of anti-inflammatory properties [56, 106]. These include (i) 9- (*S*)-dihydroerythromycin derivatives which have been demonstrated to possess impressive anti-inflammatory activity in a murine model of phorbol ester-induced ear oedema [107], and (ii) more recently, the EM900 series of novel 12-membered, erythromycin-A-derived nonantibiotic macrolides [108]. EM900 was found to promote monocyte to macrophage differentiation, while suppressing activation of NF-κB and IL-1β, IL-8, and TNF-α gene expression in a human airway epithelial cell line (A549) activated with IL-1β, as well as mucin (*muc5ac*) gene expression by HM3-muc5ac cells [58]. Although promising, the development of immunolides remains in the preclinical stages. Nonetheless, it is our belief that it is the combination of antimicrobial and immunomodulatory properties, as described previously, that is most likely to confer optimum anti-inflammatory activity on the macrolide/azalide/ketolide group of antibiotics.

5. Clinical Conditions for Which Macrolides Are Used Primarily for Their Anti-Inflammatory, Immunomodulatory Properties

Many of the medical conditions for which macrolides are used primarily for their alternative properties, rather than their antimicrobial activity, are chronic disorders of the airway, of both the upper and lower respiratory tract, in which inflammation plays a major pathogenic role [109–112]. While in some of these disorders, such as DPB and CF, evidence for macrolide use is well accepted so that these agents have been included internationally as part of the standard of care, in other conditions, however, the evidence is somewhat less well established, and here these agents are used much more selectively, and particularly in cases that are not responding adequately to more standard therapy. The alternative mechanisms by which macrolides appear to have benefit mostly relate to the cytoprotective effects of these agents on human-ciliated epithelium, their anti-inflammatory, immunomodulatory activity, and their inhibitory activity against quorum sensing mechanisms

Table 4: Conditions for which macrolide use may be beneficial, primarily as a result of their anti-inflammatory, immunomodulatory activity.

(i) Diffuse panbronchiolitis
(ii) Cystic fibrosis (CF)
(iii) Non-CF bronchiectasis
(iv) Bronchiolitis obliterans
(v) Chronic obstructive pulmonary disease
(vi) Asthma
(vii) Pneumonia

of a number of important respiratory tract pathogens as mentioned previously [69, 104, 110, 111, 113–116]. Table 4 indicates some of the more common conditions for which macrolide use has been considered. Hereinafter are brief summaries of the evidence for the possible benefits and/or roles of macrolides in various medical conditions, based on an overview of appropriate scientific studies and reviews.

5.1. Diffuse Panbronchiolitis (DPB). DPB is a chronic inflammatory disorder of the airway occurring in many population groups, but being most common among individuals of Japanese origin [109–112]. The major presentation is with cough, sputum production, and progressive shortness of breath, and patients very frequently become colonised with pseudomonal isolates. Without any treatment the outcome of DPB is dismal. Chronic low-dose macrolide therapy is the treatment of choice and has had a major positive impact on the natural history of this condition [109–112, 117–130].

5.2. Cystic Fibrosis (CF). CF is an autosomally recessive inherited disorder occurring predominantly in Caucasian populations in which abnormalities in epithelial cell ion transport occur as a consequence of defects in the CF transmembrane regulator, resulting in increased sputum viscosity, stasis of secretions, airway infection and inflammation, and progressive bronchiectasis. A myriad of studies has been conducted in the past 10 years evaluating the possible role of long-term macrolide therapy in this condition [94, 110–112, 131–153]. When evaluating these as a whole there is clear-cut evidence that long-term macrolide treatment has benefit with regard to clinically relevant end-points in patients with CF and macrolide therapy features prominently in guidelines for its management, particularly in those cases infected with *Pseudomonas aeruginosa* who have associated deterioration in lung function. It is interesting to note that the mechanisms of action of macrolides in such CF patients appear to relate not only to their antineutrophil, anti-inflammatory activities but also to their detrimental effects on the biology of *P. aeruginosa*, which have been well characterised [94, 110–112, 130–153].

5.3. Non-CF Bronchiectasis. Bronchiectasis is a condition most commonly occurring as a consequence of chronic airway infection and inflammation. In this disorder, airway obstruction mainly associated with bacterial infection, and its associated airway inflammation, leads to a "vicious circle" of chronic infection and inflammation with progressive damage to the ciliated epithelium lining the airways and subsequently its underlying structures. The condition is associated not only with airway disease punctuated by recurrent acute infective exacerbations but also with chronic systemic debility leading to considerable morbidity and even mortality. Since chronic airway inflammation is central to its pathogenesis and few other therapies have been shown to alter the natural course of the condition, it is not surprising that anti-inflammatory therapies of all sorts have been tried in this condition, of which the macrolides appear to be the most promising [36, 154–177]. Interest in macrolide use for non-CF bronchiectasis was developed following their successful use in patients with CF. Beneficial effects of long-term macrolide use for non-CF bronchiectasis have been found in small clinical trials. In most of these studies there was clear evidence of a decrease in sputum volume and, in some, a decrease in exacerbation frequency. Furthermore, in a small number in which this was tested there was an improvement in lung function parameters or a decrease in airway hyperreactivity. The common recommendation for this condition is to try macrolide therapy in selected cases for 3–6 months and to discontinue treatment if there is no clear evidence of benefit to the patient in terms of improvement in quality of life or reduction in exacerbation frequency.

5.4. Bronchiolitis Obliterans (BOs). BO is one of the manifestations of chronic rejection following lung or bone marrow transplant and is a major cause of limited survival and death in lung transplant recipients. Although the exact pathogenesis has still to be unravelled, it appears to result as a consequence of repeated insults to the airways. More recently there has been considerable interest in using macrolides for this serious condition for which other therapies have been rather disappointing or are associated with considerable side-effects [178–189]. Studies have been undertaken to investigate not only the effects of macrolides as therapy for this condition but also, more recently, its prevention. In reviewing the various therapeutic studies, it has been said that there are differences in the clinical spectrum and macrolide response of patients with BO and that those cases associated with a predominantly neutrophilic pathogenesis are macrolide responsive, while those associated with a predominantly fibroproliferative response (so-called traditional BO) are not.

5.5. Chronic Obstructive Pulmonary Disease (COPD). In more recent definitions of COPD, due recognition is given to the fact that in this condition there is an abnormal inflammatory process in the airways, which, although initially is most commonly associated with cigarette smoking, at some stage becomes self-perpetuating and contributes to the progressive deterioration that may be seen in patients with COPD, even in those that quit smoking. While macrolides may be used for the antibiotic management of acute exacerbations of COPD, studies have also been conducted wherein these agents are used for their anti-inflammatory, immunomodulatory

activities and their effects on mucus secretion. In most of these studies a reduction in sputum production, as well as improvement in the quality of the sputum, has been noted, while in some an improvement in quality of life, various clinical end-points, and occasionally in lung function parameters has been seen. Importantly, some studies have suggested that macrolide therapy may alter the course of COPD by reducing both the number and the duration of acute exacerbations [68, 109, 190–199].

5.6. Asthma. It has been recognised for a number of years that asthma is a chronic inflammatory disorder of the airways, the inflammation being mediated by a variety of cells and mediators which are responsible for the manifestations including the symptoms, the lung function abnormalities, and the airway hyperresponsiveness. Therapy is therefore primarily with anti-inflammatory agents, particularly inhaled corticosteroids, but a number of the other drugs used in asthma treatment have also been recognised to have anti-inflammatory activity. While much of the airway inflammation may be driven by allergic/atopic responses, it has also been suggested that chronic lower respiratory tract infection with *Mycoplasma pneumoniae* and *Chlamydia pneumoniae*, both microorganisms that are responsive to macrolide therapy, may initiate airway inflammation and asthma and is therefore potentially amenable to macrolide therapy. All of these considerations provide the rationale for the use of macrolides in asthma, in the hope of achieving more effective asthma control. Although a number of studies have been undertaken over more recent years using different macrolides, with some showing modest benefits, the overall data suggests that there is no role for long-term macrolide therapy in asthma, although such treatment may be of benefit in some subgroups of patients, such as those described previously [200–214].

5.7. Pneumonia. Antibiotic therapy in patients with pneumonia is short course, aimed at treating the infection and eradicating the microorganism. However, there is still considerable ongoing debate as to what antibiotic regimen constitutes optimal therapy in hospitalised cases with community-acquired pneumonia (CAP), including those that require intensive care unit (ICU) admission. A myriad of studies in more severely ill-hospitalised patients with CAP has suggested that the outcome is improved by using combination antibiotic therapy, most commonly with the addition of a macrolide to standard beta-lactam therapy [215–226]. This understanding needs to be counterbalanced by additional studies suggesting that the outcome is similar when comparing fluoroquinolone monotherapy to the beta-lactam/macrolide combination in noncritically ill-hospitalised patients [227–229]. Thus for cases not in the ICU, most guidelines recommend either option, whereas in ICU patients, combination therapy is always recommended irrespective of which of these agents is used. Interestingly, in one study in intubated patients in the ICU, the outcome was better with the use of the macrolide rather than the fluoroquinolone combination [226]. The reason that combination therapy with macrolides is associated with an improved outcome in patients with CAP is uncertain and may be multifactorial; however, many believe that it may relate to the anti-inflammatory immunomodulatory effects of these agents [229]. Two recent studies appear to support this contention [230, 231]. In the first study, macrolide use was associated with decreased mortality in patients with CAP and severe sepsis even when the infection was due to macrolide-resistant pathogens. Furthermore, a placebo-controlled, randomised, clinical trial, undertaken to investigate whether patients with sepsis and ventilator-associated pneumonia (VAP), predominantly due to Gram-negative pathogens, had improved outcome when a macrolide was added to standard antibiotic therapy, demonstrated that clarithromycin accelerated the resolution of VAP and the weaning from mechanical ventilation and delayed death in those that ultimately died of sepsis. In addition, in a very recent review of the literature, Kovaleva, et al. concluded that macrolides appear to attenuate the inflammatory response during CAP [232]. In support of this contention, Walkey and Weiner have reported, also very recently, that patients with acute lung injury (ALI), predominantly associated with pneumonia, who were treated with macrolides, had a significantly lower 180-day mortality and shorter time to successful discontinuation of mechanical ventilation relative to those patients treated with fluoroquinolones or cephalosporins [233].

5.8. Upper Respiratory Tract Disorders. A number of studies have also been undertaken investigating the use of macrolides in upper airway conditions, such as chronic rhinosinusitis, and appear to show promise [234–244]. Such studies clearly suffer from the methodological issues discussed hereinafter and need to be repeated in appropriate fashion before conclusions can be drawn about the value of macrolides and their use in upper airway diseases, although recommendations for macrolide use do appear in many of the international guidelines on rhinosinusitis management, in certain circumstances. As in many of the conditions already discussed, these potential benefits are thought to relate to the anti-inflammatory, immunomodulatory activity of macrolides and their effects on the virulence of and tissue damage caused by the chronic colonising bacteria [234–244].

6. Conclusions

It is clear from the various studies that macrolides have a clear-cut role in conditions such as DPB and CF, and possibly additional beneficial effects on morbidity, and possibly even mortality, in various other airway disorders. Furthermore, additional studies have also uncovered potential beneficial effects in various disorders unrelated to the airway. Many of these studies suffer from the fact that they are limited in terms of size, patient numbers, and length of treatment and follow-up. It is therefore clear that in many of these conditions further studies are needed in order to clarify such questions as in which patients these agents should be used, which macrolide drugs is/are best, what dosing schedules are

appropriate, for how long should treatment be continued, and what are the long-term side-effects?

References

[1] Wikipedia, the free encyclopedia, Macrolide, 2011, http://en.wikipedia.org/wiki/Macrolide.

[2] J. M. Zuckerman, F. Qamar, and B. R. Bono, "Review of macrolides (azithromycin, clarithromycin), ketolides (telithromycin) and glycylcyclines (tigecycline)," *Medical Clinics of North America*, vol. 95, no. 4, pp. 761–791, 2011.

[3] G. Foulds, R. M. Shepard, and R. B. Johnson, "The pharmacokinetics of azithromycin in human serum and tissues," *Journal of Antimicrobial Chemotherapy*, vol. 25, supplement, pp. 73–82, 1990.

[4] F. Fraschini, F. Scaglione, G. Pintucci, G. Maccarinelli, S. Dugnani, and G. Demartini, "The diffusion of clarithromycin and roxithromycin into nasal mucosa, tonsil and lung in humans," *Journal of Antimicrobial Chemotherapy*, vol. 27, pp. 61–65, 1991.

[5] J. M. Zuckerman, "Macrolides and ketolides: azithromycin, clarithromycin, telithromycin," *Infectious Disease Clinics of North America*, vol. 18, no. 3, pp. 621–649, 2004.

[6] G. L. Mandell, "Delivery of antibiotics by phagocytes," *Clinical Infectious Diseases*, vol. 19, no. 5, pp. 922–925, 1994.

[7] T. Tenson, M. Lovmar, and M. Ehrenberg, "The mechanism of action of macrolides, lincosamides and streptogramin B reveals the nascent peptide exit path in the ribosome," *Journal of Molecular Biology*, vol. 330, no. 5, pp. 1005–1014, 2003.

[8] G. Kaiser, "Protein synthesis inhibitors: macrolides mechanism of action animation. Classification of agents Pharmamotion," The Community College of Baltimore County, 2011, http://en.wikipedia.org/wiki/Protein_synthesis_inhibitor.

[9] H. M. Marriott, T. J. Mitchell, and D. H. Dockrell, "Pneumolysin: a double-edged sword during the host-pathogen interaction," *Current Molecular Medicine*, vol. 8, no. 6, pp. 497–509, 2008.

[10] H. Tsujimoto, S. Ono, P. A. Efron, P. O. Scumpia, L. L. Moldawer, and H. Mochizuki, "Role of toll-like receptors in the development of sepsis," *Shock*, vol. 29, no. 3, pp. 315–321, 2008.

[11] O. Takeuchi and S. Akira, "Pattern recognition receptors and inflammation," *Cell*, vol. 140, no. 6, pp. 805–820, 2010.

[12] P. T. Kimmitt, C. R. Harwood, and M. R. Barer, "Induction of type 2 Shiga toxin synthesis in *Escherichia coli* 0157 by 4-quinolones," *The Lancet*, vol. 353, no. 9164, pp. 1588–1589, 1999.

[13] J. Murakami, K. Kishi, K. Hirai, K. Hiramatsu, T. Yamasaki, and M. Nasu, "Macrolides and clindamycin suppress the release of Shiga-like toxins from *Escherichia coli* O157:H7 *in vitro*," *International Journal of Antimicrobial Agents*, vol. 15, no. 2, pp. 103–109, 2000.

[14] C. S. Wong, S. Jelacic, R. L. Habeeb, S. L. Watkins, and P. I. Tarr, "The risk of the hemolytic-uremic syndrome after antibiotic treatment of *Escherichia coli* O157:H7 infections," *New England Journal of Medicine*, vol. 342, no. 26, pp. 1930–1936, 2000.

[15] D. L. Stevens, Y. Ma, D. B. Salmi, E. McIndoo, R. J. Wallace, and A. E. Bryant, "Impact of antibiotics on expression of virulence-associated exotoxin genes in methicillin-sensitive and methicillin-resistant *Staphylococcus aureus*," *Journal of Infectious Diseases*, vol. 195, no. 2, pp. 202–211, 2007.

[16] O. Dumitrescu, C. Badiou, M. Bes et al., "Effect of antibiotics, alone and in combination, on Panton-Valentine leukocidin production by a *Staphylococcus aureus* reference strain," *Clinical Microbiology and Infection*, vol. 14, no. 4, pp. 384–388, 2008.

[17] A. Serna IV and E. C. Boedeker, "Pathogenesis and treatment of Shiga toxin-producing *Escherichia coli* infections," *Current Opinion in Gastroenterology*, vol. 24, no. 1, pp. 38–47, 2008.

[18] C. M. McGannon, C. A. Fuller, and A. A. Weiss, "Different classes of antibiotics differentially influence shiga toxin production," *Antimicrobial Agents and Chemotherapy*, vol. 54, no. 9, pp. 3790–3798, 2010.

[19] A. Spreer, H. Kerstan, T. Böttcher et al., "Reduced release of pneumolysin by *Streptococcus pneumoniae in vitro* and *in vivo* after treatment with nonbacteriolytic antibiotics in comparison to ceftriaxone," *Antimicrobial Agents and Chemotherapy*, vol. 47, no. 8, pp. 2649–2654, 2003.

[20] T. Böttcher, H. Ren, M. Goiny et al., "Clindamycin is neuroprotective in experimental *Streptococcus pneumoniae* meningitis compared with ceftriaxone," *Journal of Neurochemistry*, vol. 91, no. 6, pp. 1450–1460, 2004.

[21] A. Karlström, K. L. Boyd, B. K. English, and J. A. McCullers, "Treatment with protein synthesis inhibitors improves outcomes of secondary bacterial pneumonia after influenza," *Journal of Infectious Diseases*, vol. 199, no. 3, pp. 311–319, 2009.

[22] A. Spreer, R. Lugert, V. Stoltefaut, A. Hoecht, H. Eiffert, and R. Nau, "Short-term rifampicin pretreatment reduces inflammation and neuronal cell death in a rabbit model of bacterial meningitis," *Critical Care Medicine*, vol. 37, no. 7, pp. 2253–2258, 2009.

[23] R. Anderson, H. C. Steel, R. Cockeran et al., "Clarithromycin alone and in combination with ceftriaxone inhibits the production of pneumolysin by both macrolide-susceptible and macrolide-resistant strains of *Streptococcus pneumoniae*," *Journal of Antimicrobial Chemotherapy*, vol. 59, no. 2, pp. 224–229, 2007.

[24] R. Anderson, H. C. Steel, R. Cockeran et al., "Comparison of the effects of macrolides, amoxicillin, ceftriaxone, doxycycline, tobramycin and fluoroquinolones, on the production of pneumolysin by *Streptococcus pneumoniae in vitro*," *Journal of Antimicrobial Chemotherapy*, vol. 60, no. 5, pp. 1155–1158, 2007.

[25] R. A. Hirst, B. J. Mohammed, T. J. Mitchell, P. W. Andrew, and C. O'Callaghan, "*Streptococcus pneumoniae*-induced inhibition of rat ependymal cilia is attenuated by antipneumolysin antibody," *Infection and Immunity*, vol. 72, no. 11, pp. 6694–6698, 2004.

[26] K. Lagrou, W. E. Peetermans, M. Jorissen, J. Verhaegen, J. Van Damme, and J. Van Eldere, "Subinhibitory concentrations of erythromycin reduce pneumococcal adherence to respiratory epithelial cells *in vitro*," *Journal of Antimicrobial Chemotherapy*, vol. 46, no. 5, pp. 717–723, 2000.

[27] Y. Fukuda, K. Yanagihara, Y. Higashiyama et al., "Effects of macrolides on pneumolysin of macrolide-resistant *Streptococcus pneumoniae*," *European Respiratory Journal*, vol. 27, no. 5, pp. 1020–1025, 2006.

[28] R. Cockeran, H. C. Steel, N. Wolter et al., "Effects of clarithromycin at sub-minimum inhibitory concentrations on early *ermB* gene expression, metabolic activity and growth of an *ermB*-expressing, macrolide-resistant strain of *Streptococcus pneumoniae*," *Open Journal of Respiratory Diseases*, vol. 2, pp. 1–8, 2012.

[29] Y. Yasuda, K. Kasahara, F. Mizuno, K. Nishi, K. Mikasa, and E. Kita, "Roxithromycin favorably modifies the initial phase of resistance against infection with macrolide-resistant *Streptococcus pneumoniae* in a murine pneumonia model," *Antimicrobial Agents and Chemotherapy*, vol. 51, no. 5, pp. 1741–1752, 2007.

[30] S. Nakamura, K. Yanagihara, N. Araki et al., "Efficacy of clarithromycin against experimentally induced pneumonia caused by clarithromycin-resistant *Haemophilus influenzae* in mice," *Antimicrobial Agents and Chemotherapy*, vol. 54, no. 2, pp. 757–762, 2010.

[31] K. Q. Bui, M. A. Banevicius, C. H. Nightingale, R. Quintiliani, and D. P. Nicolau, "*In vitro* and *in vivo* influence of adjunct clarithromycin on the treatment of mucoid *Pseudomonas aeruginosa*," *Journal of Antimicrobial Chemotherapy*, vol. 45, no. 1, pp. 57–62, 2000.

[32] G. Tanaka, M. Shigeta, H. Komatsuzawa, M. Sugai, H. Suginaka, and T. Usui, "Effect of clarithromycin on *Pseudomonas aeruginosa* biofilms," *Chemotherapy*, vol. 46, no. 1, pp. 36–42, 2000.

[33] K. Tateda, T. J. Standiford, J. C. Pechere, and K. Yamaguchi, "Regulatory effects of macrolides on bacterial virulence: potential role as quorum-sensing inhibitors," *Current Pharmaceutical Design*, vol. 10, no. 25, pp. 3055–3065, 2004.

[34] D. J. Wozniak and R. Keyser, "Effects of subinhibitory concentrations of macrolide antibiotics on *Pseudomonas aeruginosa*," *Chest*, vol. 125, no. 2, supplement 12, pp. 62S–69S, 2004.

[35] L. Hall-Stoodley and P. Stoodley, "Evolving concepts in biofilm infections," *Cellular Microbiology*, vol. 11, no. 7, pp. 1034–1043, 2009.

[36] Y. Nalca, L. Jänsch, F. Bredenbruch, R. Geffers, J. Buer, and S. Häussler, "Quorum-sensing antagonistic activities of azithromycin in *Pseudomonas aeruginosa* PAO1: a global approach," *Antimicrobial Agents and Chemotherapy*, vol. 50, no. 5, pp. 1680–1688, 2006.

[37] A. Bala, R. Kumar, and K. Harjai, "Inhibition of quorum sensing in *Pseudomonas aeruginosa* by azithromycin and its effectiveness in urinary tract infections," *Journal of Medical Microbiology*, vol. 60, no. 3, pp. 300–306, 2011.

[38] Y. Cai, D. Chai, R. Wang, N. Bai, B. B. Liang, and Y. Liu, "Effectiveness and safety of macrolides in cystic fibrosis patients: a meta-analysis and systematic review," *Journal of Antimicrobial Chemotherapy*, vol. 66, no. 5, pp. 968–978, 2011.

[39] M. Renna, C. Schaffner, K. Brown et al., "Azithromycin blocks autophagy and may predispose cystic fibrosis patients to mycobacterial infection," *Journal of Clinical Investigation*, vol. 121, no. 9, pp. 3554–3563, 2011.

[40] K. Oishi, F. Sonoda, S. Kobayashi et al., "Role of interleukin-8 (IL-8) and an inhibitory effect of erythromycin on IL-8 release in the airways of patients with chronic airway diseases," *Infectious Immunology*, vol. 62, no. 10, pp. 4145–4152, 1994.

[41] B. M. Vanaudenaerde, W. A. Wuyts, N. Geudens et al., "Macrolides inhibit IL17-induced IL8 and 8-isoprostane release from human airway smooth muscle cells," *American Journal of Transplantation*, vol. 7, no. 1, pp. 76–82, 2007.

[42] S. Brennan, D. Cooper, and P. D. Sly, "Directed neutrophil migration to IL-8 is increased in cystic fibrosis: a study of the effect of erythromycin," *Thorax*, vol. 56, no. 1, pp. 62–64, 2001.

[43] T. Yamaryo, K. Oishi, H. Yoshimine, Y. Tsuchihashi, K. Matsushima, and T. Nagatake, "Fourteen-member macrolides promote the phosphatidylserine receptor-dependent phagocytosis of apoptotic neutrophils by alveolar macrophages," *Antimicrobial Agents and Chemotherapy*, vol. 47, no. 1, pp. 48–53, 2003.

[44] S. Hodge, G. Hodge, H. Jersmann et al., "Azithromycin improves macrophage phagocytic function and expression of mannose receptor in chronic obstructive pulmonary disease," *American Journal of Respiratory and Critical Care Medicine*, vol. 178, no. 2, pp. 139–148, 2008.

[45] E. Kita, M. Sawaki, F. Nishikawa et al., "Enhanced interleukin production after long-term administration of erythromycin stearate," *Pharmacology*, vol. 41, no. 4, pp. 177–183, 1990.

[46] A. Kamemoto, T. Ara, T. Hattori, Y. Fujinami, Y. Imamura, and P. L. Wang, "Macrolide antibiotics like azithromycin increase lipopolysaccharide-induced IL-8 production by human gingival fibroblasts," *European Journal of Medical Research*, vol. 14, no. 7, pp. 309–314, 2009.

[47] S. Bailly, J. J. Pocidalo, M. Fay, and M. A. Gougerot-Pocidalo, "Differential modulation of cytokine production by macrolides: interleukin-6 production is increased by spiramycin and erythromycin," *Antimicrobial Agents and Chemotherapy*, vol. 35, no. 10, pp. 2016–2019, 1991.

[48] S. I. Konno, M. Adachi, K. Asano, T. Kawazoe, K. I. Okamoto, and T. Takahashi, "Influences of roxithromycin on cell-mediated immune responses," *Life Sciences*, vol. 51, no. 10, pp. PL107–PL112, 1992.

[49] H. Suzaki, K. Asano, S. Ohki, K. Kanai, T. Mizutani, and T. Hisamitsu, "Suppressive activity of a macrolide antibiotic, roxithromycin, on pro- inflammatory cytokine production in vitro and in vivo," *Mediators of Inflammation*, vol. 8, no. 4-5, pp. 199–204, 1999.

[50] H. Yamasawa, K. Oshikawa, S. Ohno, and Y. Sugiyama, "Macrolides inhibit epithelial cell-mediated neutrophil survival by modulating granulocyte macrophage colony-stimulating factor release," *American Journal of Respiratory Cell and Molecular Biology*, vol. 30, no. 4, pp. 569–575, 2004.

[51] M. Bosnar, B. Bošnjak, S. Ćužić et al., "Azithromycin and clarithromycin inhibit lipopolysaccharide-induced murine pulmonary neutrophilia mainly through effects on macrophage-derived granulocyte-macrophage colony-stimulating factor and interleukin-1 beta," *Journal of Pharmacology Experimental Therapy*, vol. 331, no. 1, pp. 104–113, 2009.

[52] M. Meyer, F. Huaux, X. Gavilanes et al., "Azithromycin reduces exaggerated cytokine production by M1 alveolar macrophages in cystic fibrosis," *American Journal of Respiratory Cell and Molecular Biology*, vol. 41, no. 5, pp. 590–602, 2009.

[53] S. Abe, H. Nakamura, S. Inoue et al., "Interleukin-8 gene repression by clarithromycin is mediated by the activator protein-1 binding site in human bronchial epithelial cells," *American Journal of Respiratory Cell and Molecular Biology*, vol. 22, no. 1, pp. 51–60, 2000.

[54] T. Kikuchi, K. Hagiwara, Y. Honda et al., "Clarithromycin suppresses lipopolysaccharide-induced interleukin-8 production by human monocytes through AP-1 and NF-κB transcription factors," *Journal of Antimicrobial Chemotherapy*, vol. 49, no. 5, pp. 745–755, 2002.

[55] M. Desaki, H. Okazaki, T. Sunazuka, S. Omura, K. Yamamoto, and H. Takizawa, "Molecular mechanisms of anti-inflammatory action of erythromycin in human bronchial epithelial cells: possible role in the signaling pathway that regulates nuclear factor-kappaB activation,"

Antimicrobial Agents and Chemotherapy, vol. 48, no. 5, pp. 1581–1585, 2004.

[56] M. Shinkai, G. H. Foster, and B. K. Rubin, "Macrolide antibiotics modulate ERK phosphorylation and IL-8 and GM-CSF production by human bronchial epithelial cells," *American Journal of Physiology*, vol. 290, no. 1, pp. L75–L85, 2006.

[57] M. Shinkai, J. Tamaoki, H. Kobayashi et al., "Clarithromycin delays progression of bronchial epithelial cells from G 1 phase to S phase and delays cell growth via extracellular signal-regulated protein kinase suppression," *Antimicrobial Agents and Chemotherapy*, vol. 50, no. 5, pp. 1738–1744, 2006.

[58] C. Cigana, B. M. Assael, and P. Melotti, "Azithromycin selectively reduces tumor necrosis factor alpha levels in cystic fibrosis airway epithelial cells," *Antimicrobial Agents and Chemotherapy*, vol. 51, no. 3, pp. 975–981, 2007.

[59] M. Bosnar, S. Čužić, B. Bošnjak et al., "Azithromycin inhibits macrophage interleukin-1β production through inhibition of activator protein-1 in lipopolysaccharide-induced murine pulmonary neutrophilia," *International Immunopharmacology*, vol. 11, no. 4, pp. 424–434, 2011.

[60] K. Otsu, H. Ishinaga, S. Suzuki et al., "Effects of a novel nonantibiotic macrolide, EM900, on cytokine and mucin gene expression in a human airway epithelial cell line," *Pharmacology*, vol. 88, no. 5-6, pp. 3272–332, 2009.

[61] E. Hoffmann, O. Dittrich-Breiholz, H. Holtmann, and M. Kracht, "Multiple control of interleukin-8 gene expression," *Journal of Leukocyte Biology*, vol. 72, no. 5, pp. 847–855, 2002.

[62] C. Cigana, E. Nicolis, M. Pasetto, B. M. Assael, and P. Melotti, "Anti-inflammatory effects of azithromycin in cystic fibrosis airway epithelial cells," *Biochemical and Biophysical Research Communications*, vol. 350, no. 4, pp. 977–982, 2006.

[63] M. Shinkai, M. O. Henke, and B. K. Rubin, "Macrolide antibiotics as immunomodulatory medications: proposed mechanisms of action," *Pharmacology and Therapeutics*, vol. 117, no. 3, pp. 393–405, 2008.

[64] S. Ikegaya, K. Inai, H. Iwasaki, H. Naiki, and T. Ueda, "Azithromycin reduces tumor necrosis factor-alpha production in lipopolysaccharide-stimulated THP-1 monocytic cells by modification of stress response and p38 MAPK pathway," *Journal of Chemotherapy*, vol. 21, no. 4, pp. 396–402, 2009.

[65] K. Yamauchi, Y. Shibata, T. Kimura et al., "Azithromycin suppresses interleukin-12p40 expression in lipopolysaccharide and interferon-γ stimulated macrophages," *International Journal of Biological Sciences*, vol. 5, no. 7, pp. 667–678, 2009.

[66] G. Reato, A. M. Cuffini, V. Tullio et al., "Immunomodulating effect of antimicrobial agents on cytokine production by human polymorphonuclear neutrophils," *International Journal of Antimicrobial Agents*, vol. 23, no. 2, pp. 150–154, 2004.

[67] M. J. Parnham, O. Culić, V. Eraković et al., "Modulation of neutrophil and inflammation markers in chronic obstructive pulmonary disease by short-term azithromycin treatment," *European Journal of Pharmacology*, vol. 517, no. 1-2, pp. 132–143, 2005.

[68] J. Tamaoki, J. Kadota, and H. Takizawa, "Clinical implications of the immunomodulatory effects of macrolides," *The American Journal of Medicine*, vol. 117, supplement 9, pp. 5S–11S, 2004.

[69] C. Feldman and R. Anderson, "The cytoprotective interactions of antibiotics with human ciliated airway epithelium," in *Antibiotics as Anti-Inflammatory and Immunomodulatory Agents*, B. K. Rubin and J. Tamaoki, Eds., pp. 49–63, Birkhauser, Basel, Switzerland, 2005.

[70] H. Oda, J. I. Kadota, S. Kohno, and K. Hara, "Leukotriene B4 in bronchoalveolar lavage fluid of patients with diffuse panbronchiolitis," *Chest*, vol. 108, no. 1, pp. 116–122, 1995.

[71] N. Hashimoto, T. Kawabe, T. Hara et al., "Effect of erythromycin on matrix metalloproteinase-9 and cell migration," *Journal of Laboratory and Clinical Medicine*, vol. 137, no. 3, pp. 176–183, 2001.

[72] K. Kanai, K. Asano, T. Hisamitsu, and H. Suzaki, "Suppresion in matrix metalloproteinase production from nasal fibroblasts by macrolide antibiotics *in vitro*," *European Respiratory Journal*, vol. 23, no. 5, pp. 671–678, 2004.

[73] K. I. Kanai, K. Asano, T. Hisamitsu, and H. Suzaki, "Suppression of matrix metalloproteinase-9 production from neutrophils by a macrolide antibiotic, roxithromycin, *in vitro*," *Mediators of Inflammation*, vol. 13, no. 5-6, pp. 313–319, 2004.

[74] M. Yasutomi, Y. Ohshima, N. Omata et al., "Erythromycin differentially inhibits lipopolysaccharide- or poly(I:C)-induced but not peptidoglycan-induced activation of human monocyte-derived dendritic cells," *Journal of Immunology*, vol. 175, no. 12, pp. 8069–8076, 2005.

[75] J. Y. Park, Y. H. Kim, Y. L. Ja et al., "Macrolide-affected Toll-like receptor 4 expression from *Helicobacter pylori*-infected monocytes does not modify interleukin-8 production," *FEMS Immunology and Medical Microbiology*, vol. 44, no. 2, pp. 171–176, 2005.

[76] T. Mitsuyama, T. Tanaka, K. Hidaka, M. Abe, and N. Hara, "Inhibition by erythromycin of superoxide anion production by human polymorphonuclear leukocytes through the action of cyclic AMP-dependent protein kinase," *Respiration*, vol. 62, no. 5, pp. 269–273, 1995.

[77] R. Anderson, A. J. Theron, and C. Feldman, "Membrane-stabilizing, anti-inflammatory interactions of macrolides with human neutrophils," *Inflammation*, vol. 20, no. 6, pp. 693–705, 1996.

[78] J. P. Montenez, F. Van Bambeke, J. Piret, R. Brasseur, P. M. Tulkens, and M. P. Mingeot-Leclercq, "Interactions of macrolide antibiotics (erythromycin A, roxithromycin, erythromycylamine [dirithromycin], and azithromycin) with phospholipids: computer-aided conformational analysis and studies on acellular and cell culture models," *Toxicology and Applied Pharmacology*, vol. 156, no. 2, pp. 129–140, 1999.

[79] V. Munić, M. Banjanac, S. Koštrun et al., "Intensity of macrolide anti-inflammatory activity in J774A.1 cells positively correlates with cellular accumulation and phospholipidosis," *Pharmacological Research*, vol. 64, no. 3, pp. 298–307, 2011.

[80] J. Tamaoki, M. Kondo, K. Kohri, K. Aoshiba, E. Tagaya, and A. Nagai, "Macrolide antibiotics protect against immune complex-induced lung injury in rats: role of nitric oxide from alveolar macrophages," *Journal of Immunology*, vol. 163, no. 5, pp. 2909–2915, 1999.

[81] K. Asano, K. Kamakazu, T. Hisamitsu, and H. Suzaki, "Suppressive activity of macrolide antibiotics on nitric oxide production from nasal polyp fibroblasts *in vitro*," *Acta Oto-Laryngologica*, vol. 123, no. 9, pp. 1064–1069, 2003.

[82] R. Anderson, G. Tintinger, R. Cockeran, M. Potjo, and C. Feldman, "Beneficial and harmful interactions of antibiotics with microbial pathogens and the host innate immune system," *Pharmaceuticals*, vol. 3, no. 5, pp. 1694–1710, 2010.

[83] K. Takeyama, J. Tamaoki, A. Chiyotani, E. Tagaya, and K. Konno, "Effect of macrolide antibiotics on ciliary motility in rabbit airway epithelium in-vitro," *Journal of Pharmacy and Pharmacology*, vol. 45, no. 8, pp. 756–758, 1993.

[84] C. Feldman and R. Anderson, "Non-antimicrobial activity of macrolides: therapeutic potential in chronic inflammatory airway disorders," *South African Journal of Epidemiology Infections*, vol. 24, no. 4, pp. 21–26, 2009.

[85] T. Shimizu, S. Shimizu, R. Hattori, E. C. Gabazza, and Y. Majima, "*In vivo* and *in vitro* effects of macrolide antibiotics on mucus secretion in airway epithelial cells," *American Journal of Respiratory and Critical Care Medicine*, vol. 168, no. 5, pp. 581–587, 2003.

[86] C. M. P. Ribeiro, H. Hurd, Y. Wu et al., "Azithromycin treatment alters gene expression in inflammatory, lipid metabolism, and cell cycle pathways in well-differentiated human airway epithelia," *PLoS ONE*, vol. 4, no. 6, Article ID e5806, 2009.

[87] T. Tanabe, S. Kanoh, K. Tsushima et al., "Clarithromycin inhibits interleukin-13-induced goblet cell hyperplasia in human airway cells," *American Journal of Respiratory Cell and Molecular Biology*, vol. 45, no. 5, pp. 1075–1083, 2011.

[88] Y. Imamura, K. Yanagihara, Y. Mizuta et al., "Azithromycin inhibits MUC5AC production induced by the *Pseudomonas aeruginosa* autoinducer N-(3-oxododecanoyl) homoserine lactone in NCI-H292 cells," *Antimicrobial Agents and Chemotherapy*, vol. 48, no. 9, pp. 3457–3461, 2004.

[89] C. Feldman, R. Anderson, A. J. Theron, G. Ramafi, P. J. Cole, and R. Wilson, "Roxithromycin, clarithromycin, and azithromycin attenuate the injurious effects of bioactive phospholipids on human respiratory epithelium *in vitro*," *Inflammation*, vol. 21, no. 6, pp. 655–665, 1997.

[90] L. Wu, W. Zhang, L. Tian, K. Bao, P. Li, and J. Lin, "Immunomodulatory effects of erythromycin and its derivatives on human T-lymphocyte *in vitro*," *Immunopharmacology and Immunotoxicology*, vol. 29, no. 3-4, pp. 587–596, 2007.

[91] Y. Ishida, Y. Abe, and Y. Harabuchi, "Effects of macrolides on antigen presentation and cytokine production by dendritic cells and T lymphocytes," *International Journal of Pediatric Otorhinolaryngology*, vol. 71, no. 2, pp. 297–305, 2007.

[92] Y. Hiwatashi, M. Maeda, H. Fukushima et al., "Azithromycin suppresses proliferation, interleukin production and mitogen-activated protein kinases in human peripheral-blood mononuclear cells stimulated with bacterial superantigen," *Journal of Pharmacy and Pharmacology*, vol. 63, no. 10, pp. 1320–1326, 2011.

[93] T. Noma, K. Aoki, M. Hayashi, I. Yoshizawa, and Y. Kawano, "Effect of roxithromycin on T lymphocyte proliferation and cytokine production elicited by mite antigen," *International Immunopharmacology*, vol. 1, no. 2, pp. 201–210, 2001.

[94] A. L. Pukhalsky, G. V. Shmarina, N. I. Kapranov, S. N. Kokarovtseva, D. Pukhalskaya, and N. J. Kashirskaja, "Anti-inflammatory and immunomodulating effects of clarithromycin in patients with cystic fibrosis lung disease," *Mediators of Inflammation*, vol. 13, no. 2, pp. 111–117, 2004.

[95] K. Asano, K. Kamakazu, T. Hisamitsu, and H. Suzaki, "Modulation of Th2 type cytokine production from human peripheral blood leukocytes by a macrolide antibiotic, roxithromycin, *in vitro*," *International Immunopharmacology*, vol. 1, no. 11, pp. 1913–1921, 2001.

[96] A. C. Williams, H. F. Galley, A. M. Watt, and N. R. Webster, "Differential effects of three antibiotics on T helper cell cytokine expression," *Journal of Antimicrobial Chemotherapy*, vol. 56, no. 3, pp. 502–506, 2005.

[97] S. J. Park, Y. C. Lee, Y. K. Rhee, and H. B. Lee, "The effect of long-term treatment with erythromycin on Th1 and Th2 cytokines in diffuse panbronchiolitis," *Biochemical and Biophysical Research Communications*, vol. 324, no. 1, pp. 114–117, 2004.

[98] K. Morikawa, J. Zhang, M. Nonaka, and S. Morikawa, "Modulatory effect of macrolide antibiotics on the Th1- and Th2-type cytokine production," *International Journal of Antimicrobial Agents*, vol. 19, no. 1, pp. 53–59, 2002.

[99] T. Ito, N. Ito, H. Hashizume, and M. Takigawa, "Roxithromycin inhibits chemokine-induced chemotaxis of Th1 and Th2 cells but regulatory T cells," *Journal of Dermatological Science*, vol. 54, no. 3, pp. 185–191, 2009.

[100] Y. Ishimatsu, J. I. Kadota, T. Iwashita et al., "Macrolide antibiotics induce apoptosis of human peripheral lymphocytes *in vitro*," *International Journal of Antimicrobial Agents*, vol. 24, no. 3, pp. 247–253, 2004.

[101] S. Mizunoe, J. I. Kadota, I. Tokimatsu, K. Kishi, H. Nagai, and M. Nasu, "Clarithromycin and azithromycin induce apoptosis of activated lymphocytes via down-regulation of Bcl-xL," *International Immunopharmacology*, vol. 4, no. 9, pp. 1201–1207, 2004.

[102] J. I. Kadota, S. Mizunoe, K. Kishi, I. Tokimatsu, H. Nagai, and M. Nasu, "Antibiotic-induced apoptosis in human activated peripheral lymphocytes," *International Journal of Antimicrobial Agents*, vol. 25, no. 3, pp. 216–220, 2005.

[103] K. Asano, M. Suzuki, T. Shimane, and H. Suzaki, "Suppression of co-stimulatory molecule expressions on splenic B lymphocytes by a macrolide antibiotic, roxithromycin *in vitro*," *International Immunopharmacology*, vol. 1, no. 7, pp. 1385–1392, 2001.

[104] Y. Aoki and P. N. Kao, "Erythromycin inhibits transcriptional activation of NF-κB, but not NFAT, through calcineurin-independent signaling in T cells," *Antimicrobial Agents and Chemotherapy*, vol. 43, no. 11, pp. 2678–2684, 1999.

[105] S. Kanoh and B. K. Rubin, "Mechanisms of action and clinical application of macrolides as immunomodulatory medications," *Clinical Microbiology Reviews*, vol. 23, no. 3, pp. 590–615, 2010.

[106] R. A. Fecik, P. L. Nguyen, and L. Venkatraman, "Approaches to the synthesis of immunolides: selective immunomodulatory macrolides for cystic fibrosis," *Current Opinion in Drug Discovery and Development*, vol. 8, no. 6, pp. 741–747, 2005.

[107] A. Mereu, E. Moriggi, M. Napoletano et al., "Design, synthesis and *in vivo* activity of 9-(S)-dihydroerythromycin derivatives as potent anti-inflammatory agents," *Bioorganic and Medicinal Chemistry Letters*, vol. 16, no. 22, pp. 5801–5804, 2006.

[108] A. Sugawara, A. Sueki, T. Hirose et al., "Novel 12-membered non-antibiotic macrolides from erythromycin A; EM900 series as novel leads for anti-inflammatory and/or immunomodulatory agents," *Bioorganic & Medicinal Chemistry Letters*, vol. 21, no. 11, pp. 3373–3376, 2011.

[109] M. H. Gotfried, "Macrolides for the treatment of chronic sinusitis, asthma, and COPD," *Chest*, vol. 125, no. 2, supplement, pp. 52S–61S, 2004.

[110] P. A. J. Crosbie and M. A. Woodhead, "Long-term macrolide therapy in chronic inflammatory airway diseases," *European Respiratory Journal*, vol. 33, no. 1, pp. 171–181, 2009.

[111] A. L. Friedlander and R. K. Albert, "Chronic macrolide therapy in inflammatory airways diseases," *Chest*, vol. 138, no. 5, pp. 1202–1212, 2010.

[112] P. Zarogoulidis, N. Papanas, I. Kioumis, E. Chatzaki, E. Maltezos, and K. Zarogoulidis, "Macrolides: from *in vitro* anti-inflammatory and immunomodulatory properties to clinical practice in respiratory diseases," *European Journal of Clinical Pharmacology*, vol. 68, no. 5, pp. 479–503, 2012.

[113] K. Tateda, Y. Ishii, S. Kimura, M. Horikawa, S. Miyairi, and K. Yamaguchi, "Suppression of *Pseudomonas aeruginosa* quorum-sensing systems by macrolides: a promising strategy or an oriental mystery?" *Journal of Infection and Chemotherapy*, vol. 13, no. 6, pp. 357–367, 2007.

[114] E. J. Giamarellos-Bourboulis, "Macrolides beyond the conventional antimicrobials: a class of potent immunomodulators," *International Journal of Antimicrobial Agents*, vol. 31, no. 1, pp. 12–20, 2008.

[115] J. Altenburg, C. S. de Graaff, T. S. van der Werf, and W. G. Boersma, "Immunomodulatory effects of macrolide antibiotics—part 2: advantages and disadvantages of long-term, low-dose macrolide therapy," *Respiration*, vol. 81, no. 1, pp. 75–87, 2010.

[116] L. Guillot, O. Tabary, N. Nathan, H. Corvol, and A. Clement, "Macrolides: new therapeutic perspectives in lung diseases," *International Journal of Biochemistry and Cell Biology*, vol. 43, no. 9, pp. 1241–1246, 2011.

[117] H. Nagai, H. Shishido, R. Yoneda, E. Yamaguchi, A. Tamura, and A. Kurashima, "Long-term low-dose administration of erythromycin to patients with diffuse panbronchiolitis," *Respiration*, vol. 58, no. 3-4, pp. 145–149, 1991.

[118] H. Kobayashi, N. Ohgaki, and H. Takeda, "Therapeutic possibilities for diffuse panbronchiolitis," *International Journal of Antimicrobial Agents*, vol. 3, no. 1, pp. S81–S86, 1993.

[119] H. Koyama and D. M. Geddes, "Erythromycin and diffuse panbronchiolitis," *Thorax*, vol. 52, no. 10, pp. 915–918, 1997.

[120] S. Kudoh, "Erythromycin treatment in diffuse panbronchiolitis," *Current Opinion in Pulmonary Medicine*, vol. 4, no. 2, pp. 116–121, 1998.

[121] S. Kudoh, A. Azuma, M. Yamamoto, T. Izumi, and M. Ando, "Improvement of survival in patients with diffuse panbronchiolitis treated with low-dose erythromycin," *American Journal of Respiratory and Critical Care Medicine*, vol. 157, no. 6, pp. 1829–1832, 1998.

[122] K. Yanagihara, J. Kadoto, and S. Kohno, "Diffuse panbronchiolitis—pathophysiology and treatment mechanisms," *International Journal of Antimicrobial Agents*, vol. 18, no. 1, pp. S83–S87, 2001.

[123] N. Keicho and S. Kudoh, "Diffuse panbronchiolitis: role of macrolides in therapy," *American Journal of Respiratory Medicine*, vol. 1, no. 2, pp. 119–131, 2002.

[124] J. Kadota, H. Mukae, H. Ishii et al., "Long-term efficacy and safety of clarithromycin treatment in patients with diffuse panbronchiolitis," *Respiratory Medicine*, vol. 97, no. 7, pp. 844–850, 2003.

[125] S. Kudoh, "Applying lessons learned in the treatment of diffuse panbronchiolitis to other chronic inflammatory diseases," *The American Journal of Medicine*, vol. 117, supplement 9, pp. 12S–19S, 2004.

[126] V. Poletti, M. Chilosi, G. Casoni, and T. V. Colby, "Diffuse panbronchiolitis," *Sarcoidosis Vasculitis and Diffuse Lung Diseases*, vol. 21, no. 2, pp. 94–104, 2004.

[127] M. J. Schultz, "Macrolide activities beyond their antimicrobial effects: macrolides in diffuse panbronchiolitis and crystic fibrosis," *Journal of Antimicrobial Chemotherapy*, vol. 54, no. 1, pp. 21–28, 2004.

[128] A. Azuma and S. Kudoh, "Diffuse panbronchiolitis in East Asia," *Respirology*, vol. 11, no. 3, pp. 249–261, 2006.

[129] V. Poletti, G. Casoni, M. Chilosi, and M. Zompatori, "Diffuse panbronchiolitis," *European Respiratory Journal*, vol. 28, no. 4, pp. 862–871, 2006.

[130] M. Yang, B. R. Dong, J. Lu, X. Lin, and H. M. Wu, "Macrolides for diffuse panbronchiolitis," *Cochrane Database of Systematic Reviews (Online)*, vol. 12, Article ID CD007716, 2010.

[131] A. Jaffè, "The anti-inflammatory effects of macrolides in cystic fibrosis," *Japanese Journal of Antibiotics*, vol. 54, supplement, pp. 77–82, 2001.

[132] A. Equi, I. M. Balfour-Lynn, A. Bush, and M. Rosenthal, "Long term azithromycin in children with cystic fibrosis: a randomised, placebo-controlled crossover trial," *The Lancet*, vol. 360, no. 9338, pp. 978–984, 2002.

[133] T. Nguyen, S. G. Louie, P. M. Beringer, and M. A. Gill, "Potential role of macrolide antibiotics in the management of cystic fibrosis lung disease," *Current Opinion in Pulmonary Medicine*, vol. 8, no. 6, pp. 521–528, 2002.

[134] J. Wolter, S. Seeney, S. Bell, S. Bowler, P. Masel, and J. McCormack, "Effect of long term treatment with azithromycin on disease parameters in cystic fibrosis: a randomised trial," *Thorax*, vol. 57, no. 3, pp. 212–216, 2002.

[135] J. M. Wolter, S. L. Seeney, and J. G. McCormack, "Macrolides in cystic fibrosis: is there a role?" *American Journal of Respiratory Medicine*, vol. 1, no. 4, pp. 235–241, 2002.

[136] L. Saiman, B. C. Marshall, N. Mayer-Hamblett et al., "Azithromycin in patient with cystic fibrosis chronically infected with *Pseudomonas aeruginosa*: a randomized controlled trial," *JAMA: Journal of the American Medical Association*, vol. 290, no. 13, pp. 1749–1756, 2003.

[137] M. H. Schöni, "Macrolide antibiotic therapy in patients with cystic fibrosis," *Swiss Medical Weekly*, vol. 133, no. 21-22, pp. 297–301, 2003.

[138] B. K. Rubin and M. O. Henke, "Immunomodulatory activity and effectiveness of macrolides in chronic airway disease," *Chest*, vol. 125, no. 2, supplement, pp. 70S–78S, 2004.

[139] L. Saiman, "The use of macrolide antibiotics in patients with cystic fibrosis," *Current Opinion in Pulmonary Medicine*, vol. 10, no. 6, pp. 515–523, 2004.

[140] S. C. Bell, S. L. Senini, and J. G. McCormack, "Macrolides in cystic fibrosis," *Chronic Respiratory Disease*, vol. 2, no. 2, pp. 85–98, 2005.

[141] R. Dinwiddie, "Anti-inflammatory therapy in cystic fibrosis," *Journal of Cystic Fibrosis*, vol. 4, no. 2, supplement, pp. 45–48, 2005.

[142] C. R. Hansen, T. Pressler, C. Koch, and N. Høiby, "Long-term azitromycin treatment of cystic fibrosis patients with chronic *Pseudomonas aeruginosa* infection; an observational cohort study," *Journal of Cystic Fibrosis*, vol. 4, no. 1, pp. 35–40, 2005.

[143] W. A. Prescott Jr. and G. E. Johnson, "Antiinflammatory therapies for cystic fibrosis: past, present, and future," *Pharmacotherapy*, vol. 25, no. 4, pp. 555–573, 2005.

[144] A. Clement, A. Tamalet, E. Leroux, S. Ravilly, B. Fauroux, and J. P. Jais, "Long term effects of azithromycin in patients with cystic fibrosis: a double blind, placebo controlled trial," *Thorax*, vol. 61, no. 10, pp. 895–902, 2006.

[145] J. R. McArdle and J. S. Talwalkar, "Macrolides in cystic fibrosis," *Clinics in Chest Medicine*, vol. 28, no. 2, pp. 347–360, 2007.

[146] J. McCormack, S. Bell, S. Senini et al., "Daily versus weekly azithromycin in cystic fibrosis patients," *European Respiratory Journal*, vol. 30, no. 3, pp. 487–495, 2007.

[147] M. E. Skindersoe, M. Alhede, R. Phipps et al., "Effects of antibiotics on quorum sensing in *Pseudomonas aeruginosa*," *Antimicrobial Agents and Chemotherapy*, vol. 52, no. 10, pp. 3648–3663, 2008.

[148] G. Steinkamp, S. Schmitt-Grohe, G. Döring et al., "Once-weekly azithromycin in cystic fibrosis with chronic

Pseudomonas aeruginosa infection," *Respiratory Medicine*, vol. 102, no. 11, pp. 1643–1653, 2008.

[149] D. F. Florescu, P. J. Murphy, and A. C. Kalil, "Effects of prolonged use of azithromycin in patients with cystic fibrosis: a meta-analysis," *Pulmonology, Pharmacology and Therapy*, vol. 22, no. 6, pp. 467–472, 2009.

[150] C. Winstanley and J. L. Fothergill, "The role of quorum sensing in chronic cystic fibrosis *Pseudomonas aeruginosa* infections," *FEMS Microbiology Letters*, vol. 290, no. 1, pp. 1–9, 2009.

[151] S. K. Kabra, R. Pawaiya, R. Lodha et al., "Long-term daily high and low doses of azithromycin in children with cystic fibrosis: a randomized controlled trial," *Journal of Cystic Fibrosis*, vol. 9, no. 1, pp. 17–23, 2010.

[152] A. A. Yousef and A. Jaffe, "The role of azithromycin in patients with cystic fibrosis," *Paediatric Respiratory Reviews*, vol. 11, no. 2, pp. 108–114, 2010.

[153] K. W. Southern, P. M. Barker, A. Solis-Moya, and L. Patel, "Macrolide antibiotics for cystic fibrosis," *Cochrane Database Systematic Reviews*, vol. 12, Article ID CD002203, 2011.

[154] M. Mikami, "Clinical and pathophysiological significance of neutrophil elastase in sputum and the effect of erythromycin in chronic respiratory diseases," *Nihon Kyobu Shikkan Gakkai Zasshi*, vol. 29, no. 1, pp. 72–83, 1991.

[155] N. Ohgaki, "Bacterial biofilm in chronic airway infection," *Kansenshogaku zasshi*, vol. 68, no. 1, pp. 138–151, 1994.

[156] K. W. T. Tsang, P. Roberts, R. C. Read, F. Kees, R. Wilson, and P. J. Cole, "The concentrations of clarithromycin and its 14-hydroxy metabolite in sputum of patients with bronchiectasis following single dose oral administration," *Journal of Antimicrobial Chemotherapy*, vol. 33, no. 2, pp. 289–297, 1994.

[157] P. J. Cole, "Bronchiectasis," in *Respiratory Medicine*, R. A. L. Brewis, B. Corrin, D. M. Geddes, and G. J. Gibson, Eds., pp. 1286–1317, W. B. Saunders, London, UK, 2nd edition, 1995.

[158] Y. Y. Koh, M. H. Lee, Y. H. Sun, K. W. Sung, and J. H. Chae, "Effect of roxithromycin on airway responsiveness in children with bronchiectasis: a double-blind, placebo-controlled study," *European Respiratory Journal*, vol. 10, no. 5, pp. 994–999, 1997.

[159] H. Nakamura, S. Fujishima, T. Inoue et al., "Clinical and immunoregulatory effects of roxithromycin therapy for chronic respiratory tract infection," *European Respiratory Journal*, vol. 13, no. 6, pp. 1371–1379, 1999.

[160] K. W. T. Tsang, P. I. Ho, K. N. Chan et al., "A pilot study of low-dose erythromycin in bronchiectasis," *European Respiratory Journal*, vol. 13, no. 2, pp. 361–364, 1999.

[161] M. Gorrini, A. Lupi, S. Viglio et al., "Inhibition of human neutrophil elastase by erythromycin and flurythromycin, two macrolide antibiotics," *American Journal of Respiratory Cell and Molecular Biology*, vol. 25, no. 4, pp. 492–499, 2001.

[162] A. Jaff and A. Bush, "Anti-inflammatory effects of macrolides in lung disease," *Pediatric Pulmonology*, vol. 31, no. 6, pp. 464–473, 2001.

[163] Y. Shibuya, P. J. Wills, and P. J. Cole, "The effect of erythromycin on mucociliary transportability and rheology of cystic fibrosis and bronchiectasis sputum," *Respiration*, vol. 68, no. 6, pp. 615–619, 2001.

[164] E. Tagaya, J. Tamaoki, M. Kondo, and A. Nagai, "Effect of a short course of clarithromycin therapy on sputum production in patients with chronic airway hypersecretion," *Chest*, vol. 122, no. 1, pp. 213–218, 2002.

[165] A. Bush and B. K. Rubin, "Macrolides as biological response modifiers in cystic fibrosis and bronchiectasis," *Seminars in Respiratory and Critical Care Medicine*, vol. 24, no. 6, pp. 737–747, 2003.

[166] G. Davies and R. Wilson, "Prophylactic antibiotic treatment of bronchiectasis with azithromycin," *Thorax*, vol. 59, no. 6, pp. 540–541, 2004.

[167] A. A. Cymbala, L. C. Edmonds, M. A. Bauer et al., "The disease-modifying effects of twice-weekly oral azithromycin in patients with bronchiectasis," *Treatments in Respiratory Medicine*, vol. 4, no. 2, pp. 117–122, 2005.

[168] L. Máiz Carro, "Long-term treatment with azithromycin in a patient with idiopathic bronchiectasis," *Archivos de Bronconeumologia*, vol. 41, no. 5, p. 295, 2005.

[169] G. M. Verleden, L. J. Dupont, J. Vanhaecke, W. Daenen, and D. E. M. Van Raemdonck, "Effect of azithromycin on bronchiectasis and pulmonary function in a heart-lung transplant patient with severe chronic allograft dysfunction: a case report," *Journal of Heart and Lung Transplantation*, vol. 24, no. 8, pp. 1155–1158, 2005.

[170] E. Yalcin, N. Kiper, and U. Ozcelik, "Effects of clarithromycin on inflammatory parameters and clinical conditions in children with bronchiectasis," *Journal of Clinical Pharmacology and Therapeutics*, vol. 31, no. 1, pp. 49–55, 2006.

[171] M. Vila-Justribo, J. Dorca-Sargatal, and S. Bello-Dronda, "Bronchiectasis and macrolides," *Archivos de Bronconeumologia*, vol. 42, no. 4, p. 206, 2006.

[172] P. King, "Is there a role for inhaled corticosteroids and macrolide therapy in bronchiectasis?" *Drugs*, vol. 67, no. 7, pp. 965–974, 2007.

[173] G. A. Anwar, S. C. Bourke, G. Afolabi, P. Middleton, C. Ward, and R. M. Rutherford, "Effects of long-term low-dose azithromycin in patients with non-CF bronchiectasis," *Respiratory Medicine*, vol. 102, no. 10, pp. 1494–1496, 2008.

[174] K. W. Tsang and D. Bilton, "Clinical challenges in managing bronchiectasis series," *Respirology*, vol. 14, no. 5, pp. 637–650, 2009.

[175] M. L. Metersky, "New treatment options for bronchiectasis," *Therapeutic Advances in Respiratory Disease*, vol. 4, no. 2, pp. 93–99, 2010.

[176] M. Bochet, N. Garin, J. P. Janssens, and E. Gerstel, "Is there a role for prophylactic antibiotic treatment with macrolides in bronchiectasis?" *Revue Medicale Suisse*, vol. 7, no. 280, pp. 308–312, 2011.

[177] D. J. Serisier and M. L. Martin, "Long-term, low-dose erythromycin in bronchiectasis subjects with frequent infective exacerbations," *Respiratory Medicine*, vol. 105, no. 6, pp. 946–949, 2011.

[178] S. G. Gerhardt, J. F. McDyer, R. E. Girgis, J. V. Conte, S. C. Yang, and J. B. Orens, "Maintenance azithromycin therapy for bronchiolitis obliterans syndrome: results of a pilot study," *American Journal of Respiratory and Critical Care Medicine*, vol. 168, no. 1, pp. 121–125, 2003.

[179] G. M. Verleden and L. J. Dupont, "Azithromycin therapy for patients with bronchiolitis obliterans syndrome after lung transplantation," *Transplantation*, vol. 77, no. 9, pp. 1465–1467, 2004.

[180] S. Crowley and J. J. Egan, "Macrolide antibiotics and bronchiolitis obliterans following lung transplantation," *Expert Review of Anti-Infective Therapy*, vol. 3, no. 6, pp. 923–930, 2005.

[181] B. Yates, D. M. Murphy, I. A. Forrest et al., "Azithromycin reverses airflow obstruction in established bronchiolitis obliterans syndrome," *American Journal of Respiratory and Critical Care Medicine*, vol. 172, no. 6, pp. 772–775, 2005.

[182] A. M. Fietta and F. Meloni, "Lung transplantation: the role of azithromycin in the management of patients with bronchiolitis obliterans syndrome," *Current Medicinal Chemistry*, vol. 15, no. 7, pp. 716–723, 2008.

[183] J. Gottlieb, J. Szangolies, T. Koehnlein, H. Golpon, A. Simon, and T. Welte, "Long-term azithromycin for bronchiolitis obliterans syndrome after lung transplantation," *Transplantation*, vol. 85, no. 1, pp. 36–41, 2008.

[184] N. R. Porhownik, W. Batobara, W. Kepron, H. W. Unruh, and Z. Bshouty, "Effect of maintenance azithromycin on established bronchiolits obliterans syndrome in lung transplant patients," *Canadian Respiratory Journal*, vol. 15, no. 4, pp. 199–202, 2008.

[185] B. M. Vanaudenaerde, I. Meyts, R. Vos et al., "A dichotomy in bronchiolitis obliterans syndrome after lung transplantation revealed by azithromycin therapy," *European Respiratory Journal*, vol. 32, no. 4, pp. 832–843, 2008.

[186] C. Benden and A. Boehler, "Long-term clarithromycin therapy in the management of lung transplant recipients," *Transplantation*, vol. 87, no. 10, pp. 1538–1540, 2009.

[187] N. Maimon, J. H. Lipton, C. K. N. Chan, and T. K. Marras, "Macrolides in the treatment of bronchiolitis obliterans in allograft recipients," *Bone Marrow Transplantation*, vol. 44, no. 2, pp. 69–73, 2009.

[188] R. Jain, R. R. Hachem, M. R. Morrell et al., "Azithromycin is associated with increased survival in lung transplant recipients with bronchiolitis obliterans syndrome," *Journal of Heart and Lung Transplantation*, vol. 29, no. 5, pp. 531–537, 2010.

[189] R. Vos, B. M. Vanaudenaerde, A. Ottevaere et al., "Long-term azithromycin therapy for bronchiolitis obliterans syndrome: divide and conquer?" *Journal of Heart and Lung Transplantation*, vol. 29, no. 12, pp. 1358–1368, 2010.

[190] A. J. Fisher, "Azithromycin and bronchiolitis obliterans syndrome after lung transplantation: is prevention better than cure?" *European Respiratory Journal*, vol. 37, no. 1, pp. 10–12, 2011.

[191] R. Vos, B. M. Vanaudenaerde, S. E. Verleden et al., "A randomised controlled trial of azithromycin to prevent chronic rejection after lung transplantation," *European Respiratory Journal*, vol. 37, no. 1, pp. 164–172, 2011.

[192] J. Tamaoki, K. Takeyama, E. Tagaya, and K. Konno, "Effect of clarithromycin on sputum production and its rheological properties in chronic respiratory tract infections," *Antimicrobial Agents and Chemotherapy*, vol. 39, no. 8, pp. 1688–1690, 1995.

[193] W. E. Swords and B. K. Rubin, "Macrolide antibiotics, bacterial populations and inflammatory airway disease," *The Netherlands Journal of Medicine*, vol. 61, no. 7, pp. 242–248, 2003.

[194] D. Banerjee, D. Honeybourne, and O. A. Khair, "The effect of oral clarithromycin on bronchial airway inflammation in moderate-to-severe stable COPD: a randomized controlled trial," *Treatments in Respiratory Medicine*, vol. 3, no. 1, pp. 59–65, 2004.

[195] I. Basyigit, F. Yildiz, S. K. Ozkara, E. Yildirim, H. Boyaci, and A. Ilgazli, "The effect of clarithromycin on inflammatory markers in chronic obstructive pulmonary disease: preliminary data," *Annals of Pharmacotherapy*, vol. 38, no. 9, pp. 1400–1405, 2004.

[196] P. J. Barnes, "Mediators of chronic obstructive pulmonary disease," *Pharmacological Reviews*, vol. 56, no. 4, pp. 515–548, 2004.

[197] D. Banerjee, O. A. Khair, and D. Honeybourne, "The effect of oral clarithromycin on health status and sputum bacteriology in stable COPD," *Respiratory Medicine*, vol. 99, no. 2, pp. 208–215, 2005.

[198] W. R. Bishai, "Macrolide immunomodulatory effects and symptom resolution in acute exacerbation of chronic bronchitis and acute maxillary sinusitis: a focus on clarithromycin," *Expert Review of Anti-Infective Therapy*, vol. 4, no. 3, pp. 405–416, 2006.

[199] D. M. Murphy, I. A. Forrest, D. Curran, and C. Ward, "Macrolide antibiotics and the airway: antibiotic or non-antibiotic effects?" *Expert Opinion on Investigational Drugs*, vol. 19, no. 3, pp. 401–414, 2010.

[200] H. Amayasu, S. Yoshida, S. Ebana et al., "Clarithromycin suppresses bronchial hyperresponsiveness associated with eosinophilic inflammation in patients with asthma," *Annals of Allergy, Asthma and Immunology*, vol. 84, no. 6, pp. 594–598, 2000.

[201] M. Cazzola, A. Salzillo, and F. Diamare, "Potential role of macrolides in the treatment of asthma," *Monaldi Archives for Chest Disease*, vol. 55, no. 3, pp. 231–236, 2000.

[202] A. Ekici, M. Ekici, and A. Kemal Erdemoğlu, "Effect of azithromycin on the severity of bronchial hyperresponsiveness in patients with mild asthma," *Journal of Asthma*, vol. 39, no. 2, pp. 181–185, 2002.

[203] D. A. Beuther and R. J. Martin, "Antibiotics in asthma," *Current Allergy and Asthma Reports*, vol. 4, no. 2, pp. 132–138, 2004.

[204] U. Hatipoğlu and I. Rubinstein, "Low-dose, long-term macrolide therapy in asthma: an overview," *Clinical and Molecular Allergy*, vol. 2, no. 1, article 4, 2004.

[205] E. Kostadima, S. Tsiodras, E. I. Alexopoulos et al., "Clarithromycin reduces the severity of bronchial hyperresponsiveness in patients with asthma," *European Respiratory Journal*, vol. 23, no. 5, pp. 714–717, 2004.

[206] G. Ferrara, M. Losi, F. Franco, L. Corbetta, L. M. Fabbri, and L. Richeldi, "Macrolides in the treatment of asthma and cystic fibrosis," *Respiratory Medicine*, vol. 99, no. 1, pp. 1–10, 2005.

[207] L. Richeldi, G. Ferrara, L. M. Fabbri, T. J. Lasserson, and P. G. Gibson, "Macrolides for chronic asthma," *Cochrane Database of Systematic Reviews (Online)*, vol. 4, no. 3, Article ID CD002997, 2005.

[208] L. Richeldi, G. Ferrara, L. M. Fabbri, T. J. Lasserson, and P. G. Gibson, "Macrolides for chronic asthma," *Cochrane Database of Systematic Reviews (Online)*, vol. 3, Article ID CD002997, 2005.

[209] P. N. Black, "Antibiotics for the treatment of asthma," *Current Opinion in Pharmacology*, vol. 7, no. 3, pp. 266–271, 2007.

[210] S. Sharma, A. Jaffe, and G. Dixon, "Immunomodulatory effects of macrolide antibiotics in respiratory disease: therapeutic implications for asthma and cystic fibrosis," *Pediatric Drugs*, vol. 9, no. 2, pp. 107–118, 2007.

[211] V. Hernando-Sastre, "Macrolide antibiotics in the treatment of asthma. An update," *Allergologia et Immunopathologia*, vol. 38, no. 2, pp. 92–98, 2010.

[212] D. R. Rollins, D. A. Beuther, and R. J. Martin, "Update on infection and antibiotics in asthma," *Current Allergy and Asthma Reports*, vol. 10, no. 1, pp. 67–73, 2010.

[213] R. Oliveinstein, H. A. Jahdali, N. Alkhamis, R. Halwani, S. Al-Muhsen, and Q. Hamid, "Challenges in the management of severe asthma: role of current and future therapies," *Current Pharmaceutical Design*, vol. 17, no. 7, pp. 703–711, 2011.

[214] J. T. Good Jr., D. R. Rollins, and R. J. Martin, "Macrolides in the treatment of asthma," *Current Opinion in Pulmonary Medicine*, vol. 18, no. 1, pp. 76–84, 2012.

[215] P. P. Gleason, T. P. Meehan, J. M. Fine, D. H. Galusha, and M. J. Fine, "Associations between initial antimicrobial therapy and medical outcomes for hospitalized elderly patients with pneumonia," *Archives of Internal Medicine*, vol. 159, no. 21, pp. 2562–2572, 1999.

[216] G. W. Waterer, G. W. Somes, and R. G. Wunderink, "Monotherapy may be suboptimal for severe bacteremic pneumococcal pneumonia," *Archives of Internal Medicine*, vol. 161, no. 15, pp. 1837–1842, 2001.

[217] R. B. Brown, P. Iannini, P. Gross, and M. Kunkel, "Impact of initial antibiotic choice on clinical outcomes in community-acquired pneumonia: analysis of a hospital claims-made database," *Chest*, vol. 123, no. 5, pp. 1503–1511, 2003.

[218] J. A. Martinez, J. P. Horcajada, M. Almela et al., "Addition of a macrolide to a beta-lactam-based empirical antibiotic regimen is associated with lower in-hospital mortality for patients with bacteremic pneumococcal pneumonia," *Clinical Infectious Diseases*, vol. 36, no. 4, pp. 389–395, 2003.

[219] L. M. Baddour, V. L. Yu, K. P. Klugman et al., "Combination antibiotic therapy lowers mortality among severely ill patients with pneumococcal bacteremia," *American Journal of Respiratory and Critical Care Medicine*, vol. 170, no. 4, pp. 440–444, 2004.

[220] E. M. Mortensen, M. I. Restrepo, A. Anzueto, and J. Pugh, "The impact of empiric antimicrobial therapy with a β-lactam and fluoroquinolone on mortality for patients hospitalized with severe pneumonia," *Critical Care*, vol. 10, no. 1, article R8, 2005.

[221] M. L. Metersky, A. Ma, P. M. Houck, and D. W. Bratzler, "Antibiotics for bacteremic pneumonia: improved outcomes with macrolides but not fluoroquinolones," *Chest*, vol. 131, no. 2, pp. 466–473, 2007.

[222] A. Rodriguez, A. Mendia, J. M. Sirvent et al., "Combination antibiotic therapy improves survival in patients with community-acquired pneumonia and shock," *Critical Care Medicine*, vol. 35, no. 6, pp. 1493–1498, 2007.

[223] C. Feldman and R. Anderson, "Therapy for pneumococcal bacteremia: monotherapy or combination therapy?" *Current Opinion in Infectious Diseases*, vol. 22, no. 2, pp. 137–142, 2009.

[224] M. I. Restrepo, E. M. Mortensen, G. W. Waterer, R. G. Wunderink, J. J. Coalson, and A. Anzueto, "Impact of macrolide therapy on mortality for patients with severe sepsis due to pneumonia," *European Respiratory Journal*, vol. 33, no. 1, pp. 153–159, 2009.

[225] A. Tessmer, T. Welte, P. Martus, M. Schnoor, R. Marre, and N. Suttorp, "Impact of intravenous β-lactam/macrolide versus β-lactam monotherapy on mortality in hospitalized patients with community-acquired pneumonia," *Journal of Antimicrobial Chemotherapy*, vol. 63, no. 5, pp. 1025–1033, 2009.

[226] I. Martin-Loeches, T. Lisboa, A. Rodriguez et al., "Combination antibiotic therapy with macrolides improves survival in intubated patients with community-acquired pneumonia," *Intensive Care Medicine*, vol. 36, no. 4, pp. 612–620, 2010.

[227] D. W. Bratzler, A. Ma, and W. Nsa, "Initial antibiotic selection and patient outcomes: observations from the National Pneumonia Project," *Clinical Infectious Diseases*, vol. 47, pp. S193–201, 2008.

[228] A. Torres, J. Garau, P. Arvis et al., "Moxifloxacin monotherapy is effective in hospitalized patients with community-acquired pneumonia: the MOTIV study—a randomized clinical trial," *Clinical Infectious Diseases*, vol. 46, no. 10, pp. 1499–1509, 2008.

[229] S. Ewig, H. Hecker, N. Suttorp, R. Marre, and T. Welte, "Moxifloxacin monotherapy versus β-lactam mono- or combination therapy in hospitalized patients with community-acquired pneumonia," *Journal of Infection*, vol. 62, no. 3, pp. 218–225, 2011.

[230] B. J. Epstein and J. G. Gums, "Optimal pharmacological therapy for community-acquired pneumonia the role of dual antibacterial therapy," *Drugs*, vol. 65, no. 14, pp. 1949–1971, 2005.

[231] E. J. Giamarellos-Bourboulis, J. C. Pechère, C. Routsi et al., "Effect of clarithromycin in patients with sepsis and ventilator-associated pneumonia," *Clinical Infectious Diseases*, vol. 46, no. 8, pp. 1157–1164, 2008.

[232] A. Kovaleva, H. H. F. Remmelts, G. T. Rijkers et al., "Immunomodulatory effects of macrolides during community-acquired pneumonia: a literature review," *Journal of Antimicrobial Chemotherapy*, vol. 67, no. 3, pp. 5305–540, 2012.

[233] A. J. Walkey and R. S. Wiener, "Macrolide antibiotics and survival in patients with acute lung injury," *Chest*, vol. 141, no. 5, pp. 1153–1159, 2012.

[234] A. Cervin, "The anti-inflammatory effect of erythromycin and its derivatives, with special reference to nasal polyposis and chronic sinusitis," *Acta Oto-Laryngologica*, vol. 121, no. 1, pp. 83–92, 2001.

[235] H. Suzuki and K. Ikeda, "Mode of action of long-term low-dose macrolide therapy for chronic sinusitis in the light of neutrophil recruitment," *Current Drug Targets—Inflammation & Allergy*, vol. 1, no. 1, pp. 117–126, 2002.

[236] K. W. Garey, A. Alwani, L. H. Danziger, and I. Rubinstein, "Tissue reparative effects of macrolide antibiotics in chronic inflammatory sinopulmonary diseases," *Chest*, vol. 123, no. 1, pp. 261–265, 2003.

[237] Y. Majima, "Clinical implications of the immunomodulatory effects of macrolides on sinusitis," *The American Journal of Medicine*, vol. 117, supplement 9, pp. 20S–25S, 2004.

[238] A. Cervin and B. Wallwork, "Anti-inflammatory effects of macrolide antibiotics in the treatment of chronic rhinosinusitis," *Otolaryngologic Clinics of North America*, vol. 38, no. 6, pp. 1339–1350, 2005.

[239] U. Hatipoglu and I. Rubinstein, "Treatment of chronic rhinosinusitis with low-dose, long-term macrolide antibiotics: an evolving paradigm," *Current Allergy and Asthma Reports*, vol. 5, no. 6, pp. 491–494, 2005.

[240] B. Wallwork, W. Coman, A. Mackay-Sim, L. Greiff, and A. Cervin, "A double-blind, randomized, placebo-controlled trial of macrolide in the treatment of chronic rhinosinusitis," *Laryngoscope*, vol. 116, no. 2, pp. 189–193, 2006.

[241] A. Cervin and B. Wallwork, "Macrolide therapy of chronic rhinosinusitis," *Rhinology*, vol. 45, no. 4, pp. 259–267, 2007.

[242] R. J. Harvey, B. D. Wallwork, and V. J. Lund, "Anti-inflammatory effects of macrolides: applications in chronic rhinosinusitis," *Immunology and Allergy Clinics of North America*, vol. 29, no. 4, pp. 689–703, 2009.

[243] E. O. Meltzer and D. L. Hamilos, "Rhinosinusitis diagnosis and management for the clinician: a synopsis of recent

consensus guidelines," *Mayo Clinic Proceedings*, vol. 86, no. 5, pp. 427–443, 2011.

[244] P. Piromchai, S. Thanaviratananich, and M. Laopaiboon, "Systemic antibiotics for chronic rhinosinusitis without nasal polyps in adults," *Cochrane Database of Systematic Reviews (Online)*, vol. 5, Article ID CD008233, 2011.

Hindawi Publishing Corporation
Mediators of Inflammation
Volume 2012, Article ID 528568, 7 pages
doi:10.1155/2012/528568

Research Article

Clarithromycin Suppresses Human Respiratory Syncytial Virus Infection-Induced *Streptococcus pneumoniae* Adhesion and Cytokine Production in a Pulmonary Epithelial Cell Line

Shin-ichi Yokota,[1] Tamaki Okabayashi,[1] Satoshi Hirakawa,[2] Hiroyuki Tsutsumi,[2] Tetsuo Himi,[3] and Nobuhiro Fujii[1]

[1] *Departments of Microbiology, Sapporo Medical University School of Medicine, South-1, West-17, Chuo-ku, Sapporo 060-8556, Japan*
[2] *Department of Pediatrics, Sapporo Medical University School of Medicine, South-1, West-16, Chuo-ku, Sapporo 060-8543, Japan*
[3] *Department of Otolaryngology, Sapporo Medical University School of Medicine, South-1, West-16, Chuo-ku, Sapporo 060-8543, Japan*

Correspondence should be addressed to Nobuhiro Fujii, fujii@sapmed.ac.jp

Received 17 January 2012; Revised 9 February 2012; Accepted 14 February 2012

Academic Editor: Kazuhito Asano

Human respiratory syncytial virus (RSV) sometimes causes acute and severe lower respiratory tract illness in infants and young children. RSV strongly upregulates proinflammatory cytokines and the platelet-activating factor (PAF) receptor, which is a receptor for *Streptococcus pneumoniae*, in the pulmonary epithelial cell line A549. Clarithromycin (CAM), which is an antimicrobial agent and is also known as an immunomodulator, significantly suppressed RSV-induced production of interleukin-6, interleukin-8, and regulated on activation, normal T-cell expressed and secreted (RANTES). CAM also suppressed RSV-induced PAF receptor expression and adhesion of fluorescein-labeled *S. pneumoniae* cells to A549 cells. The RSV-induced *S. pneumoniae* adhesion was thought to be mediated by the host cell's PAF receptor. CAM, which exhibits antimicrobial and immunomodulatory activities, was found in this study to suppress the RSV-induced adhesion of respiratory disease-causing bacteria, *S. pneumoniae*, to host cells. Thus, CAM might suppress immunological disorders and prevent secondary bacterial infections during RSV infection.

1. Introduction

Human respiratory syncytial virus (RSV) is one of the most important infectious agents causing acute lower respiratory tract illness, such as bronchiolitis and pneumonia, in infants and young children [1, 2]. Viral RNA generated during RSV replication is recognized by host pattern recognition molecules, such as Toll-like receptor 3 (TLR3) and retinoic acid inducible gene-I (RIG-I), and it induces type I and type III interferon [3, 4]. Transcriptional induction of proinflammatory cytokines, chemokines, and interferons is mediated by NF-κB and interferon regulatory factors (IRFs) [5, 6]. These mediators are believed to contribute to the pathophysiology of RSV infection, such as mucous hypersecretion, swelling of submucous, and infiltration of lymphocytes, neutrophils, eosinophils, and macrophages [7].

Frequently, there are coinfections with respiratory viruses, including RSV, and bacteria that cause community-acquired respiratory diseases, such as *Streptococcus pneumoniae* and *Haemophilus influenzae*. There is evidence for a positive correlation between infections with *S. pneumoniae* and RSV in the pathogenesis of otitis media, pneumonia, and meningitis [8–11]. *S. pneumoniae* and *H. influenzae* colonize to the host respiratory epithelium via host cell surface receptors, such as the platelet-activating factor (PAF) receptor [12–14]. These bacteria interact with the PAF receptor via phosphorylcholine, which is a component of the bacterial cell surface. Both live and heat-killed *S. pneumoniae* cells show an increased adhesion to human epithelial cells infected with RSV [15]. The upregulation of PAF receptor expression that is induced by respiratory virus infections, including those caused by RSV, results in the enhanced

adhesion of *S. pneumoniae* and *H. influenzae* to respiratory epithelial cells [15–17]. PAF receptor expression and *S. pneumoniae* cell adhesion are also upregulated by exposure to acid, which causes tissue injury and an inflammatory response [18].

Clarithromycin (CAM) is 14-membered ring macrolide antibiotic that also acts as a biological reaction modifier with anti-inflammatory properties. In Japan, CAM is applied to diffuse panbronchiolitis, chronic bronchiolitis, otitis media, and chronic sinusitis as an immunomodulator [19–21]. The anti-inflammatory mechanism of CAM has not yet been completely clarified, but one of the important mechanisms for its anti-inflammatory action is considered to be the suppression of NF-κB [22–24].

Recently, we reported that fosfomycin, which is an antibiotic, suppressed RSV-induced interleukin (IL)-8, regulated on activation, normal T-cell expressed and secreted (RANTES), and the PAF receptor by suppressing NF-κB activity [25, 26]. On the other hand, Wang et al. report that CAM suppressed rhinovirus-induced *Staphylococcus aureus* and *H. influenzae* adhesions to nasal epithelial cells [27]. So we anticipate that CAM suppresses RSV-induced bacterial adhesion to epithelial cells, because expression of PAF receptor is controlled by NF-κB [28, 29]

In the present study, we examined the effect of CAM on cytokine production, PAF receptor expression, and RSV infection-induced *S. pneumoniae* adhesion to respiratory epithelial cells.

2. Materials and Methods

2.1. Viruses, Cell Lines, Bacteria, and Reagents. RSV strain Long, human type II pulmonary epithelial cell line A549 and *S. pneumoniae* strain R6 were obtained from the American Type Culture Collection (ATCC, Manassas, VA). RSV was grown in HEp-2 cells. The virus titer of RSV was determined using a plaque-forming assay with HEp-2 cells as the indicator cells [25]. RSV infection to A549 cells was performed at multiplicity of infection (MOI) of 1. CAM was donated by Abbott Japan (Tokyo, Japan). A PAF receptor antagonist, 1-*O*-hexadecyl-2-acetyl-*sn*-glycero-3-phospho(N,N,N,-trimethyl)-hexanolamine, was purchased from Calbiochem-Merck (Darmstadt, Germany). An NF-κB inhibitor, pyrrolidine dithiocarbamate (PDTC), was purchased from Sigma-Aldrich (St. Louis, MO).

2.2. Measurement of Cytokine Production. A549 cells were infected with RSV at MOI of 1. After 24-hour infection, culture supernatants of RSV-infected and -uninfected cells were collected. The amounts of IL-6, IL-8, and RANTES in the culture supernatants were determined by enzyme-linked immunosorbent assay (ELISA) (DuoSet ELISA development kit, R&D systems, Minneapolis, MN).

2.3. Reverse Transcription-Polymerase Chain Reaction (RT-PCR). Semiquantitative RT-PCR was carried out as described previously [4, 30].

2.4. Flow Cytometry. The cell surface expression of the PAF receptor was examined by flow cytometry as previously described [26]. The cells were harvested from culture flasks using a cell scraper and then incubated with 2.5 μg/mL of mouse anti-PAF receptor monoclonal antibody (11A4 (clone 21); Cayman Chemical, Ann Arbor, MI) or mouse IgG2a, κ isotype control antibody (eBioscience, San Diego, CA). After incubation at 4°C for 30 min, cells were collected by centrifugation and washed once with Dulbecco's phosphate-buffered saline (PBS (−)). Cell suspensions were incubated with a phycoerythrin-conjugated goat anti-mouse IgG $F(ab)_2$ fragment antibody (1 : 100 dilution) (Abcam, Cambridge, UK) at 4°C for 30 min, and the stained cells were assessed with FACSCalibur (BD Bioscience, San Jose, CA).

2.5. Bacterial Adhesion Assay. S. pneumoniae adhesion was assayed using fluorescein-isothiocyanate- (FITC-) labeled *S. pneumoniae* as previously described [26]. Briefly, a bacterial suspension in 0.1 M NaCl-50 mM sodium carbonate buffer (pH9.5) at 1×10^8 CFU/mL was prepared. FITC isomer-I (Dojindo Laboratories, Kumamoto, Japan) was added at a concentration of 1 mg/mL, and the mixture was incubated at 4°C for 1 h. The cells were washed three times with PBS (−).

CAM was added to monolayers of A549 cells 1 h prior to RSV infection. The A549 cells infected with RSV at an MOI of 1 for 24 h and uninfected A549 cells were incubated with FITC-labeled *S. pneumoniae* cells at MOI of 10 for 30 min at 37°C. For the control experiments, either 20 μg/mL of the PAF receptor antagonist or 10 μg/mL of the mouse anti-PAF receptor monoclonal antibody (11A4(clone 21)) was added to the A549 cells 1 h prior to the addition of the FITC-labeled bacteria. The cell monolayer was gently washed three times with PBS (−) and observed by fluorescence microscopy. Alternatively, the cells were harvested with cell scraper and then assessed by flow cytometry as previously described [26].

3. Results

First, we examined the effect of CAM on RSV replication in A549 cells. RSV infection to A549 cells was performed at MOI of 1. After 24 and 36 h of infection, significant alterations of the RSV titers or expression levels of G mRNA were not observed by the addition of CAM even at a concentration of 100 μg/mL (Figure 1).

When A549 cells were infected with RSV at MOI of 1, RANTES, IL-8, and IL-6 were markedly induced. These cytokine inductions were significantly suppressed in the presence of CAM in a dose-dependent manner (Figure 2). The degree of suppression by CAM was less than that by an NF-κB inhibitor, PDTC.

PAF receptor expression on the cell surface is upregulated during RSV infection in A549 cells [26]. The RSV-induced upregulation of the PAF receptor was significantly suppressed by CAM and PDTC in a dose-dependent manner (Figure 3). The degree of suppression by CAM was slightly less than that by PDTC. Suppression of the PAF receptor expression was also observed when A549 cells were posttreated with CAM (4 or 12 h after RSV infection) (data not shown).

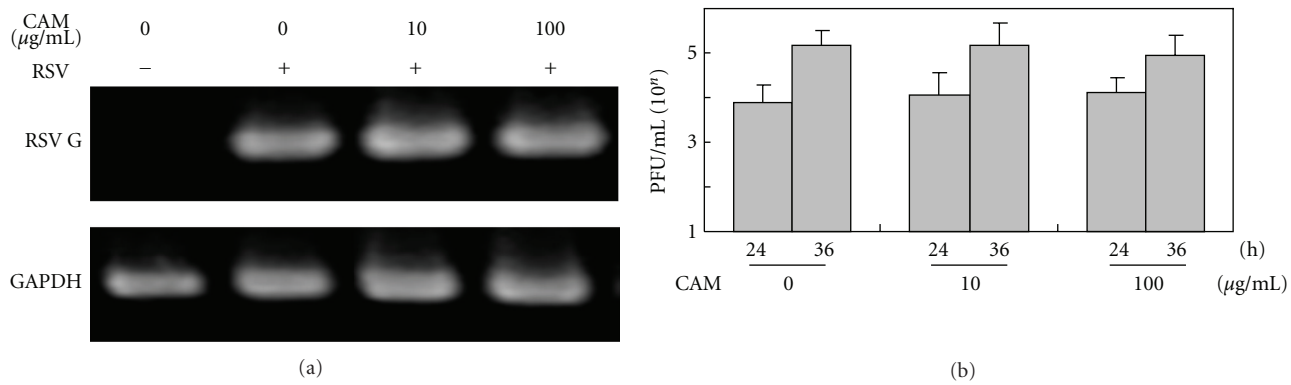


Figure 1: Effects of CAM on RSV G mRNA expression (a) and production of infectious virus particles (b) in A549 cells infected with RSV. One hour before RSV infection, CAM was added to A549 cell culture at the indicated concentration. A549 cells were infected with the RSV at MOI of 1. (a) RT-PCR. After 24 h of infection, total RNAs were extracted from the cells. The mRNA levels of RSV G were determined by RT-PCR. The mRNA levels of human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were carried out as a control. (b) Plaque-forming assay. After 24 h and 36 h infection, the culture supernatants were corrected. Virus titers in the supernatants were determined by plaque-forming assay using Hep-2 cells as the indicator cell. Each experiment was performed in quadruplicate. The mean value and standard deviation are shown.

We examined the adhesion of FITC-labeled *S. pneumoniae* cells to A549 cells by fluorescence microscopy (Figure 4) and flow cytometry (Figure 5). RSV infection significantly enhanced the adhesion of *S. pneumoniae* to A549 cells, and this enhancement was suppressed by adding a PAF receptor antagonist (Figures 4 and 5) or anti-PAF receptor monoclonal antibody (data not shown). This result indicated that the RSV-induced *S. pneumoniae* adhesion occurs via the PAF receptor on A549 cells. The bacterial adhesion was significantly suppressed by CAM, as well as PDTC.

These lines of evidence confirmed that the expression of the PAF receptor was induced by RSV infection and indicated that this induction, and subsequent RSV-induced *S. pneumoniae* adhesion, can be suppressed by CAM treatment.

4. Discussion

Macrolides, with the exception of the 16-membered ring type, have both anti-inflammatory and antibacterial functions [20, 21]. One of the important mechanisms of anti-inflammatory action is the suppression of NF-κB activation [22–24]. Our recent studies show that RSV upregulates proinflammatory cytokines, such as IL-6, and chemokines, such as IL-8 and RANTES, in the respiratory epithelial cell line A549. Furthermore, the induction of chemokines by RSV is significantly suppressed by an antibiotic, fosfomycin, via suppression of NF-κB activation [25]. In the present study, CAM was shown to suppress IL-6, IL-8, and RANTES, which are induced by RSV infection, at concentrations of 10 and 100 μg/mL. Patel et al. reported that the concentration of CAM in fluid of the bronchopulmonary epithelial lining was 34.2 ± 5.16 μg/mL at 4 h, 23.01 ± 11.9 μg/mL at 12 h in healthy adults orally administered CAM 500 mg [31]. We observed that CAM did not affect RSV replication even at a concentration of 100 μg/mL. However, it is reported that respiratory virus, such as RSV [32], rhinovirus [33, 34], and influenza virus [35], replication is suppressed by 14-membered ring macrolides, including CAM. The reasons of contradictory results between the report of Asada et al. [32] and our present study have been unclear. These two studies used different types of epithelial cells and different experimental conditions of RSV infection. Asada et al. used primary human tracheal epithelial cells, and in contrast we used A549 cell line. Asada et al. carry out infection at a lower titer of RSV (10^{-3} $TCID_{50}$/cell) and measuring virus titer at a longer period (3–5 days) after infection. Our results indicated that suppression of the RSV-induced cytokines by CAM was not caused by the amount of replicated RSV. In other words, CAM was suggested to have suppressive activity of cytokine production independent of viral replication. Both IL-8 and RANTES, which are strongly upregulated during RSV infection, play important roles in pathogenesis [36, 37]. IL-8 primarily activates neutrophils and promotes their migration. RANTES is secreted from respiratory epithelial cells and promotes migration of eosinophils, basophils, monocytes, and neutrophils. In particular, RANTES is an efficient eosinophil chemoattractant involved in the pathogenesis of asthma [38]. CAM has been suggested to suppress the inflammatory disorders induced by RSV.

In the present study, we also observed that CAM suppressed enhanced *S. pneumoniae* adhesion by RSV infection in A549 cells. The RSV-induced *S. pneumoniae* adhesion was mainly mediated by host PAF receptor, as indicated by that suppressed by the PAF receptor antagonist and anti-PAF receptor monoclonal antibody. The PAF receptor acts as a receptor for *S. pneumoniae* and *H. influenzae* [12–14]. Transcription of the PAF receptor gene is controlled by NF-κB [28, 29]. We confirmed it by that the RSV-induced PAF receptor expression and *S. pneumoniae* adhesion were suppressed by an NF-κB inhibitor, PDTC. We revealed that CAM also suppressed PAF receptor expression induced by RSV infection and *S. pneumoniae* adhesion to RSV-infected A549 cells. It should be caused by the suppression of

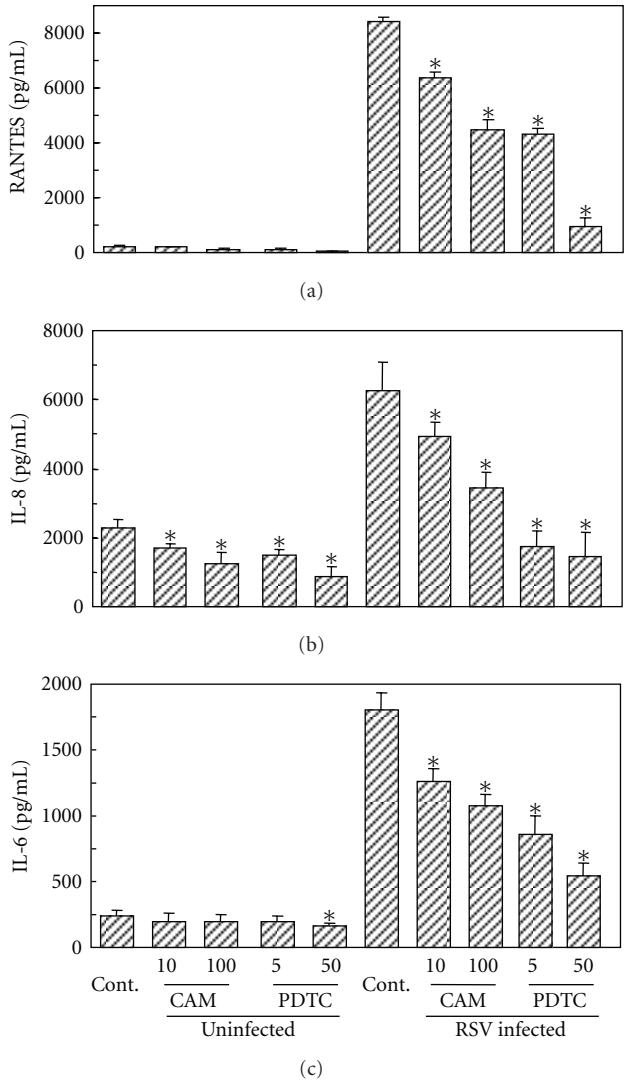


Figure 2: Effects of CAM and PDTC on RSV-induced RANTES (a), IL-8 (b), and IL-6 (c) production in A549 cells. One hour before RSV infection, CAM or PDTC is added to A549 cell culture at the indicated concentration. A549 cells were infected with the RSV at MOI of 1. After 24 h of infection, the culture supernatants were collected, and each cytokine in the supernatants was determined by ELISA. The experiments were performed in triplicate. The mean value and standard deviation were calculated. Statistical difference was examined by Student's t-test. $^*P < 0.01$ compared to cytokine production without any reagent treatment in uninfected cells and RSV-infected cells, respectively.

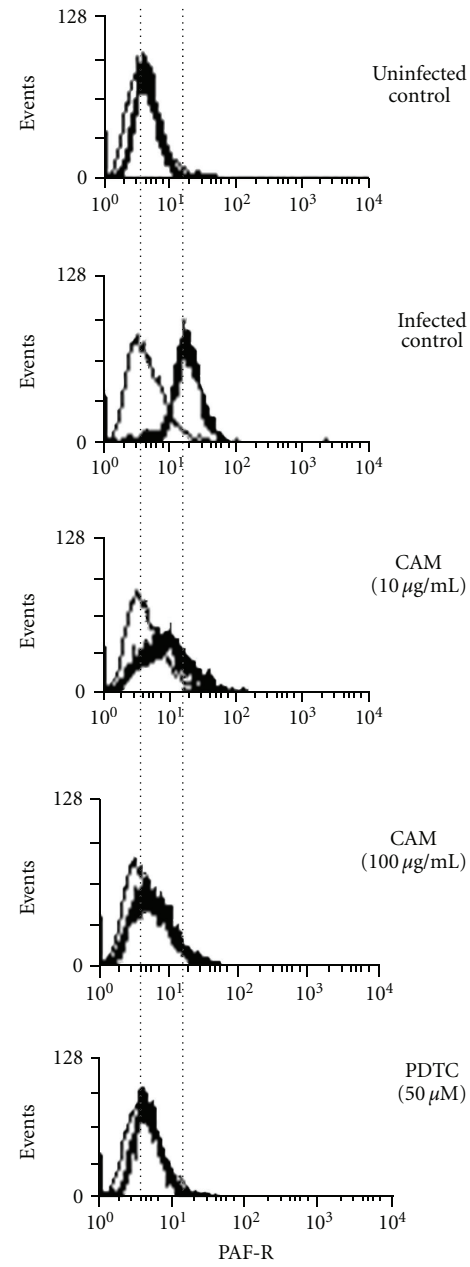


Figure 3: Effect of CAM and PDTC on RSV-induced PAF receptor expression in A549 cells. One hour before RSV infection, CAM or PDTC is added to A549 cell culture at the indicated concentration. The cells were infected with the RSV at MOI of 1. After 24 h of infection, the cells were collected and then stained with an anti-PAF receptor antibody and phycoerythrin-labeled anti-mouse IgG antibody (thick lines). The stained cells were analyzed by flow cytometry. Thin lines indicate the cells stained with an unrelated isotype control antibody instead of the anti-PAF receptor antibody.

NF-κB activated by RSV infection. Recently, Wang et al. [27] reported that CAM suppressed rhinovirus-induced *S. aureus* and *H. influenzae* adhesions to nasal epithelial cells. They show that the expressions of fibronectin and carcinoembryonic antigen-related cell adhesion molecule (CEACAM), which act as receptors for *S. aureus* and *H. influenza,* respectively, are induced by rhinovirus and suppressed by CAM. The present study indicated that CAM suppressed the PAF receptor-phosphorylcholine (host-bacteria) interaction, which is enhanced by RSV infection, by inhibiting PAF receptor expression. CAM showed more potent suppression of RSV-induced *S. pneumoniae* adhesion and production of proinflammatory cytokines and chemokines than fosfomycin, as we reported previously [25, 26]. Notably, CAM significantly suppressed RSV-induced IL-6 production, whereas fosfomycin did not significantly [25].

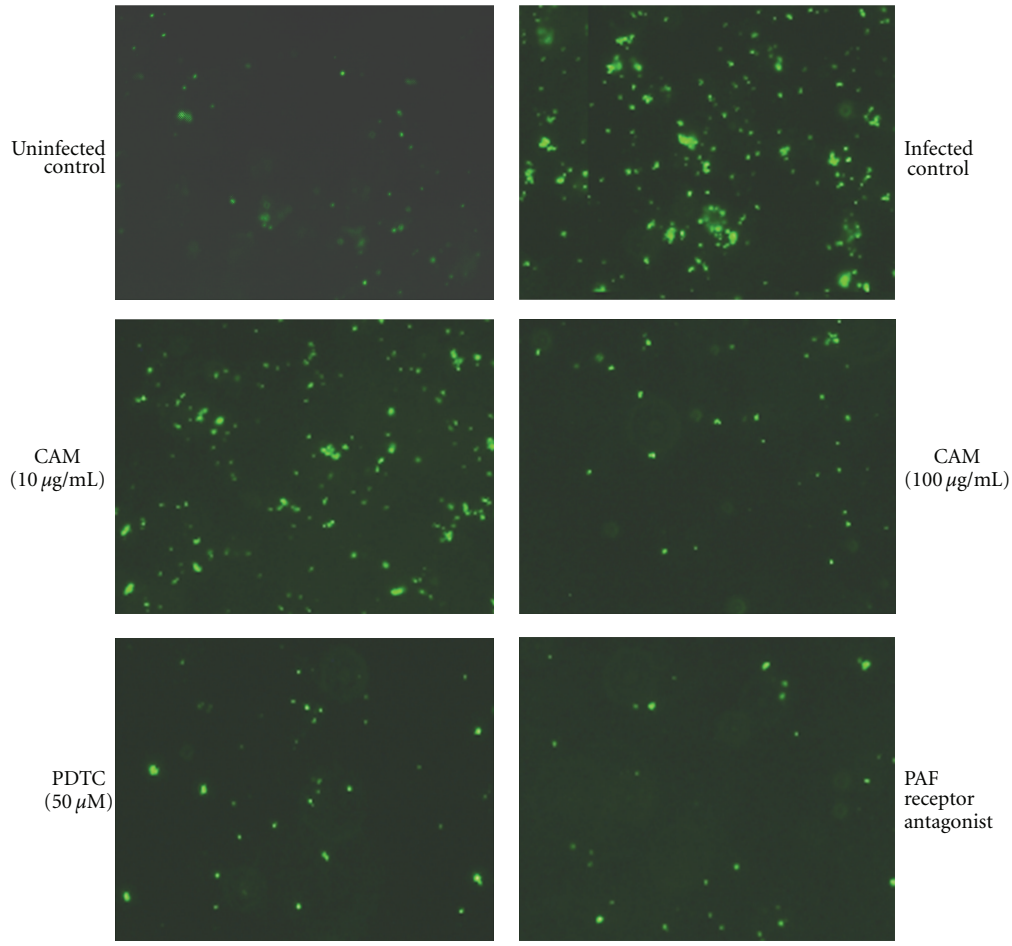


Figure 4: Suppression by CAM of RSV-induced adhesion of FITC-labeled *S. pneumoniae* to A549 cells, as observed by fluorescence microscopy. One hour before RSV infection, CAM (10 or 100 μg/mL) or PDTC (50 μM) was added to A549 cell monolayer. The cells were infected with RSV at MOI of 1. After 24 h of infection, FITC-labeled bacterial cells were added to the cell monolayer at MOI of 10, and incubation was continued at 37°C for 30 min. A PAF receptor antagonist (20 μg/mL) was added to the cell monolayer 1 h before the addition of labeled bacterial cells. The bacteria adhering to the A549 cell monolayer were visualized by fluorescence microscopy.

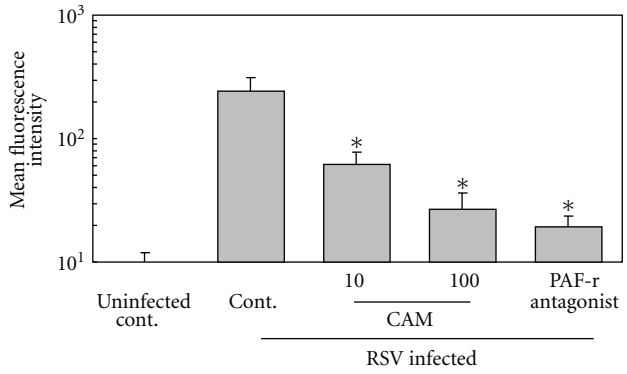


Figure 5: Suppression by CAM of RSV-induced adhesion of FITC-labeled *S. pneumoniae* to A549 cells, as observed by flow cytometry. Experiments were performed as in Figure 5. The A549 cell monolayer incubated with FITC-labeled *S. pneumoniae* cells was harvested by cell scraper and then applied to flow cytometry. Each experiment was performed in triplicate. The data present as mean value ± standard deviation of the mean relative fluorescence intensity. $^*P < 0.01$ compared to RSV-infected cells without any reagent treatment.

This finding may be caused by that CAM is more potent than fosfomycin; however, the actual reason for this disparity is not clear. The upregulation of PAF receptor expression and the enhanced adhesion of pathogenic bacteria, such as *S. pneumoniae*, to respiratory epithelial cells are considered to be a major risk factor for secondary bacterial infections after primary respiratory viral infections. CAM may suppress both secondary bacterial infections and immunological disorders induced by RSV, without suppressing viral replication. Infection with other respiratory viruses, such as human parainfluenza virus 3 [16] and rhinovirus [17], also upregulates known receptors for the pathogenic bacteria, including PAF receptor and *S. pneumoniae* adhesion. On the other hand, influenza virus does not upregulate the known receptors for bacteria, whereas bacterial adhesion is increased by the infection [16]. McCullers [39] reported that influenza-induced bacterial adhesion to A549 cells was not inhibited by PAF receptor antagonist, and the PAF receptor knock-out mice did not show lower susceptibility to experimental secondary pneumonia caused by *S. pneunimoae* following influenza infection compared to the parent mice. Lines of evidence

suggest that adherent inducing mechanisms of *S. pneumoniae* to host respiratory epithelial cells are varied among viruses. So CAM may not always suppress virus-induced pathogenic bacteria adhesion.

5. Conclusions

We proposed that clarithromycin efficiently suppressed PAF receptor-mediated *Streptococcus pneumoniae* adhesion to respiratory epithelial cells as well as RSV-induced proinflammatory cytokine and chemokine production. Clarithromycin may suppress secondary bacterial infections and immunological disorders during RSV infection.

Abbreviations

CAM:	Clarithromycin
ELISA:	Enzyme-linked immunosorbent assay
FITC:	Fluorescein isothiocyanate
IL:	Interleukin
MOI:	Multiplicity of infection
PAF:	Platelet-activating factor
PDTC:	Pyrrolidine dithiocarbamate
RANTES:	Regulated on activation, normal T-cell expressed and secreted
RSV:	Human respiratory syncytial virus
RT-PCR:	Reverse transcription-polymerase chain reaction.

Conflict of Interests

All the authors declare that there is no conflict of interests.

Acknowledgment

This work was supported in part by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science.

References

[1] C. B. Hall, G. A. Weinberg, M. K. Iwane et al., "The burden of respiratory syncytial virus infection in young children," *The New England Journal of Medicine*, vol. 360, no. 6, pp. 588–598, 2009.

[2] A. Greenough, "Respiratory syncytial virus infection: clinical features, management, and prophylaxis," *Current Opinion in Pulmonary Medicine*, vol. 8, no. 3, pp. 214–217, 2002.

[3] P. Liu, M. Jamaluddin, K. Li, R. P. Garofalo, A. Casola, and A. R. Brasier, "Retinoic acid-inducible gene I mediates early antiviral response and Toll-like receptor 3 expression in respiratory syncytial virus-infected airway epithelial cells," *Journal of Virology*, vol. 81, no. 3, pp. 1401–1411, 2007.

[4] T. Okabayashi, T. Kojima, T. Masaki et al., "Type-III interferon, not type-I, is the predominant interferon induced by respiratory viruses in nasal epithelial cells," *Virus Research*, vol. 160, no. 1-2, pp. 360–366, 2011.

[5] M. Matsumoto and T. Seya, "TLR3: interferon induction by double-stranded RNA including poly(I:C)," *Advanced Drug Delivery Reviews*, vol. 60, no. 7, pp. 805–812, 2008.

[6] K. Onomoto, M. Yoneyama, and T. Fujita, "Regulation of antiviral innate immune responses by RIG-I family of RNA helicases," *Current Topics in Microbiology and Immunology*, vol. 316, pp. 193–205, 2007.

[7] H. Tsutsumi, R. Takeuchi, and S. Chiba, "Activation of cellular genes in the mucosal epithelium by respiratory syncytial virus: implications in disease and immunity," *Pediatric Infectious Disease Journal*, vol. 20, no. 10, pp. 997–1001, 2001.

[8] P. E. Kim, D. M. Musher, W. P. Glezen, M. C. Rodriguez-Barradas, W. K. Nahm, and C. E. Wright, "Association of invasive pneumococcal disease with season, atmospheric conditions, air pollution, and the isolation of respiratory viruses," *Clinical Infectious Diseases*, vol. 22, no. 1, pp. 100–106, 1996.

[9] J. M. Hament, J. L. L. Kimpen, A. Fleer, and T. F. W. Wolfs, "Respiratory viral infection predisposing for bacterial disease: a concise review," *FEMS Immunology and Medical Microbiology*, vol. 26, no. 3-4, pp. 189–195, 1999.

[10] T. Chonmaitree and T. Heikkinen, "Viruses and acute otitis media," *Pediatric Infectious Disease Journal*, vol. 19, no. 10, pp. 1005–1007, 2000.

[11] M. A. Andrade, A. Hoberman, J. Glustein, J. L. Paradise, and E. R. Wald, "Acute otitis media in children with bronchiolitis," *Pediatrics*, vol. 101, no. 4, pp. 617–619, 1998.

[12] D. R. Cundell, C. Gerard, I. Idanpaan-Heikkila, E. I. Tuomanen, and N. P. Gerard, "PAF receptor anchors *Streptococcus pneumoniae* to activated human endothelial cells," *Advances in Experimental Medicine and Biology*, vol. 416, pp. 89–94, 1997.

[13] D. R. Cundell, N. P. Gerard, C. Gerard, I. Idanpaan-Heikkila, and E. I. Tuomanen, "*Streptococcus pneumoniae* anchor to activated human cells by the receptor for platelet-activating factor," *Nature*, vol. 377, no. 6548, pp. 435–438, 1995.

[14] W. E. Swords, B. A. Buscher, K. Ver Steeg Li et al., "Nontypeable *Haemophilus influenzae* adhere to and invade human bronchial epithelial cells via an interaction of lipooligosaccharide with the PAF receptor," *Molecular Microbiology*, vol. 37, no. 1, pp. 13–27, 2000.

[15] J. M. Hament, P. C. Aerts, A. Fleer et al., "Enhanced adherence of *Streptococcus pneumoniae* to human epithelial cells infected with respiratory synctial virus," *Pediatric Research*, vol. 55, no. 6, pp. 972–978, 2004.

[16] V. Avadhanula, C. A. Rodriguez, J. P. De Vincenzo et al., "Respiratory viruses augment the adhesion of bacterial pathogens to respiratory epithelium in a viral species- and cell type-dependent manner," *Journal of Virology*, vol. 80, no. 4, pp. 1629–1636, 2006.

[17] S. Ishizuka, M. Yamaya, T. Suzuki et al., "Effects of rhinovirus infection on the adherence of *Streptococcus pneumoniae* to cultured human airway epithelial cells," *Journal of Infectious Diseases*, vol. 188, no. 12, pp. 1928–1939, 2003.

[18] S. Ishizuka, M. Yamaya, T. Suzuki et al., "Acid exposure stimulates the adherence of *Streptococcus pneumoniae* to cultured human airway epithelial cells: effects on platelet-activating factor receptor expression," *American Journal of Respiratory Cell and Molecular Biology*, vol. 24, no. 4, pp. 459–468, 2001.

[19] N. Keicho and S. Kudoh, "Diffuse panbronchiolitis: role of macrolides in therapy," *American Journal of Respiratory Medicine*, vol. 1, no. 2, pp. 119–131, 2002.

[20] J. Tamaoki, J. Kadota, and H. Takizawa, "Clinical implications of the immunomodulatory effects of macrolides," *The American Journal of Medicine*, vol. 117, supplement 9, pp. 5S–11S, 2004.

[21] Y. S. López-Boado and B. K. Rubin, "Macrolides as immunomodulatory medications for the therapy of chronic lung

diseases," *Current Opinion in Pharmacology*, vol. 8, no. 3, pp. 286–291, 2008.

[22] T. Ichiyama, M. Nishikawa, T. Yoshitomi et al., "Clarithromycin inhibits NF-κB activation in human peripheral blood mononuclear cells and pulmonary epithelial cells," *Antimicrobial Agents and Chemotherapy*, vol. 45, no. 1, pp. 44–47, 2001.

[23] T. Kikuchi, K. Hagiwara, Y. Honda et al., "Clarithromycin suppresses lipopolysaccharide-induced interleukin-8 production by human monocytes through AP-1 and NF-κB transcription factors," *Journal of Antimicrobial Chemotherapy*, vol. 49, no. 5, pp. 745–755, 2002.

[24] T. Miyanohara, M. Ushikai, S. Matsune, K. Ueno, S. Katahira, and Y. Kurono, "Effects of clarithromycin on cultured human nasal epithelial cells and fibroblasts," *Laryngoscope*, vol. 110, no. 1, pp. 126–131, 2000.

[25] T. Okabayashi, S. Yokota, Y. Yoto, H. Tsutsumi, and N. Fujii, "Fosfomycin suppresses chemokine induction in airway epithelial cells infected with respiratory syncytial virus," *Clinical and Vaccine Immunology*, vol. 16, no. 6, pp. 859–865, 2009.

[26] S. Yokota, T. Okabayashi, Y. Yoto, T. Hori, H. Tsutsumi, and N. Fujii, "Fosfomycin suppresses RS-virus-induced *Streptococcus pneumoniae* and *Haemophilus influenzae* adhesion to respiratory epithelial cells via the platelet-activating factor receptor," *FEMS Microbiology Letters*, vol. 310, no. 1, pp. 84–90, 2010.

[27] J. H. Wang, S. H. Lee, H. J. Kwon, and Y. J. Jang, "Clarithromycin inhibits rhinovirus-induced bacterial adhesions to nasal epithelial cells," *Laryngoscope*, vol. 120, no. 1, pp. 193–199, 2010.

[28] H. Mutoh, S. Ishii, T. Izumi, S. Kato, and T. Shimizu, "Platelet-activating factor (PAF) positively auto-regulates the expression of human PAF receptor transcript 1 (leukocyte-type) through NF-κB," *Biochemical and Biophysical Research Communications*, vol. 205, no. 2, pp. 1137–1142, 1994.

[29] T. Shimizu and H. Mutoh, "Structure and regulation of platelet activating factor receptor gene," *Advances Experimental Medicine and Biology*, vol. 416, pp. 197–204, 1997.

[30] T. Okabayashi, H. Kariwa, S. Yokota et al., "Cytokine regulation in SARS coronavirus infection compared to other respiratory virus infections," *Journal of Medical Virology*, vol. 78, no. 4, pp. 417–424, 2006.

[31] K. B. Patel, D. Xuan, P. R. Tessier, J. H. Russomanno, R. Quintiliani, and C. H. Nightingale, "Comparison of bronchopulmonary pharmacokinetics of clarithromycin and azithromycin," *Antimicrobial Agents and Chemotherapy*, vol. 40, no. 10, pp. 2375–2379, 1996.

[32] M. Asada, M. Yoshida, T. Suzuki et al., "Macrolide antibiotics inhibit respiratory syncytial virus infection in human airway epithelial cells," *Antiviral Research*, vol. 83, no. 2, pp. 191–200, 2009.

[33] T. Suzuki, M. Yamaya, K. Sekizawa et al., "Erythromycin inhibits rhinovirus infection in cultured human tracheal epithelial cells," *American Journal of Respiratory and Critical Care Medicine*, vol. 165, no. 8, pp. 1113–1118, 2002.

[34] Y. J. Jang, H. J. Kwon, and B. J. Lee, "Effect of clarithromycin on rhinovirus-16 infection in A549 cells," *European Respiratory Journal*, vol. 27, no. 1, pp. 12–19, 2006.

[35] M. Tsurita, M. Kurokawa, M. Imakita, Y. Fukuda, Y. Watanabe, and K. Shiraki, "Early augmentation of interleukin (IL)-12 level in the airway of mice administered orally with clarithromycin or intranasally with IL-12 results in alleviation of influenza infection," *Journal of Pharmacology and Experimental Therapeutics*, vol. 298, no. 1, pp. 362–368, 2001.

[36] F. J. Culley, A. M. J. Pennycook, J. S. Tregoning et al., "Role of CCL5 (RANTES) in viral lung disease," *Journal of Virology*, vol. 80, no. 16, pp. 8151–8157, 2006.

[37] J. S. Yoon, H. H. Kim, Y. Lee, and J. S. Lee, "Cytokine induction by respiratory syncytial virus and adenovirus in bronchial epithelial cells," *Pediatric Pulmonology*, vol. 42, no. 3, pp. 277–282, 2007.

[38] J. Venge, M. Lampinen, L. Håkansson, S. Rak, and P. Venge, "Identification of IL-5 and RANTES as the major eosinophil chemoattractants in the asthmatic lung," *Journal of Allergy and Clinical Immunology*, vol. 97, no. 5, pp. 1110–1115, 1996.

[39] J. A. Mccullers, A. R. Iverson, and P. J. Murray, "The platelet activating factor receptor is not required for exacerbation of bacterial pneumonia following influenza," *Scandinavian Journal of Infectious Diseases*, vol. 40, no. 1, pp. 11–17, 2008.

Hindawi Publishing Corporation
Mediators of Inflammation
Volume 2012, Article ID 649570, 9 pages
doi:10.1155/2012/649570

Review Article

Macrolide Therapy in Respiratory Viral Infections

Jin-Young Min and Yong Ju Jang

Department of Otolaryngology, Asan Medical Center, University of Ulsan College of Medicine, 388-1 Pungnap-2dong, Songpa-gu, Seoul 138-736, Republic of Korea

Correspondence should be addressed to Yong Ju Jang, jangyj@amc.seoul.kr

Received 20 February 2012; Revised 8 April 2012; Accepted 10 April 2012

Academic Editor: Kazuhito Asano

Background. Macrolides have received considerable attention for their anti-inflammatory and immunomodulatory actions beyond the antibacterial effect. These two properties may ensure some efficacy in a wide spectrum of respiratory viral infections. We aimed to summarize the properties of macrolides and their efficacy in a range of respiratory viral infection. *Methods*. A search of electronic journal articles through PubMed was performed using combinations of the following keywords including macrolides and respiratory viral infection. *Results*. Both *in vitro* and *in vivo* studies have provided evidence of their efficacy in respiratory viral infections including rhinovirus (RV), respiratory syncytial virus (RSV), and influenza virus. Much data showed that macrolides reduced viral titers of RV ICAM-1, which is the receptor for RV, and RV infection-induced cytokines including IL-1β, IL-6, IL-8, and TNF-α. Macrolides also reduced the release of proinflammatory cytokines which were induced by RSV infection, viral titers, RNA of RSV replication, and the susceptibility to RSV infection partly through the reduced expression of activated RhoA which is an RSV receptor. Similar effects of macrolides on the influenza virus infection and augmentation of the IL-12 by macrolides which is essential in reducing virus yield were revealed. *Conclusion*. This paper provides an overview on the properties of macrolides and their efficacy in various respiratory diseases.

1. Introduction

Macrolides are a group of antibiotics whose activity stems from the presence of the macrolide ring to which one or more deoxy sugars, usually cladinose and desosamine, may be attached. Lactone rings are usually 14, 15 or 16 membered. Macrolides which tend to accumulate within leukocytes and are transported into the site of infection are used to treat respiratory and soft-tissue infections caused by Gram-positive bacteria such as *Streptococcus pneumoniae* and *Haemophilus influenzae*. In addition to the typical antibiotic effect, two properties including the anti-inflammatory and the immunomodulatory actions are inherent in this group of drugs. These anti-inflammatory and immunomodulatory actions of macrolides encouraged a number of researchers to explore a potential application of macrolides even for respiratory viral infection [1–5].

The purpose of this paper is to summarize the properties of macrolides and their efficacy in a range of respiratory viral infection.

2. Search Strategy

We performed an electronic article search through PubMed using combinations of the following keywords: macrolides (azithromycin, clarithromycin, dirithromycin, erythromycin, roxithromycin, and telithromycin) and respiratory viral infection (respiratory syncytial virus, rhinovirus, adenovirus, metapneumovirus, influenza virus, and parainfluenza virus). All types of articles such as randomized controlled trials, clinical observational cohort studies, review articles, and case reports were included.

3. Anti-Inflammatory and Immune Modulation Effects of Macrolides

At present, macrolides are known to possess anti-inflammatory and immunomodulatory actions extending beyond their antibacterial activity in pulmonary inflammatory disorders such as diffuse panbronchiolitis (DPB), asthma, and

cystic fibrosis. Both *in vitro* and *in vivo* data show macrolides to downregulate prolonged inflammatory response, reduce airway mucus secretion, inhibit the bacterial adhesion biofilm, reduce the production of reactive oxygen species, inhibit neutrophil activation and mobilization with an acceleration of the apoptotic process, and also block the activation of nuclear transcription factors [6–11]. After macrolides accumulating within cells, they may interact with receptors or second messengers responsible for the regulation of cell cycle and cellular immunity.

However, the anti-inflammatory effects observed with macrolides are modest if compared to the anti-inflammatory effects of corticosteroids and require much higher doses, questioning their real use as an anti-inflammatory agent. Further studies are needed.

4. Macrolides and Respiratory Viral Infections

As macrolides have anti-inflammatory and immunomodulatory effect, the scenario thus depicted is sufficiently suggestive to consider the possible use of these drugs in respiratory viral infection presenting an inflammatory basis. The common causes of respiratory viral infection include rhinovirus (RV), respiratory syncytial virus (RSV), adenovirus, metapneumovirus, influenza virus, and parainfluenza virus. Recent studies have shown that the high mortality rate of respiratory virus infections is a result of an overactive inflammatory response. Respiratory viral infections are characterized by the appearance of cytokine storms which is extreme production and secretion of numerous proinflammatory cytokines. Severity of infection is closely related with virus-induced cytokine dysregulation which is responsible for the development of fatal clinical symptoms, such as massive pulmonary edema, acute bronchopneumonia, alveolar hemorrhage, reactive hemophagocytosis, and acute respiratory distress syndrome. Numerous *in vitro*, *in vivo,* and clinical studies have established that viruses are potent inducers of various cytokines and chemokines including TNF-α, interferon (IFN)-γ, IFN-α/β, IL-6, IL-1, MIP (macrophage inflammatory protein)-1, MIG (monokine induced by IFN-γ), IP (interferon-gamma-inducible protein)-10, MCP (monocyte chemoattractant protein)-1, RANTES, and IL-8 [12–17].

It is known that macrolides downregulate the inflammatory cascade, they attenuate excessive cytokine production in viral infections, and they may reduce virus-related exacerbation. Furthermore, macrolides may influence phagocyte activity by modifying their miscellaneous functions including chemotaxis, phagocytosis, oxidative burst, bacterial killing, and cytokine production [18]. It has also been reported that macrolides could interfere with the influenza virus replication cycle, resulting in the inhibition of virus production from infected cells, mainly by inhibiting intracellular hemagglutinin HA0 proteolysis [19, 20]. There are still controversies in the effects of macrolides in respiratory viral infections. The following review will introduce recent research findings regarding the effectiveness of macrolides antibiotic on different forms of respiratory viral infections (Table 1).

4.1. Cell Culture Studies. Among *in vitro*, *in vivo*, and clinical studies, *in vitro* studies, especially cell culture studies, were most frequently performed to evaluate the effect of macrolides on respiratory viral infection. Numerous *in vitro* studies with various respiratory virus revealed that macrolides are effective on respiratory viral infections.

RV is the most common cause of viral upper respiratory tract infections (URIs) and is responsible for about one half of all cases of the common cold. Although RV does not cause necrosis of epithelial cells or substantial histological changes in nasal mucosa, RV infection induces the hypersecretion of mucus, as well as the increased expression and secretion of various cytokines, including interleukin (IL)-6, IL-8, IL-9, IL-1b, IL-11, and TNF-α, and the influx of neutrophils, which correlate with the severity of cold symptoms [35, 36]. It is well known that approximately 90% of more than 100 different RV serotypes bind to ICAM-1, and RV infection upregulates ICAM-1 expression on airway epithelial cells, thus facilitating further viral attachment and entry [36, 37]. As ICAM-1 is the receptor for the major RV and since IL-1b, IL-6, and IL-8 play significant roles in the pathophysiology of RV infection, macrolides which are known to have inhibitory effect on those cytokines may be able to modulate inflammatory processes during RV infection. Studies have been done to determine anti-inflammatory properties of macrolide antibiotics against RV infection.

Among these macrolides, erythromycin is the first drug which was studied about their efficacy on RV. Erythromycin is a macrolide antibiotic with potent anti-inflammatory effects that is used for treating chronic lower respiratory tract infections. Suzuki et al. examined the effects of erythromycin on RV (RV2 and RV14) infection in airway epithelium [23]. In their study, erythromycin reduced the supernatant RV14 titers, RV14 RNA, the susceptibility to RV14 infection, and the production of ICAM-1 and cytokines which was upregulated by RV14. Erythromycin also reduced the supernatant RV2 titers, RV2 RNA, the susceptibility to RV2 infection, and cytokine production, although the inhibitory effects of erythromycin on the expression of the low-density lipoprotein receptor, the minor RV receptor, were small. In addition, erythromycin may also modulate airway inflammation by reducing the production of proinflammatory cytokines and ICAM-1 induced by RV infection. Erythromycin reduced the NF-κB activation by RV14 and decreased the number of acidic endosomes in the epithelial cells.

Another type of macrolide antibiotics, bafilomycin A1 also inhibits infection of RV, in human airway epithelial cells by the reduction of ICAM-1 and by affecting the acidification of endosomes, where RV RNA enters into the cytoplasm of infected cells [22]. Bafilomycin A1 and erythromycin could reduce proinflammatory cytokines including IL-6 after RV infection in airway epithelial cells [22, 38].

Jang et al. investigated the effect of clarithromycin on RV infection in A549 cells [24]. In their study, clarithromycin treatment inhibited the RV-induced increase in ICAM-1 mRNA and protein, as well as the RV induced secretion of IL-1β, IL-6, and IL-8. These effects were greater in cells treated with 10 μM than in those treated with 100 μM CM, and the maximum effect was observed 3 days after viral

Table 1: Macrolide studies evaluating efficacy on various respiratory viral infection.

Virus	Study	Macrolide	Dose	Method	Parameter	Results	Ref
Rhinovirus							
RV16	Abisheganaden et al. (2000)	Clarithromycin	500 mg	Clinical trial	Symptom, nasal peak flow, weight of nasal secretion, cytokines (IL-6, IL-8 in nasal lavage fluid)	No effect	[21]
RV14	Suzuki et al. (2001)	Bafilomycin A1	0.1 μM	*In vitro* (human tracheal epithelial cells)	RV titer, ICAM-1, cytokines (IL-1β, IL-6, IL-8, TNF-α)	Inhibition	[22]
RV14RV2	Suzuki et al. (2002)	Erythromycin	10 μM	*In vitro* (human tracheal epithelial cells)	RV titer, ICAM-1, cytokines (IL-1β, IL-6, IL-8, TNF-α)	Inhibition	[23]
RV16	Jang et al. (2006)	Clarithromycin	1, 10, 100 μM	*In vitro* (A549 cells)	RV titer, ICAM-1, cytokines (IL-1β, IL-6, IL-8)	Inhibition	[24]
RV14	Inoue et al. (2008)	Erythromycin	10 μM	*In vitro* (human tracheal epithelial cells)	MUC5AC	inhibition	[25]
RV16	Wang et al. (2010)	Clarithromycin	10 μM	*In vitro* (Nasal epithelial cell)	Fn, CEACAM, bacterial adhesion (*S. aureus*, *H. influenza*)	Inhibition	[26]
RV16RV1b	Gielen et al. (2010)	Azithromycin, Erythromycin, Telithromycin	10 μM	*In vitro* (Human bronchial epithelial cells)	mRNA of antiviral genes, type I IFN-β, type III IFN-λ1, IFN-λ2/3, IFN-stimulated genes, cytokines (IL-6, IL-8), RV replication, RV release	Azithromycin: inhibition, erythromycin, telithromycin:no effect	[27]
Respiratory syncytial virus							
RSV	Tahan et al. (2007)	Clarithromycin	15 mg/kg	Clinical trial	Cytokines (IL-4, IL-8, eotaxin, IFN-γ) duration of hospitalization, duration of need for supplemental oxygen, β2-agonist	Effective	[28]
RSV	Kneyber et al. (2008)	Azithromycin	10 mg/kg	Clinical trial	duration of hospitalization, duration of oxygen supplementation and nasogastric tube feeding, RSV symptom score, number of PICU referrals number of patients who received additional antibiotic treatment	No effect	[29]
RSV	Asada et al. (2009)	Bafilomycin A1 Clarithromycin	10 μM	*In vitro* (human tracheal epithelial cells)	Viral titers, cytokines (IL-1β, IL-6, IL-8)	Inhibition	[30]
Influenza virus							
A/Kumamoto/Y5/67	Sato et al. (1998)	Erythromycin	1.0 or 3.3 mg/kg	*In vivo* (Mice)	Survival rate, body weight, cytokines (IFN-γ, TNF-α, IL-1β), NO_2^- /NO_3^-	Inhibition	[31]
A/PR/8/34 (H1N1)	Tsurita et al. (2001)	Clarithromycin	20 mg	*In vivo* (Mice)	Virus yield, severity of pneumonia, cytokines (IL-4, 6, 10, 12)	Inhibition IL-12: elevation	[32]

Table 1: Continued.

Virus	Study	Macrolide	Dose	Method	Parameter	Results	Ref
A/PR/8/34 (H1N1) A/Aichi/2/68 (H3N2) A/Memphis/1/71 (H3N2)	Miyamoto et al. (2008)	Clarithromycin	25 mg/mL	*In vitro* (MDCK cells, human lung epithelial A549 cells)	Multiple infection assay	Inhibition (middle to late stage of the viral replication cycle)	[20]
A/WSN/33 (H1N1) influenza A (H1N1) and (H3N2)	Sawabuchi et al. (2009)	Clarithromycin	5 mg/kg	Clinical trial	Antiviral sIgA, numbers of viral RNA copies, symptom	Inhibition	[33]
type A influenza virus (H3N2)	Yamaya et al. (2010)	Clarithromycin	10 μM	*In vitro* (human tracheal epithelial cells)	Viral titer, cytokines (IL-1β, IL-6), viral RNA	Inhibition	[34]

RV: rhinovirus, IL: interleukin, ICAM-1: intercellular adhesion molecule-1, TNF-α: tumour necrosis factor alpha, Fn: fibronectin, CEACAM: carcinoembryonic antigen-related cell adhesion molecules, IFN: interferon, RSV: respiratory syncytial virus, and MDCK: Mardin-Darby canine kidney.

infection. In contrast, secretion of IL-8 was not inhibited significantly when clarithromycin was added at the time of viral infection. In their study, RV titer, as measured by culture on MRC-5 cells, was reduced by clarithromycin, with the degree of reduction being greater when clarithromycin was added 3 days before infection than it was added at the time of infection. Through these findings, they suggested that, in A549 cells, clarithromycin inhibits the induction of ICAM-1 expression, cytokine elaboration, and viral infection.

Secondary bacterial infection by respiratory viral infection is important pathogenic mechanism in rhinosinusitis. Wang et al. investigated the inhibitory effects of clarithromycin on secondary bacterial infection after RV infection [26]. RV-induced URIs may enhance secondary bacterial infections via upregulation of cell adhesion molecules in the nasal mucosa, leading to acute bacterial rhinosinusitis. *Staphylococcus aureus* binds to human fibronectin (Fn) and *Haemophilus influenza* adheres to the carcinoembryonic antigen-related cell adhesion molecules (CEACAMs) of epithelial cells. In their study, clarithromycin treatment alone had no effect on the baseline levels of mRNA and protein expression of Fn and CEACAM, but significantly reduced the RV-induced increases in the mRNA and protein levels of Fn and CEACAM to the levels found in noninfected controls. They also demonstrated clarithromycin treatment-induced reduction of bacterial adhesion to RV-infected human nasal epithelial cells. Thus, they suggested that clarithromycin may be effective at preventing secondary acute bacterial RS following RV infection.

Several macrolide antibiotics are reported to inhibit airway mucus hypersecretion induced by several stimuli. The main component of mucus is mucin. MUC5AC and MUC5B are reported to constitute 95–98% of secreted mucin in airways. Mucus with a high concentration of MUC5AC or MUC5B has a high viscosity and is likely to cause airway narrowing. Erythromycin attenuated RV14-induced MUC5AC production and secretion in cultured human tracheal epithelial cells [25]. MUC5AC mRNA expression was also attenuated by erythromycin treatment, suggesting that erythromycin affects pretranscriptional mechanisms. Furthermore, erythromycin attenuated RV14-induced p44/42 MAPK activation.

Gielen et al. investigated the anti-RV (RV 1B and RV16) potential of macrolides including azithromycin, erythromycin, and telithromycin, through the induction of antiviral gene mRNA and protein [27]. Azithromycin, but not erythromycin or telithromycin, significantly increased RV 1B- and RV 16-induced IFNs and IFN-stimulated gene mRNA expression and protein production. Furthermore, azithromycin significantly reduced RV replication and release. RV-induced IL-6 and IL-8 protein and mRNA expressions were not significantly reduced by azithromycin before treatment. These results demonstrated that azithromycin has antirhinoviral activity in bronchial epithelial cells by increasing the production of IFN-stimulated genes.

In addition, the duration of macrolide therapy could affect the immune response. *Ex-vivo* studies seem to indicate that short-term administration of macrolides may enhance the immune response, whereas long-term administration results in immunosuppression [39].

RSV bronchiolitis is the most common lower respiratory tract infection in infancy, occurring in 90% of children of 2 yrs or under. Development of an effective therapy against the short-term morbidity by RSV bronchiolitis could be important in reducing subsequent morbidity. RSV causes widespread damage to bronchial epithelium and stimulates epithelial cells to secrete a wide range of pro-inflammatory cytokines and chemokines. IL-8 is a key chemokine produced by RSV-infected airway cells and is involved in the activation and recruitment of neutrophils. Neutrophils play a major role in the pathophysiology of RSV bronchiolitis.

Several reports showed that macrolide antibiotics may also modulate airway inflammation induced by RSV infection [28–30]. Suppressive effects of macrolides on the plasma IL-4, IL-8, and eotaxin levels may have a role in suppression of airway hyperresponsiveness or may inhibit cholinergic neuroeffector transmission in human airway smooth muscle, thereby influencing bronchial tone [31, 39–43]. Macrolides attenuate the release of eotaxin, granulocyte-macrophage colony-stimulating factor (GMCSF), and RANTES. It may also protect epithelial cells at inflamed sites by inhibiting the release of reactive oxygen species from eosinophils [32, 44].

In the RSV infection, RhoA, isoform A of the Ras-homologus (Rho) family, has various functions including stimulus-evoked cell adhesion and motility, enhancement of contractile response, and cytokine production. The activated form of RhoA moves to the cell membrane and is implicated in the RSV infection [30, 45, 46]. Asada et al. reported that bafilomycin A1 and clarithromycin inhibit infection by RSV and decrease the susceptibility of cultured human tracheal epithelial cells to RSV infection, partly through the reduced expression of activated RhoA which is an RSV F protein receptor [30]. Because activated RhoA interacts with the RSV F protein, these findings suggest that clarithromycin may inhibit RSV infection, partly through the reduction of activated RhoA in the cells. Clarithromycin also reduced baseline and RSV infection-induced release of proinflammatory cytokines in supernatant fluids including IL-1β, IL-6, and IL-8 [30]. It has been shown that viral titers in supernatant fluids and RNA of RSV in the human tracheal epithelial cells increased with time, and clarithromycin reduced viral titers of RSV in supernatant fluids concentration-dependently, RNA of RSV replication, and the susceptibility to RSV infection.

Influenza virus is another common cause of respiratory viral infection. Human influenza virus infection causes rapid onset constitutional symptoms, including fever and lower respiratory tract symptoms, and also induces exacerbations of bronchial asthma and chronic obstructive pulmonary disease (COPD) in the winter. Human influenza viruses attach to sialic acid with an α2,6linkage (SAα2,6Gal) on the airway epithelial cells. The viruses are then delivered into the cytoplasm, and ribonucleoproteins (RNPs) of viruses, which include viral RNA, are released from acidic endosomes into the cytoplasm of the cells. There are several reports which showed the efficacy of macrolide antibiotics on influenza virus infection. Miyamoto et al.

showed the ability of clarithromycin in inhibition of human influenza A virus production *in vitro* at a middle-to-late stage of viral replication cycle [20]. They found that treatment with clarithromycin at a final concentration of 25 μg/mL had a strong inhibitory effect on plaque reduction of the tested human influenza A viruses. In addition to decrease of progeny virus production, clarithromycin decreased apoptotic cell numbers of infected host cells. These findings suggested that clarithromycin acts directly on virus-infected cells and contributes to the prevention of virus production by inhibiting viral replication in infected host cells. The influenza virus replication cycle can be divided into 5 steps: (1) binding of viral hemagglutinin to sialic acid receptor on host cell surface (adsorption step), (2) internalization of virus by receptor-mediated endocytosis and fusion of viral HA2 with endosomal membranes triggered by influx of protons through M2 channel (endocytosis and fusion step), (3) release of viral genes into the cytoplasm (uncoating step), (4) packaging of viral proteins with viral genes after viral RNA replication, transcription and translation, and budding of new viruses (packaging and budding step), and (5) release of new viruses by sialidase cleaving sialic acid receptors (release step) [20]. Clarithromycin had no or little inhibitory effect on hemagglutination, hemolysis activity (membrane fusion), and sialidase activity. These results suggest that decrease of progeny virus production is not due to inhibition of viral hemagglutinin and sialidase activities, which play an important role at the beginning and the end of viral replication, respectively. After clarithromycin was incubated with virus-infected cells at different times, it has been found that clarithromycin predominantly inhibited viral replication after viral adsorption to host cells at about the 4–7th hour [20]. Clarithromycin therefore might act on middle-to-late stage of viral replication cycle, presumably *via* blockage of producing viral protein. These findings strongly encourage the potential use of clarithromycin as an anti-influenza virus chemotherapeutic agent.

4.2. Animal Studies. Compare to *in vitro* studies, *in vivo* studies were relatively rare. Further *in vivo* animal studies are needed with various respiratory viruses.

There were several reports which evaluated the effects of macrolide on influenza-virus-induced respiratory infection. Sato et al. evaluated the effects of erythromycin on influenza-virus-induced pneumonia in mice infected with a lethal dose of influenza virus A/Kumamoto/Y5/67 (H2N2) [31]. In their report, erythromycin may have substantial therapeutic value for various acute inflammatory disorders such as influenza-virus-induced pneumonia. The effects were by inhibiting inflammatory cell responses and suppressing nitric oxide (NO) which plays critical role in the pathologic events of various inflammatory diseases, overproduced in the lung. Regarding the NO, erythromycin treatment resulted in a dose-dependent decrease in the level of nitrite/nitrate (metabolites of NO) in the serum and the NO-synthase-(NOS-) inducting potential in the lungs of the virus-infected mice. As a result, administration of erythromycin significantly improved the survival rate of mice infected with influenza virus, and the survival rate of the virus-infected mice increased in a dose-dependent fashion. It has also been found in their study that the induction of IFN-γ in the mouse lung was inhibited and the number of inflammatory cells after virus infection was significantly reduced by erythromycin treatment on day 6 after infection.

In addition to being an antibiotic able to prevent complications and aggravation of symptoms, clarithromycin has been reported to alleviate pneumonia secondary to influenza virus infection in mice [32]. In their study, clarithromycin has been shown to suppress the inflammatory cytokines such as TNF-α, but augment IL-12 production, resulting in alleviation of influenza infection itself in infected mice [32]. These studies indicated that clarithromycin may play a role *in vivo* as an immunomodulator for influenza virus infection.

The protective role of IL-12 against influenza infection was assessed by analyzing the efficacies of orally administered clarithromycin as an immunomodulator and intranasal administration of recombinant IL-12 in influenza-virus-infected mice. Tsurita et al. reported that, in infected mice, clarithromycin at 20 mg/mouse/day significantly elevated the levels of IL-12 and IFN-γ in the bronchoalveolar lavage on days 2 and 3, respectively, but the levels in the sera were not affected [32]. In accordance with the locally elevated level of f IL-12, clarithromycin reduced virus yield and the number of infiltrated cells, the severity of pneumonia, and mortality of the treated mice. Thus, the augmentation of IL-12 production in the respiratory tract was essential in reducing virus yield in the early phase of influenza and may be crucial for recovery from influenza infection [32].

There is another report which revealed the effect of macrolides on reducing the receptor for virus on the airway epithelial cells and reducing entry of virus into the cytoplasm. Human seasonal influenza viruses and classical H1N1 swine influenza viruses bind to SAα2,6Gal, and most avian and equine viruses bind to SAα2,3Gal [47]. Clarithromycin reduced the expression of SAα2,6Gal, a receptor for human influenza, on the mucosal surface of human tracheae, and reduced the number of acidic endosomes from which viral RNPs enter into the cytoplasm. These findings suggest that a clinically used clarithromycin may inhibit type A seasonal human influenza virus infection via reducing its receptor on the airway epithelial cells and reducing entry of viral RNPs, into the cytoplasm. Although the mechanisms for the reduction of SAα2,6Gal expression by clarithromycin are uncertain, these effects are similar to those of clarithromycin on the reduced expression of activated RhoA, one of receptors for RSV, and on inhibition of RSV infection [30]. These effects are also similar to those of erythromycin on the reduced expression of ICAM-1, a receptor for RV, and on inhibition of the RV infection.

Recently, Yamaya et al. demonstrated that clarithromycin reduces FluA viral titers and cytokines secretion in supernatant fluids and susceptibility of the cells to infection by the virus [34].

4.3. Clinical Studies. Although numerous *in vivo* studies have established that macrolides have inhibitory effects on respiratory viral infections, the outcomes of clinical studies

are controversial and the clinical benefits of macrolides in respiratory virus infection are still uncertain.

In *in vitro* study, Jang et al. reported that clarithromycin inhibits the RV-induced induction of ICAM-1 expression, cytokine elaboration, and viral infection in A549 cells [24]. However, there is a controversial report performed in a double-blinded clinical trial showing that clarithromycin treatment had little or no effect on the severity of cold symptoms or the intensity of neutrophilic nasal inflammation [21]. The discrepancy between the results of *in vitro* study by Jang et al. and those of the *in vivo* clinical trial may be due to differences in dosage or mode of treatment. For example, in the clinical trial, 1,000 $mg \cdot day^{-1}$ of clarithromycin, a higher dose than the 250 $mg \cdot day^{-1}$ usually used for low-dose, long-term treatment [48], was started 24 h before inoculation of RV. However, it was found that clarithromycin started 3 days before RV infection was more effective than clarithromycin started at the time of infection and that 10 μM clarithromycin, the usual blood level in clinical use, was more effective than 100 μM in reducing viral titer and cytokine secretion.

In addition, there are controversies about the effective duration of macrolide therapy. *Ex vivo* studies seem to indicate that short-term administration of macrolides may enhance the immune response, whereas long-term administration results in immunosuppression [39]. However, other study described that short-term administration of a macrolide is not beneficial for acute uncomplicated colds caused by RV infection [21].

Severe RSV infections during early infancy are associated with the excessive production of Th2 cytokines, which has been suggested as a risk factor for the later development of asthma and allergic sensitization [49]. Macrolides may normalize the Th1/Th2 lymphocyte balance [50]. They regulate immunologic activities by enhancing production of IFN-γ and by reducing production of IL-4 and IL-5. Treatment that restores the Th1/Th2 cytokine balance to the relative type 1 predominance may ameliorate short- and long-term effects of RSV disease. Tahan et al. studied the use of 3 weeks of macrolide therapy in the treatment of RSV bronchiolitis in a double-blind, randomized, placebo-controlled trial [28]. In their study, treatment with clarithromycin daily for 3 weeks was associated with a statistically significant reduction in the length of hospital stay, the duration of need for supplemental oxygen, the need for β2-agonist treatment, and readmission to the hospital within 6 months after discharge. Furthermore, there were significant decreases in plasma IL-4, IL-8, and eotaxin levels after 3 weeks of treatment with clarithromycin. As previously described, RSV is the leading cause of viral lower respiratory tract disease (LRTD) in infants and young children. Nearly half of all hospitalized infants with RSV LRTD are treated with antibiotics. In contrast to favorable effects of macrolides on RSV infection reported in number of papers, Kneyber et al., however, reported that the use of macrolide antibiotics would not lead to a reduced duration of hospitalization in mild-to-moderate RSV LRTD [29]. In their study, azithromycin was not associated with a stronger resolution of clinical symptoms represented by the RSV symptom score.

Various inflammatory mediators are suggested to be associated with the pathogenesis and severity of influenza virus infection [42]. Increases in proinflammatory cytokines and monokines, including interleukin IL-1, IL-6, and IL-8, are observed in the serum in the patients and in the lung of mice infected with influenza virus [41, 42]. Although the clinical benefits of macrolides in influenza virus infection are still uncertain, reduction of proinflammatory cytokines by clarithromycin may modulate influenza-virus-induced inflammation and severity of the disease and may prevent COPD exacerbations. Clarithromycin inhibits the activation of NF-κB, migration of neutrophils, and the production of proinflammatory cytokines by interfering with extracellular signal-regulated kinases [39]. It also promotes the induction of sIgA and IgG in the airway fluids of mice infected with influenza A virus [51]. Sawabuchi et al. investigated the immunomodulatory effects of clarithromycin on mucosal immune responses in the nasopharyngeal aspiration of pediatric patients with influenza [33]. In their study, low induction of antiviral sIgA which represents the first immunological barrier to pathogens was observed in the oseltamivir, an antiviral neuraminidase inhibitor, treatment group. However, the addition of clarithromycin to oseltamivir augmented sIgA production and restored local mucosal sIgA levels, indicating that clarithromycin boosted the nasopharyngeal mucosal immune response in children presenting with influenza A, even in those treated with oseltamivir who had low production of mucosal anti-viral sIgA [33].

5. Conclusions

Macrolides possess anti-inflammatory and immunomodulatory properties extending beyond their antibacterial activity. They downregulate the inflammatory cascade, attenuate excessive cytokine production in viral infections, and they may reduce virus-related exacerbations. Based on existing evidence, macrolides may be considered as promising treatment option in treatment of respiratory viral infections. However, confirmation in larger series, as well as identification of their precise mechanism affecting virus-induced inflammation or viral replicationn, is still awaited.

References

[1] F. Blasi, M. Cazzola, P. Tarsia, S. Aliberti, C. Baldessari, and V. Valenti, "Telithromycin in lower respiratory tract infections," *Future microbiology*, vol. 1, supplement 1, pp. 7–16, 2006.

[2] A. G. Buret, "Immuno-modulation and anti-inflammatory benefits of antibiotics: the example of tilmicosin," *Canadian Journal of Veterinary Research*, vol. 74, no. 1, pp. 1–10, 2010.

[3] S. Kanoh and B. K. Rubin, "Mechanisms of action and clinical application of macrolides as immunomodulatory medications," *Clinical Microbiology Reviews*, vol. 23, no. 3, pp. 590–615, 2010.

[4] H. Nakamura, S. Fujishima, T. Inoue et al., "Clinical and immunoregulatory effects of roxithromycin therapy for chronic respiratory tract infection," *European Respiratory Journal*, vol. 13, no. 6, pp. 1371–1379, 1999.

[5] D. Wales and M. Woodhead, "The anti-inflammatory effects of macrolides," *Thorax*, vol. 54, supplement 2, pp. S58–S62, 1999.

[6] E. Tagaya, J. Tamaoki, M. Kondo, and A. Nagai, "Effect of a short course of clarithromycin therapy on sputum production in patients with chronic airway hypersecretion," *Chest*, vol. 122, no. 1, pp. 213–218, 2002.

[7] T. Shimizu, S. Shimizu, R. Hattori, E. C. Gabazza, and Y. Majima, "In vivo and in vitro effects of macrolide antibiotics on mucus secretion in airway epithelial cells," *American Journal of Respiratory and Critical Care Medicine*, vol. 168, no. 5, pp. 581–587, 2003.

[8] T. Mitsuyama, T. Tanaka, K. Hidaka, M. Abe, and N. Hara, "Inhibition by erythromycin of superoxide anion production by human polymorphonuclear leukocytes through the action of cyclic AMP-dependent protein kinase," *Respiration*, vol. 62, no. 5, pp. 269–273, 1995.

[9] K. Aoshiba, A. Nagai, and K. Konno, "Erythromycin shortens neutrophil survival by accelerating apoptosis," *Antimicrobial Agents and Chemotherapy*, vol. 39, no. 4, pp. 872–877, 1995.

[10] T. Yamaryo, K. Oishi, H. Yoshimine, Y. Tsuchihashi, K. Matsushima, and T. Nagatake, "Fourteen-member macrolides promote the phosphatidylserine receptor-dependent phagocytosis of apoptotic neutrophils by alveolar macrophages," *Antimicrobial Agents and Chemotherapy*, vol. 47, no. 1, pp. 48–53, 2003.

[11] Y. Aoki and P. N. Kao, "Erythromycin inhibits transcriptional activation of NF-κB, but not NFAT, through calcineurin-independent signaling in T cells," *Antimicrobial Agents and Chemotherapy*, vol. 43, no. 11, pp. 2678–2684, 1999.

[12] J. A. Belser, H. Zeng, J. M. Katz, and T. M. Tumpey, "Infection with highly pathogenic H7 influenza viruses results in an attenuated proinflammatory cytokine and chemokine response early after infection," *Journal of Infectious Diseases*, vol. 203, no. 1, pp. 40–48, 2011.

[13] J. A. Belser, D. A. Wadford, C. Pappas et al., "Pathogenesis of pandemic influenza A (H1N1) and triple-reassortant swine influenza A (H1) viruses in mice," *Journal of Virology*, vol. 84, no. 9, pp. 4194–4203, 2010.

[14] P. C. Y. Woo, E. T. K. Tung, K. H. Chan, C. C. Y. Lau, S. K. P. Lau, and K. Y. Yuen, "Cytokine profiles induced by the novel swine-origin influenza A/H1N1 virus: implications for treatment strategies," *Journal of Infectious Diseases*, vol. 201, no. 3, pp. 346–353, 2010.

[15] S. M. Lee, J. L. Gardy, C. Y. Cheung et al., "Systems-level comparison of host-responses elicited by avian H5N1 and seasonal H1N1 influenza viruses in primary human macrophages," *PloS ONE*, vol. 4, no. 12, Article ID e8072, 2009.

[16] C. Zhang, Y. Xu, L. Jia et al., "A new therapeutic strategy for lung tissue injury induced by influenza with CR2 targeting complement inhibitor," *Virology Journal*, vol. 7, p. 30, 2010.

[17] L. N. Shishkina, V. E. Nebolsin, M. O. Skarnovich et al., "In vivo efficacy of Ingavirin against pandemic A(H1N1/09)v influenza virus," *Antibiotiki i Khimioterapiya*, vol. 55, no. 5-6, pp. 32–35, 2010.

[18] P. Zarogoulidis, N. Papanas, I. Kioumis, E. Chatzaki, E. Maltezosand, and K. Zarogoulidis, "Macrolides: from in vitro anti-inflammatory and immunomodulatory properties to clinical practice in respiratory diseases," *European Journal of Clinical Pharmacology*. In press.

[19] O. Zhirnov and H. D. Klenk, "Human influenza A viruses are proteolytically activated and do not induce apoptosis in CACO-2 cells," *Virology*, vol. 313, no. 1, pp. 198–212, 2003.

[20] D. Miyamoto, S. Hasegawa, N. Sriwilaijaroen et al., "Clarithromycin inhibits progeny virus production from human influenza virus-infected host cells," *Biological and Pharmaceutical Bulletin*, vol. 31, no. 2, pp. 217–222, 2008.

[21] J. A. Abisheganaden, P. C. Avila, J. L. Kishiyama et al., "Effect of clarithromycin on experimental rhinovirus-16 colds: a randomized, double-blind, controlled trial," *American Journal of Medicine*, vol. 108, no. 6, pp. 453–459, 2000.

[22] T. Suzuki, M. Yamaya, K. Sekizawa et al., "Bafilomycin A1 inhibits rhinovirus infection in human airway epithelium: effects on endosome and ICAM-1," *American Journal of Physiology*, vol. 280, no. 6, pp. L1115–L1127, 2001.

[23] T. Suzuki, M. Yamaya, K. Sekizawa et al., "Erythromycin inhibits rhinovirus infection in cultured human tracheal epithelial cells," *American Journal of Respiratory and Critical Care Medicine*, vol. 165, no. 8, pp. 1113–1118, 2002.

[24] Y. J. Jang, H. J. Kwon, and B. J. Lee, "Effect of clarithromycin on rhinovirus-16 infection in A549 cells," *European Respiratory Journal*, vol. 27, no. 1, pp. 12–19, 2006.

[25] D. Inoue, H. Kubo, T. Sasaki et al., "Erythromycin attenuates MUC5AC synthesis and secretion in cultured human tracheal cells infected with RV14," *Respirology*, vol. 13, no. 2, pp. 215–220, 2008.

[26] J. H. Wang, S. H. Lee, H. J. Kwon, and Y. J. Jang, "Clarithromycin inhibits rhinovirus-induced bacterial adhesions to nasal epithelial cells," *Laryngoscope*, vol. 120, no. 1, pp. 193–199, 2010.

[27] V. Gielen, S. L. Johnston, and M. R. Edwards, "Azithromycin induces anti-viral responses in bronchial epithelial cells," *European Respiratory Journal*, vol. 36, no. 3, pp. 646–654, 2010.

[28] F. Tahan, A. Ozcan, and N. Koc, "Clarithromycin in the treatment of RSV bronchiolitis: a double-blind, randomised, placebo-controlled trial," *European Respiratory Journal*, vol. 29, no. 1, pp. 91–97, 2007.

[29] M. C. J. Kneyber, J. B. M. Van Woensel, E. Uijtendaal, C. S. P. M. Uiterwaal, and J. L. L. Kimpen, "Azithromycin does not improve disease course in hospitalized infants with respiratory syncytial virus (RSV) lower respiratory tract disease: a randomized equivalence trial," *Pediatric Pulmonology*, vol. 43, no. 2, pp. 142–149, 2008.

[30] M. Asada, M. Yoshida, T. Suzuki et al., "Macrolide antibiotics inhibit respiratory syncytial virus infection in human airway epithelial cells," *Antiviral Research*, vol. 83, no. 2, pp. 191–200, 2009.

[31] K. Sato, M. Suga, T. Akaike et al., "Therapeutic effect of erythromycin on influenza virus-induced lung injury in mice," *American Journal of Respiratory and Critical Care Medicine*, vol. 157, no. 3, pp. 853–857, 1998.

[32] M. Tsurita, M. Kurokawa, M. Imakita, Y. Fukuda, Y. Watanabe, and K. Shiraki, "Early augmentation of interleukin (IL)-12 level in the airway of mice administered orally with clarithromycin or intranasally with IL-12 results in alleviation of influenza infection," *Journal of Pharmacology and Experimental Therapeutics*, vol. 298, no. 1, pp. 362–368, 2001.

[33] T. Sawabuchi, S. Suzuki, K. Iwase et al., "Boost of mucosal secretory immunoglobulin A response by clarithromycin in paediatric influenza," *Respirology*, vol. 14, no. 8, pp. 1173–1179, 2009.

[34] M. Yamaya, K. Shinya, Y. Hatachi et al., "Clarithromycin inhibits type A seasonal influenza virus infection in human airway epithelial cells," *Journal of Pharmacology and Experimental Therapeutics*, vol. 333, no. 1, pp. 81–90, 2010.

[35] A. Yuta, W. J. Doyle, E. Gaumond et al., "Rhinovirus infection induces mucus hypersecretion," *American Journal of Physiology*, vol. 274, no. 6, pp. L1017–L1023, 1998.

[36] M. Terajima, M. Yamaya, K. Sekizawa et al., "Rhinovirus infection of primary cultures of human tracheal epithelium: role of ICAM-1 and IL-1β," *American Journal of Physiology*, vol. 273, no. 4, pp. L749–L759, 1997.

[37] A. Papi, N. G. Papadopoulos, L. A. Stanciu et al., "Reducing agents inhibit rhinovirus-induced up-regulation of the rhinovirus receptor intercellular adhesion molecule-1 (ICAM-1) in respiratory epithelial cells," *The FASEB journal*, vol. 16, no. 14, pp. 1934–1936, 2002.

[38] M. Yamaya and H. Sasaki, "The pathogenesis and therapy of virus infection-induced senile bronchial asthma," *Japanese Journal of Geriatrics*, vol. 37, no. 6, pp. 464–468, 2000.

[39] M. T. Labro, "Anti-inflammatory activity of macrolides: a new therapeutic potential?" *Journal of Antimicrobial Chemotherapy*, vol. 41, supplement 2, pp. 37–46, 1998.

[40] J. Schwarze, G. Cieslewicz, A. Joetham et al., "Critical roles for interleukin-4 and interleukin-5 during respiratory syncytial virus infection in the development of airway hyperresponsiveness after airway sensitization," *American Journal of Respiratory and Critical Care Medicine*, vol. 162, no. 2 I, pp. 380–386, 2000.

[41] A. P. Konstantinos and J. F. Sheridan, "Stress and influenza viral infection: modulation of proinflammatory cytokine responses in the lung," *Respiration Physiology*, vol. 128, no. 1, pp. 71–77, 2001.

[42] R. Deng, M. Lu, C. Korteweg et al., "Distinctly different expression of cytokines and chemokines in the lungs of two H5N1 avian influenza patients," *Journal of Pathology*, vol. 216, no. 3, pp. 328–336, 2008.

[43] B. P. Arulanandam, M. O'Toole, and D. W. Metzger, "Intranasal interleukin-12 is a powerful adjuvant for protective mucosal immunity," *Journal of Infectious Diseases*, vol. 180, no. 4, pp. 940–949, 1999.

[44] E. Sato, D. K. Nelson, S. Koyama, J. C. Hoyt, and R. A. Robbins, "Erythromycin modulates eosinophil chemotactic cytokine production by human lung fibroblasts in vitro," *Antimicrobial Agents and Chemotherapy*, vol. 45, no. 2, pp. 401–406, 2001.

[45] T. Ishizaki, M. Maekawa, K. Fujisawa et al., "The small GTP-binding protein Rho binds to and activates a 160 kDa Ser/Thr protein kinase homologous to myotonic dystrophy kinase," *EMBO Journal*, vol. 15, no. 8, pp. 1885–1893, 1996.

[46] Q. Lu, E. O. Harrington, C. M. Hai et al., "Isoprenylcysteine carboxyl methyltransferase modulates endothelial monolayer permeability: involvement of RhoA carboxyl methylation," *Circulation Research*, vol. 94, no. 3, pp. 306–315, 2004.

[47] G. N. Rogers and J. C. Paulson, "Receptor determinants of human and animal influenza virus isolates: differences in receptor specificity of the H3 hemagglutinin based on species of origin," *Virology*, vol. 127, no. 2, pp. 361–373, 1983.

[48] A. Cervin, O. Kalm, P. Sandkull, and S. Lindberg, "One-year low-dose erythromycin treatment of persistent chronic sinusitis after sinus surgery: clinical outcome and effects on mucociliary parameters and nasal nitric oxide," *Otolaryngology*, vol. 126, no. 5, pp. 481–489, 2002.

[49] N. Sigurs, P. M. Gustafsson, R. Bjarnason et al., "Severe respiratory syncytial virus bronchiolitis in infancy and asthma and allergy at age 13," *American Journal of Respiratory and Critical Care Medicine*, vol. 171, no. 2, pp. 137–141, 2005.

[50] M. J. Parnham, "Immunomodulatory effects of antimicrobials in the therapy of respiratory tract infections," *Current Opinion in Infectious Diseases*, vol. 18, no. 2, pp. 125–131, 2005.

[51] H. Kido, Y. Okumura, H. Yamada, T. Q. Le, and M. Yano, "Proteases esesential for human influenza virus entry into cells and their inhibitors as potential therapeutic agents," *Current Pharmaceutical Design*, vol. 13, no. 4, pp. 405–414, 2007.

Hindawi Publishing Corporation
Mediators of Inflammation
Volume 2012, Article ID 410232, 9 pages
doi:10.1155/2012/410232

Research Article

Erythromycin Enhances $CD4^+Foxp3^+$ Regulatory T-Cell Responses in a Rat Model of Smoke-Induced Lung Inflammation

Jing Bai, Shi-Lin Qiu, Xiao-Ning Zhong, Qiu-Ping Huang, Zhi-Yi He, Jian-Quan Zhang, Guang-Nan Liu, Mei-Hua Li, and Jing-Min Deng

Department of Respiratory Medicine, The First Affiliated Hospital of Guangxi Medical University, Nanning 530000, China

Correspondence should be addressed to Xiao-Ning Zhong, xnzhong101@sina.com

Received 17 January 2012; Revised 1 April 2012; Accepted 1 April 2012

Academic Editor: Kazuhito Asano

Heavy smoking can induce airway inflammation and emphysema. Macrolides can modulate inflammation and effector T-cell response in the lungs. However, there is no information on whether erythromycin can modulate regulatory T-cell (Treg) response. This study is aimed at examining the impact of erythromycin on Treg response in the lungs in a rat model of smoking-induced emphysema. Male Wistar rats were exposed to normal air or cigarette smoking daily for 12 weeks and treated by gavage with 100 mg/kg of erythromycin or saline daily beginning at the forth week for nine weeks. The lung inflammation and the numbers of inflammatory infiltrates in bronchoalveolar lavage fluid (BALF) were characterized. The frequency, the number of Tregs, and the levels of Foxp3 expression in the lungs and IL-8, IL-35, and TNF-α in BALF were determined by flow cytometry, RT-PCR and ELISA, respectively. Treatment with erythromycin reduced smoking-induced inflammatory infiltrates, the levels of IL-8 and TNF-α in the BALF and lung damages but increased the numbers of $CD4^+Foxp3^+$ Tregs and the levels of Foxp3 transcription in the lungs, accompanied by increased levels of IL-35 in the BALF of rats. Our novel data indicated that erythromycin enhanced Treg responses, associated with the inhibition of smoking-induced inflammation in the lungs of rats.

1. Introduction

Chronic obstructive pulmonary disease (COPD) is one of the most prevalent illnesses worldwide and is estimated as the third leading cause of mortality in 2020 [1]. COPD is characterised by airflow limitation that is poorly reversible. The pathogenesis of COPD is usually progressive and associated with an abnormal inflammatory response in the lungs, particularly in response to noxious particles or gases, such as cigarette smoke [2]. Recently, COPD-associated inflammation is thought to be an autoimmune response induced by smoking or pathogenic microbials that activate lymphocytes and antigen-presenting cells [3]. Previous studies have shown that Th1 cells are predominantly associated with the development of emphysematous lungs, leading to the progression of COPD although the mechanisms by which tobacco smoke is associated with Th1 immunity remain unclear [4–7].

$CD4^+CD25^+Foxp3^+$ regulatory T cells (Tregs) are crucial regulators of the maintenance of peripheral immunologic tolerance, and Tregs can suppress effectors Th1, Th2, and Th17 responses, inflammation, and autoimmune responses [8, 9]. Tregs can secrete IL-35, which inhibits inflammatory responses [10]. A deficiency in Treg regulation has been associated with the development of many Th1-mediated chronic inflammation and autoimmune disorders, including type 1 diabetes, multiple sclerosis, atherosclerosis, and rheumatoid arthritis [11–14]. Interestingly, decreased numbers of Tregs were detected in the lungs of subjects with emphysema [15], suggesting that Tregs participate in the regulation of emphysema-related inflammation in the lungs. However, little is known on what therapeutic strategies could increase the number of Tregs and IL-35 responses in the lungs of subjects with emphysema-related inflammation. Currently, anti-inflammatory steroids have been often used for the treatment of COPD patients with acute exacerbation, but the therapeutic efficacy of steroids is limited [16, 17]. Therefore, discovery of new therapeutic reagents will be of great significance in the management of patients with COPD.

Erythromycin is a 14-membered ring macrolide antibiotic and has been prescribed for the treatment of various respiratory infections. Erythromycin can inhibit mitogen-stimulated human T-cell proliferation and cytokine production, which are associated with inhibition of the MAPK and NF-κB activation [18, 19]. Furthermore, erythromycin can ameliorate chronic inflammation in various animal models [20, 21]. In addition, long-term treatment with low doses of a 14-membered ring macrolide is beneficial for patients with airway inflammatory diseases, such as diffuse panbronchiolitis (DPB) [22], cystic fibrosis [23, 24], bronchiectasis [25], and bronchial asthma [26, 27]. Our previous study has reported that treatment with erythromycin reduces the number of smoking-induced airway inflammatory infiltrates and airway remodelling in the lungs of rodents [28]. However, little is known on whether treatment with erythromycin could modulate Treg and IL-35 responses in the lungs.

In this study, we evaluated the impact of treatment with erythromycin for nine weeks on cigarette smoking-induced inflammation in a rat model of emphysema. Our findings indicated that treatment with erythromycin not only reduced smoking-induced airway inflammation and emphysema but also increased Treg infiltrates and IL-35 production in the lungs of rats.

2. Materials and Methods

2.1. Animals and Treatments. Male Wistar rats at 12 weeks of age were obtained from the Animal Research Center of Guangxi Medical University. The animals were housed individually in standard laboratory cages with free access to standard food and tap water *ad libitum*. The experimental protocols were established, according to the guidelines of NIH Animal Research Care and were approved by the Animal Research Care Committee of Guangxi Medical University.

Individual rats ($n = 40$) were exposed either to room air (control) or to cigarette smoke, as described previously [28]. Briefly, groups of rats ($n = 20$ per group) were exposed to tobacco smoke with 20 cigarettes (Nanning Jiatianxia unfiltered cigarettes: 12 mg of tar and 0.9 mg of nicotine) in a closed 0.54 m^3 space for 2 hours daily for six consecutive days per week for 12 consecutive weeks. As a result, an optimal ratio of smoking to air at 1 : 6 was obtained and the levels of oxygen exposed by the rats were kept at a $21 \pm 1\%$, which is similar to atmospheric oxygen concentrations. The rats tolerated the cigarette smoke without evidence of toxicity (the levels of serum carboxyhemoglobin in rats were at ~10%, and no weight loss in the rats was observed). The levels of serum carboxyhemoglobin in the smoking rats ($n = 20$) were $8.3 \pm 1.4\%$, as compared with $1.0 \pm 0.2\%$ in the control rats ($n = 20$), which were similar to the concentrations of blood carboxyhemoglobin of human smokers [29].

Three weeks after exposure to cigarette smoke, the rats were randomized and treated by gavage with 100 mg/kg/d of erythromycin (Meichuang Pharmaceuticals, Dailian, China) in saline (1 mL) or saline alone daily for nine weeks, respectively. We used this dose based on our previous findings to show that treatment with 100 mg/kg/d of erythromycin inhibits smoke-related lung inflammation without obvious adverse effect [28]. The rats that exposed to regular air were randomized and treated with erythromycin or saline in the same manner. Accordingly, there were four groups of rats ($n = 10$ per group). The normal group of rats were exposed to regular air and treated with saline (group N); the smoking group of rats were exposed to smoking air for 12 weeks and treated with saline (group S); the erythromycin group of rats were exposed to smoking air for 12 weeks and treated with erythromycin (group E); the control group of rats were exposed to regular air and treated with erythromycin (group C).

One day after the last smoking, animals were injected intraperitoneally with 20 mg/kg pentobarbital and subjected to a thoracotomy. Their left lungs were lavaged through an intratracheal cannula three times with 2 mL of cold saline, and the bronchoalveolar lavage fluid (BALF) samples were collected. The left lungs were used for the preparation of single cell suspension. The lower lobes of their right lungs were fixed in 10% formalin for pathological examination.

2.2. Histology. The fixed lower lobes of the right lungs were embedded in paraffin, and the midsagittal sections of the lungs were stained with hematoxylin and eosin (H&E), followed by examining under a light microscope. Three nonconsecutive lung sections from each animal and three nonoverlapping random fields from each section were examined for the quantification of lung damages. Alveolar airspace enlargement was assessed by the mean linear intercept (MLI) by two independent individuals in a blinded manner, as described previously [30]. Briefly, multiple digital images of histological sections were systematically captured at $100 \times$ magnification. Images were overlaid with a 10×10 grid (1 mm^2), and the MLI was established from every second image (i.e., in a checkerboard fashion, averaging six images for each rat). The distribution of the MLI values of all the digital photographs was assessed using frequency distribution analysis and characterized using a Gaussian model.

2.3. Characterization of Inflammatory Cells in BALF. The collected BALF samples from the left lung tissues were centrifuged, and their supernatants were stored at -80°C for ELISA analysis. The pelleted cells were resuspended in PBS and a portion of the cells (1×10^5 cells) was subjected to cytospin centrifugation on glass slides and fixed with methanol, followed by staining with May-Grünwald-Giemsa solution, and a differential cell count was performed under a light microscope, according to morphological characteristics.

2.4. Measurement of IL-8, IL-35, and TNF-α in BALF. The concentrations of IL-8, IL-35, and TNF-α in BALF were measured with a multiplex-enzyme-linked immunosorbent assay (ELISA) system, according to the manufacturers' instructions (Lincoplex Systems, St Charles, MO, USA).

2.5. Lung Cell Preparation. A single-cell suspension of whole left lung tissue was prepared by combined procedures of

mechanical fragmentation, enzymatic digestion, and centrifugation, as described in previous studies [5, 15]. The prepared lung cells were used for flow cytometry analyses. Briefly, lungs were flushed via the right ventricle with 10 mL of warm (37°C) HBSS (calcium and magnesium free) containing 5% fetal bovine serum (FBS, Sigma, Beijing, China), 100 U/mL of penicillin, and 100 μg/mL of streptomycin (Gibco BRL). The lungs were then cut into small pieces (~2 mm in diameter) and digested with 150 U/mL of collagenase (Worthington Biochemical, Freehold, NJ, USA) in HBSS with being shaken at 37°C for 1 h. Using a plunger from a 5-mL syringe, the lung pieces were triturated through a mess of 100 μM into HBSS, and the resulting cell suspension was filtered through nylon mesh. The cells were washed twice, and mononuclear cells were isolated using density centrifugation in 30% percoll (Pharmacia, Uppsala, Sweden). The total numbers of cells were counted. The collected leukocytes (1×10^6 cells) were used for flow cytometry analysis and the remaining cells were used for the extraction of total RNA for RT-PCR analysis.

2.6. Flow Cytometry. The collected cells (1×10^6) from individual rats were stained with PE-Cy5-conjugated anti-CD4 (clone: OX-35) or its isotype control (BD Pharmingen, San Diego, CA, USA) at 4°C for 45 minutes, fixed, permeabilized, and stained with PE-conjugated anti-Foxp3 (clone: FJK16s) or its isotype control (eBioscience, Wembley, UK) at 4°C for another 40 minutes. The frequency and the number of Tregs were determined by flow cytometry on a FACSCalibur (BD PharMingen) and analysed by FCS Express software.

2.7. RNA Isolation and RT-PCR. Total RNA was extracted from the lung cells of individual rats with TRIzol reagent, according to the manufacturers' instructions (Invitrogen, Carlsbad, CA, USA). The quality and quantity of total RNA were analysed by a spectrophotometer. The RNA samples were reversely transcribed into cDNA using a reverse transcription kit (Finn-zymes, Espoo, Finland) and oligo (dT) primers. The relative levels of Foxp3 mRNA transcripts to control β-actin in individual samples were characterized by quantitative RT-PCR using SYBR Green on a LightCycler (iCycler IQ, BioRad, USA) and the specific primers. The sequences of primers were forward 5′-GGAGATTACTGCCCTGGCTCCTA-3′, and reverse 5′-GACTCATCGTACTCCTGCTTGCTG-3′ for β-actin and forward 5′-TGAGCTGGCTGCAATTCTGG-3′ and reverse 5′-ATCTAGCTGCTCTGCATGAGGTGA-3′ for Foxp3. The PCR amplifications were performed in triplicate at 95°C for 30 sec and subjected to 40 cycles of 95°C for 5 sec and 60°C for 30 sec. The values of Foxp3 mRNA transcripts in each sample were normalized to that of β-actin and the relative levels of Foxp3 mRNA transcripts were calculated.

2.8. Statistical Analysis. Data are expressed as means ± SD. Differences among groups were analysed using the analysis of variance (ANOVA) and post hoc Student's t-test, the Kruskal-Wallis test, and the Mann-Whitney U-test where applicable using statistical package SPSS 11.0 (SPSS, Chicago, IL, USA). The association between two variants was analyzed using Spearman's rank method. A P value of <0.05 was considered statistically significant.

3. Results

3.1. Treatment with Erythromycin Reduces the Smoking-Induced Lung Damages in Rats. Following smoking for 12 weeks and treatment with erythromycin for 9 weeks, the lung tissue sections of the different groups of rats were stained with H&E and subjected to quantitative analysis of the lung airspace (Figure 1). We observed the enlargement of air spaces and many inflammatory infiltrates in the lungs of the smoking rats. Quantitative analysis indicated that there was no significant difference in the MLI values between the N and C groups of rats. In contrast, the MLI values in the S and E group of rats were significantly greater than that in the N and C groups of rats ($P < 0.05$), demonstrating that long-term heavy smoking-induced lung emphysema in rats. Interestingly, the MLI values in the E groups of rats were significantly less than that in the S group of rats although they remained greater than that in controls. In addition, treatment with erythromycin mitigated smoke-induced histological damage in the lungs of rats, consistent with our previous observation [28]. These data indicated that treatment with erythromycin significantly diminished smoking-related emphysema in the lungs of rats.

3.2. Treatment with Erythromycin Modulates the Smoking-Induced Inflammatory Infiltrates in BALF. To quantify the airway inflammation response, we evaluated the numbers of inflammatory infiltrates in BALF and found significantly increased numbers of total infiltrates, particularly macrophages, lymphocytes, and neutrophils in the BALF from the smoking rats, as compared with that in the N and C groups of rats ($P < 0.05$, Figure 2). In contrast, the total numbers of inflammatory infiltrates, macrophages, lymphocytes, and neutrophils in the BALF from the erythromycin-treated smoking rats were reduced significantly, as compared with those in the smoking rats without erythromycin treatment. In addition, treatment with erythromycin did not cause obvious adverse effect in rats, consistent with our previous findings [28]. These data demonstrated that treatment with erythromycin significantly mitigated smoking-induced inflammatory cell infiltration in the lungs of rats.

3.3. Treatment with Erythromycin Alters the Levels of TNF-α and IL-8 in BALF. Analysis of the concentrations of TNF-α and IL-8 in the BALF indicated that significantly higher levels of TNF-α and IL-8 were detected in BLAF from the smoking rats, as compared with that in the N and C groups of rats (Figure 3). Furthermore, the levels of TNF-α and IL-8 in BALF from the smoking rats that had been treated with erythromycin were significantly lower than that in the smoking rats without erythromycin treatment. Apparently, treatment with erythromycin inhibited the smoking-induced proinflammatory cytokine production in the lungs.

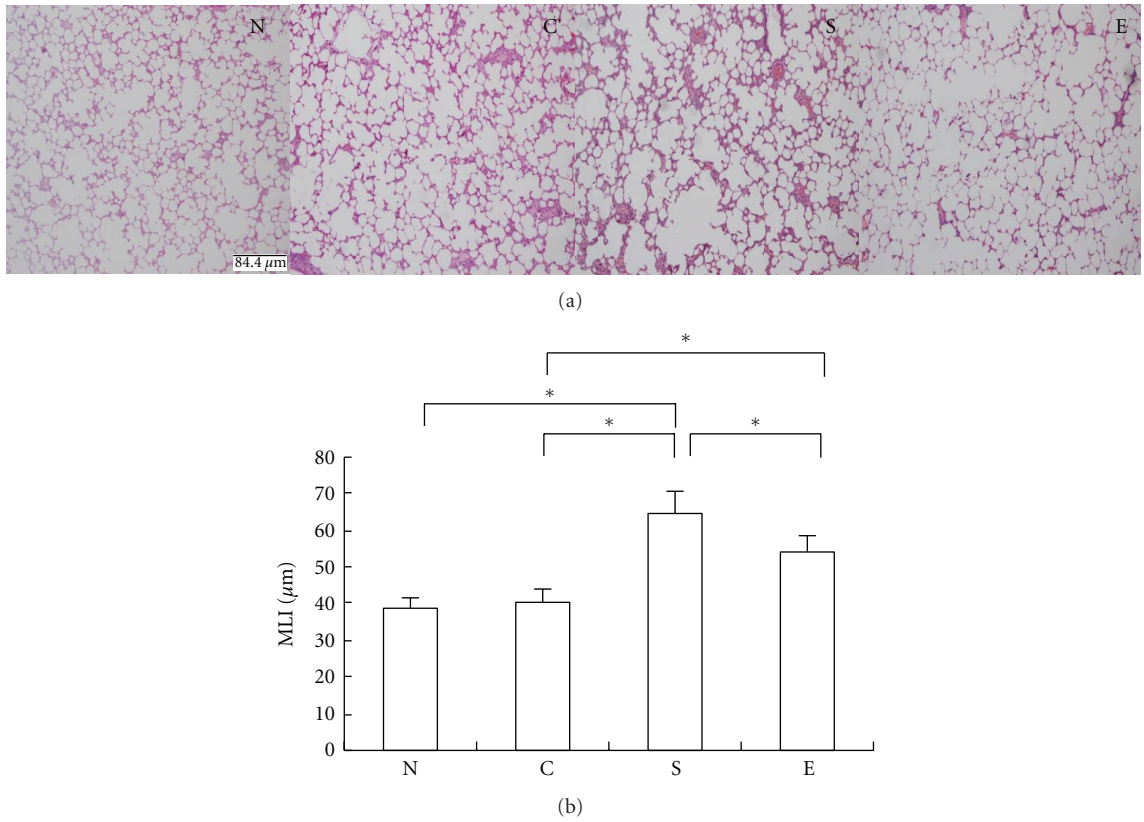


Figure 1: Treatment with erythromycin protects against the smoking-induced emphysema in rats. The lung tissue sections from different groups of rats were subjected to H&E staining, and the alveolar airspace enlargement was assessed using MLI by two independent individuals in a blinded manner. Data are representative images or expressed as mean value ± SD of each group of rats ($n = 10$) from five separate experiments. (a) Morphological changes in the lungs of rats (magnification × 100). (b) Quantitative analysis of alveolar airspace. Group N: rats exposed to regular air without any special treatment; Group C: rats exposed to regular air and were treated with erythromycin; Group S: rats exposed to smoking air and were treated with saline; Group E: rats exposed to smoking air and were treated with erythromycin daily for nine weeks beginning at the forth weeks smoking. $^*P < 0.05$.

3.4. Treatment with Erythromycin Alters the Numbers of Tregs in the Lungs of Rats. Flow cytometry analysis revealed that the frequency and the number of Tregs in the lung parenchyma of smoking rats were significantly lower than that of the N and C groups of control rats ($P < 0.01$, Figure 4), while the frequency and the number of Tregs in the erythromycin-treated group of rats were higher than that of the S group of rats ($P < 0.05$). A similar pattern of the relative levels of Foxp3 mRNA transcripts was detected in the different groups of rats. Apparently, treatment with erythromycin mitigated heavy smoking-induced reduction in the numbers of Tregs in the lungs of rats.

3.5. Treatment with Erythromycin Alters the Levels of IL-35 in BALF. IL-35 is an inhibitory cytokine and is predominantly secreted by Tregs. Next, we determined the levels of IL-35 in BALF from different groups of rats. The concentrations of IL-35 in the BALF from the S group of rats were significantly lower than that in the N and C groups of control rats (Figure 5). Interestingly, the levels of IL-35 in the BALF from E group of rats were similar to that in the N and C groups of rats and were significantly higher than that in the S group of rats. Apparently, treatment with erythromycin increased the levels of IL-35 responses in the lungs of rats.

4. Discussion

COPD and emphysema are common destructive inflammatory diseases that are leading causes of mortality worldwide. The smoking-induced emphysema is thought to be an autoimmune disease and is mediated predominantly by Th1 responses in the lung [15]. In this study, we employed a rat model of smoking-related airway inflammation and emphysema to test the therapeutic effect of treatment with erythromycin and the potential mechanisms. Our data showed that treatment with erythromycin significantly reduced smoking-induced lung inflammation and damages, consistent with our previous findings [28]. Furthermore, treatment with erythromycin increased the numbers of Tregs, accompanied by increased levels of inhibitory IL-35 in the lungs of rats. The increased levels of IL-35 may contribute to the inhibition of erythromycin on smoking-related inflammation. Our novel findings extend previous observations and suggest that erythromycin may be valuable for the intervention of

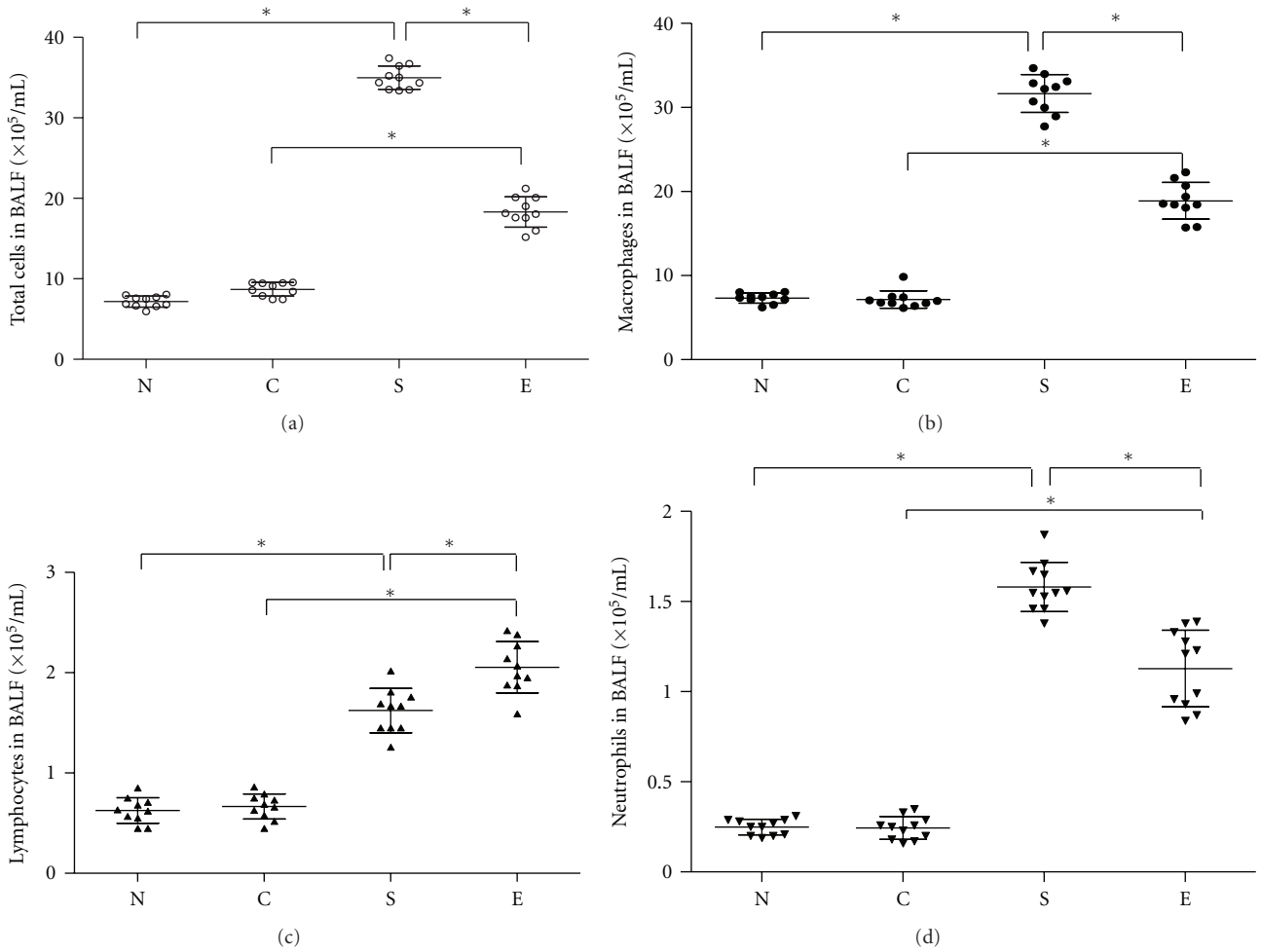


Figure 2: Treatment with erythromycin reduces the numbers of inflammatory infiltrates in the lungs of rats. BALF samples were collected from individual rats and the cells were stained with May-Grünwald-Giemsa. The numbers of total inflammatory infiltrates, macrophages, lymphocytes, and neutrophils were analyzed, according to their morphological characters. Data are expressed as mean numbers of individual samples and mean values (lines) for each group ($n = 10$). Group N: rats exposed to regular air without any special treatment; Group C: rats exposed to regular air and were treated with erythromycin; Group S: rats exposed to smoking air and were treated with saline; Group E: rats exposed to smoking air and were treated with erythromycin daily for nine weeks beginning at the forth weeks smoking. $^*P < 0.05$.

airway inflammation by upregulating Treg responses in patients with COPD in the clinic.

Macrolide antibiotics have been used for the treatment of lung inflammation in patients with COPD in the clinic [31]. Previous studies have shown that macrolides, especially for erythromycin, can modulate immune responses and inhibit inflammation in patients with DB and CF [32]. Indeed, long-term treatment with a low dose of macrolide benefits patients with COPD by its anti-inflammatory activities. In this study, we employed a well-known cigarette-smoking-inuced rat emphysema model and examined the effect of treatment with erythromycin on the airway inflammation and lung damages. We detected high values of MLI, great numbers of inflammatory infiltrates, and high levels of TNF-α and IL-8 in the lungs of smoking rats, demonstrating that heavy smoking-inuced emphysema and airway inflammation in the lungs of rats. Furthermore, we found that treatment with erythromycin mitigated the smoking-induced emphysema and reduced the numbers of inflammatory infiltrates and levels of TNF-α and IL-8 in the lungs of rats. Our data were consistent with a previous report that treatment with clarithromycin for six months decreases airspace enlargement in the smoke-induced emphysema in mice [33]. Our findings support the notion that erythromycin inhibits airway inflammation [28].

Heavy smoking can modulate the function of antigen-presenting cells, which may induce T-cell autoimmunity against the lungs and Th1 immunity has been thought to be related to the pathogenic process of COPD [15, 34]. Microbials, such as erythromycin, can modulate T-cell responses and inhibit airway inflammation [23, 27, 35]. Notably, Tregs are potent regulators of T-cell autoimmunity and inflammation and IL-35 is predominantly produced by Tregs and contributes to regulatory T-cell function [8, 9]. We found

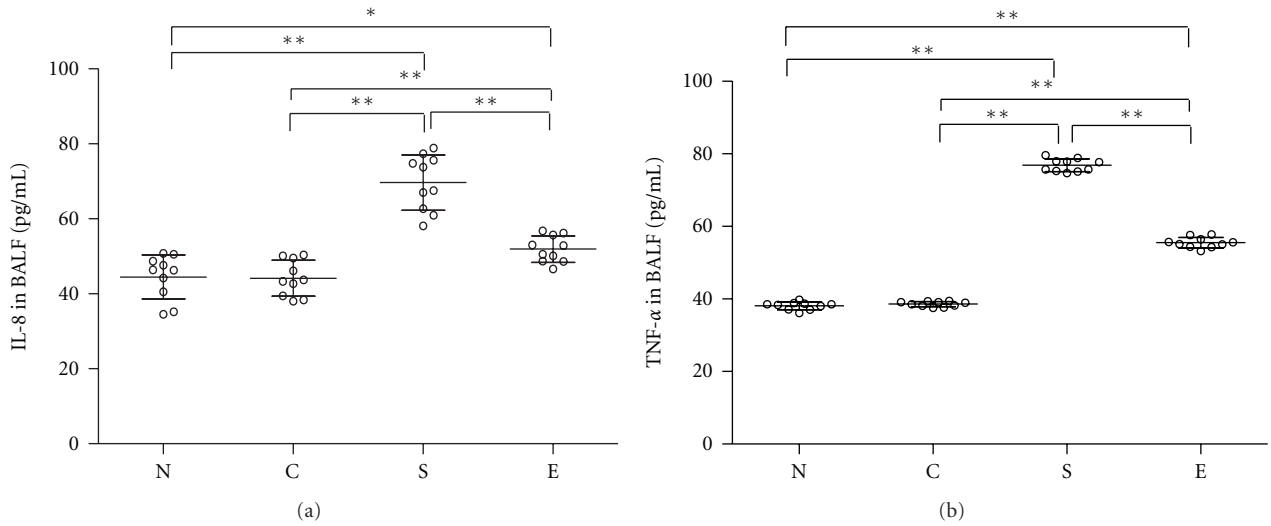


Figure 3: Treatment with erythromycin decreases the levels of TNF-α, IL-8 in the lungs of rats. The levels of TNF-α and IL-8 in BALF of individual rats were analyzed by ELISA. Data shown are mean values of individual samples from three separate experiments and mean values for each group of rats ($n = 10$). Group N: rats exposed to regular air without any special treatment; Group C: rats exposed to regular air and were treated with erythromycin; Group S: rats exposed to smoking air and were treated with saline; and Group E: rats exposed to smoking air and were treated with erythromycin daily for nine weeks beginning at the forth weeks smoking. $^{*}P < 0.05$, $^{**}P < 0.01$.

that treatment with erythromycin enhanced Treg responses, which may contribute to the inhibition of airway inflammation. Evidentially, in comparison with that in the smoking rats, treatment with erythromycin significantly increased the frequency and the numbers of Treg infiltrates in the lungs. Furthermore, treatment with erythromycin upregulated the levels of Foxp3 mRNA transcripts in the lungs. In addition, treatment with erythromycin increased the levels of IL-35 in the BALF, given that Tregs can inhibit pathogenic T-cell responses and IL-35 is crucial for the function of Tregs [10]. Although the increased Treg responses in the lungs by treatment with erythromycin were moderate the significantly reduced inflammation suggests that marginal effect of erythromycin on increasing Treg response in the lung may be sufficient in suppressing smoking-related inflammation. We understand that our data did not demonstrate that the increased Treg responses were responsible for the inhibition of smoke-related lung inflammation. We are interested in further investigation of whether adoptive transfer of Tregs or inactivation of Tregs could modulate smoke-induced inflammation and examining whether neutralization of IL-35 could change the effect of treatment with erythromycin on smoke-induced lung damage in rats.

While there is clear evidence that treatment with macrolide antibiotics inhibits effector T-cell proliferation and cytokine production there currently is little information on how macrolide antibiotics modulate T-cell immunity. Erythromycin may modulate the components of gut microbiota and promote the development of Tregs. Indeed, the components of gut microbiota are crucial for the development of Tregs in rodents. Furthermore, a previous study has shown that Roxithromycin inhibits chemokine-induced chemotaxis of Th1 and Th2 cells but does not affect regulatory T-cell migration [36]. Erythromycin may act, like Roxithromycin, and inhibit the migration of effector T cells, but not Tregs, leading to relative increase in the numbers of Tregs in the lungs of rats. In addition, erythromycin has been shown to downregulate dendritic cell function and cytokine production, particularly for LPS-stimulated dendritic cell maturation and activation [37]. However, treatment with erythromycin does not affect peptidoglycan-induced dendritic cell activation [37]. It is possible that erythromycin may modulate dendritic cell function toward to promoting Treg development. Indeed, we found that treatment with erythromycin upregulated Foxp3 transcription and IL-35 production. Given that IL-35 has been shown to promote Treg proliferation the increased levels of IL-35 may feedback enhance Treg responses in the lungs of rats. We are interested in further investigating the mechanisms underlying the role of erythromycin in regulating Treg responses.

5. Conclusions

In summary, treatment of COPD currently remains a significant challenge, and pharmacological understanding of drugs for the treatment of COPD is crucial for the control of disease progression. Our data indicated that treatment with erythromycin significantly reduced smoking-related lung inflammation and damages and modulated Treg and IL-35 responses in the lungs of rats. Therefore, our findings may provide new insights into understanding the pharmacological action of erythromycin in the management of COPD in the clinic.

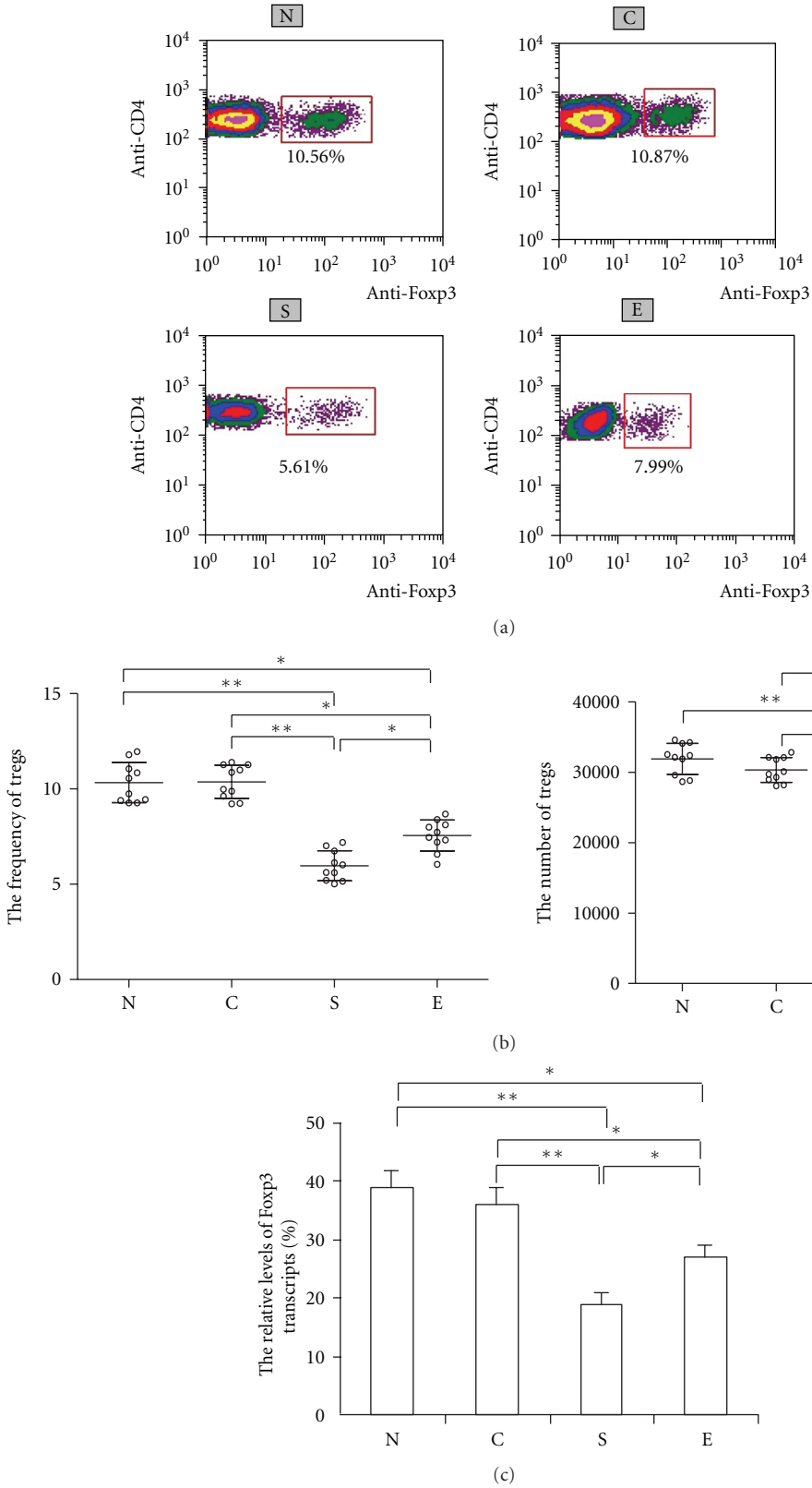


Figure 4: Treatment with erythromycin modulates the frequency and the number of Treg and Foxp3 transcription in the lungs of rats. The frequency of Tregs, the number of Tregs (b), and the relative levels of Foxp3 mRNA transcripts to β-actin in the lungs (c) were analyzed by flow cytometry (a) and RT-PCR, respectively. The isolated lung cells were stained with anti-CD4 and anti-Foxp3 and subjected to flow cytometry analysis. Data are expressed as mean numbers of individual samples and mean values (lines) of each group or the mean ± SD of the relative levels of Foxp3 mRNA transcripts of each group ($n = 10$ per group) of rats from three separate experiments. Group N: rats exposed to regular air without any special treatment; Group C: rats exposed to regular air and were treated with erythromycin; Group S: rats exposed to smoking air and were treated with saline; and Group E: rats exposed to smoking air and were treated with erythromycin daily for nine weeks beginning at the forth weeks smoking. $^{*}P < 0.05$, $^{**}P < 0.01$.

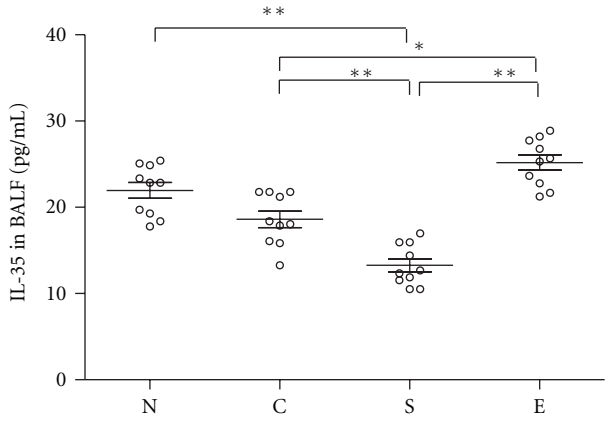


Figure 5: Treatment with erythromycin increases the levels of IL-35 in the lungs of rats. The levels of IL-35 in BALF of individual rats were analyzed by ELISA. Data shown are mean values of individual samples from three separate experiments and mean values (lines) of each group of rats ($n = 10$). Group N: rats exposed to regular air without any special treatment; Group C: rats exposed to regular air and were treated with erythromycin; Group S: rats exposed to smoking air and were treated with saline; and Group E: rats exposed to smoking air and were treated with erythromycin daily for nine weeks beginning at the forth weeks smoking. $^*P < 0.05$, $^{**}P < 0.01$.

Acknowledgments

The authors would like to thank Dr. Hui Chen (the Fifth Affiliated Hospital of Guangxi Medical University) for her excellent assistance in statistical analyses. This study was supported by a grant from the National Nature Science Foundation of China (no. 30860106).

References

[1] R. A. Pauwels, A. S. Buist, P. M. A. Calverley, C. R. Jenkins, and S. S. Hurd, "Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: NHLBI/WHO Global Initiative for Chronic Obstructive Lung Disease (GOLD) workshop summary," *American Journal of Respiratory and Critical Care Medicine*, vol. 163, no. 5, pp. 1256–1276, 2001.

[2] K. F. Rabe, S. Hurd, A. Anzueto et al., "Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary," *American Journal of Respiratory and Critical Care Medicine*, vol. 176, no. 6, pp. 532–555, 2007.

[3] A. Agustí, W. MacNee, K. Donaldson, and M. Cosio, "Hypothesis: does COPD have an autoimmune component?" *Thorax*, vol. 58, no. 10, pp. 832–834, 2003.

[4] M. Saetta, M. Mariani, P. Panina-Bordignon et al., "Increased expression of the chemokine receptor CXCR3 and its ligand CXCL10 in peripheral airways of smokers with chronic obstructive pulmonary disease," *American Journal of Respiratory and Critical Care Medicine*, vol. 165, no. 10, pp. 1404–1409, 2002.

[5] S. Grumelli, D. B. Corry, L. Z. Song et al., "An immune basis for lung parenchymal destruction in chronic obstructive pulmonary disease and emphysema," *PLoS Medicine*, vol. 1, article e8, 2004.

[6] M. G. Cosio and A. Guerassimov, "Chronic obstructive pulmonary disease: inflammation of small airways and lung parenchyma," *American Journal of Respiratory and Critical Care Medicine*, vol. 160, no. 5, pp. S21–S25, 1999.

[7] E. M. Shevach, "Regulatory T cells in autoimmmunity," *Annual Review of Immunology*, vol. 18, pp. 423–449, 2000.

[8] C. Dejaco, C. Duftner, B. Grubeck-Loebenstein, and M. Schirmer, "Imbalance of regulatory T cells in human autoimmune diseases," *Immunology*, vol. 117, no. 3, pp. 289–300, 2006.

[9] H. Von Boehmer, "Mechanisms of suppression by suppressor T cells," *Nature Immunology*, vol. 6, no. 4, pp. 338–344, 2005.

[10] L. W. Collison, C. J. Workman, T. T. Kuo et al., "The inhibitory cytokine IL-35 contributes to regulatory T-cell function," *Nature*, vol. 450, no. 7169, pp. 566–569, 2007.

[11] H. Ait-Oufella, B. L. Salomon, S. Potteaux et al., "Natural regulatory T cells control the development of atherosclerosis in mice," *Nature Medicine*, vol. 12, no. 2, pp. 178–180, 2006.

[12] J. A. Bluestone and Q. Tang, "How do $CD4^+CD25^+$ regulatory T cells control autoimmunity?" *Current Opinion in Immunology*, vol. 17, no. 6, pp. 638–642, 2005.

[13] H. Jiang and L. Chess, "Regulation of immune responses by T cells," *The New England Journal of Medicine*, vol. 354, no. 11, pp. 1116–1176, 2006.

[14] M. Möttönen, J. Heikkinen, L. Mustonen, P. Isomäki, R. Luukkainen, and O. Lassila, "$CD4^+$ $CD25^+$ T cells with the phenotypic and functional characteristics of regulatory T cells are enriched in the synovial fluid of patients with rheumatoid arthritis," *Clinical and Experimental Immunology*, vol. 140, no. 2, pp. 360–367, 2005.

[15] S. H. Lee, S. Goswami, A. Grudo et al., "Antielastin autoimmunity in tobacco smoking-induced emphysema," *Nature Medicine*, vol. 13, no. 5, pp. 567–569, 2007.

[16] L. P. McGarvey, M. John, J. A. Anderson, M. Zvarich, and R. A. Wise, "Ascertainment of cause-specific mortality in COPD: operations of the TORCH Clinical Endpoint Committee," *Thorax*, vol. 62, no. 5, pp. 411–415, 2007.

[17] J. Bourbeau, P. Christodoulopoulos, F. Maltais, Y. Yamauchi, R. Olivenstein, and Q. Hamid, "Effect of salmeterol/fluticasone propionate on airway inflammation in COPD: a randomised controlled trial," *Thorax*, vol. 62, no. 11, pp. 938–943, 2007.

[18] S. Harita, S. Kuyama, T. Okada, and Y. Tanizaki, "Effect of long-term and low-dose administration of erythromycin on proliferation of T lymphocytes stimulated with mitogens," *Journal of Chemotherapy*, vol. 20, no. 5, pp. 604–608, 2008.

[19] L. Sun, J. Liu, D. Cui et al., "Anti-inflammatory function of withangulatin a by targeted inhibiting COX-2 expression via MAPK and NF-κB pathways," *Journal of Cellular Biochemistry*, vol. 109, no. 3, pp. 532–541, 2010.

[20] M. Shinkai, G. H. Foster, and B. K. Rubin, "Macrolide antibiotics modulate ERK phosphorylation and IL-8 and GM-CSF production by human bronchial epithelial cells," *American Journal of Physiology*, vol. 290, no. 1, pp. L75–L85, 2006.

[21] T. Suzuki, M. Yamaya, K. Sekizawa et al., "Erythromycin inhibits rhinovirus infection in cultured human tracheal epithelial cells," *American Journal of Respiratory and Critical Care Medicine*, vol. 165, no. 8, pp. 1113–1118, 2002.

[22] H. Nagai, H. Shishido, R. Yoneda, E. Yamaguchi, A. Tamura, and A. Kurashima, "Long-term low-dose administration of erythromycin to patients with diffuse panbronchiolitis," *Respiration*, vol. 58, no. 3-4, pp. 145–149, 1991.

[23] N. Keicho and S. Kudoh, "Diffuse panbronchiolitis: role of macrolides in therapy," *American Journal of Respiratory Medicine*, vol. 1, no. 2, pp. 119–131, 2002.
[24] A. Azuma and S. Kudoh, "Diffuse panbronchiolitis in East Asia," *Respirology*, vol. 11, no. 3, pp. 249–261, 2006.
[25] P. King, "Is there a role for inhaled corticosteroids and macrolide therapy in bronchiectasis?" *Drugs*, vol. 67, no. 7, pp. 965–974, 2007.
[26] J. L. Simpson, H. Powell, M. J. Boyle, R. J. Scott, and P. G. Gibson, "Clarithromycin targets neutrophilic airway inflammation in refractory asthma," *American Journal of Respiratory and Critical Care Medicine*, vol. 177, no. 2, pp. 148–155, 2008.
[27] D. L. Hahn, "Macrolide therapy in asthma: limited treatment, long-term improvement," *European Respiratory Journal*, vol. 33, no. 5, article 1239, 2009.
[28] X. N. Zhong, J. Bai, H. Z. Shi, C. Wu, G. R. Liang, and Z. B. Feng, "An experimental study on airway inflammation and remodeling in a rat model of chronic bronchitis and emphysema," *Zhonghua Jie He He Hu Xi Za Zhi*, vol. 26, no. 12, pp. 750–755, 2003.
[29] M. Sorsa, P. Einisto, and K. Husgafvel-Pursiainen, "Passive and active exposure to cigarette smoke in a smoking experiment," *Journal of Toxicology and Environmental Health*, vol. 16, no. 3-4, pp. 523–534, 1985.
[30] W. M. Thurlbeck, "The internal surface area of nonemphysematous lungs," *American Review of Respiratory Disease*, vol. 95, no. 5, pp. 765–773, 1967.
[31] T. A. R. Seemungal, T. M. A. Wilkinson, J. R. Hurst, W. R. Perera, R. J. Sapsford, and J. A. Wedzicha, "Long-term erythromycin therapy is associated with decreased chronic obstructive pulmonary disease exacerbations," *American Journal of Respiratory and Critical Care Medicine*, vol. 178, no. 11, pp. 1139–1147, 2008.
[32] B. K. Rubin and M. O. Henke, "Immunomodulatory activity and effectiveness of macrolides in chronic airway disease," *Chest*, vol. 125, no. 2, pp. 70S–78S, 2004.
[33] Y. Nakanishi, D. Kobayashi, Y. Asano et al., "Clarithromycin prevents smoke-induced emphysema in mice," *American Journal of Respiratory and Critical Care Medicine*, vol. 179, no. 4, pp. 271–278, 2009.
[34] A. M. Stefanska and P. T. Walsh, "Chronic obstructive pulmonary disease: evidence for an autoimmune component," *Cellular and Molecular Immunology*, vol. 6, no. 2, pp. 81–86, 2009.
[35] Y. Ishida, Y. Abe, and Y. Harabuchi, "Effects of macrolides on antigen presentation and cytokine production by dendritic cells and T lymphocytes," *International Journal of Pediatric Otorhinolaryngology*, vol. 71, no. 2, pp. 297–305, 2007.
[36] T. Ito, N. Ito, H. Hashizume, and M. Takigawa, "Roxithromycin inhibits chemokine-induced chemotaxis of Th1 and Th2 cells but regulatory T cells," *Journal of Dermatological Science*, vol. 54, no. 3, pp. 185–191, 2009.
[37] M. Yasutomi, Y. Ohshima, N. Omata et al., "Erythromycin differentially inhibits lipopolysaccharide- or poly(I:C)-induced but not peptidoglycan-induced activation of human monocyte-derived dendritic cells," *The Journal of Immunology*, vol. 175, no. 12, pp. 8069–8076, 2005.

Hindawi Publishing Corporation
Mediators of Inflammation
Volume 2012, Article ID 159354, 7 pages
doi:10.1155/2012/159354

Review Article

Macrolides in Chronic Inflammatory Skin Disorders

Abdullateef A. Alzolibani[1] and Khaled Zedan[2]

[1] *Dermatology Department, College of Medicine, Qassim University, P.O. Box 30109, Buraidah 51477, Saudi Arabia*
[2] *Pediatric Department, College of Medicine, Qassim University, P.O. Box 30109, Buraidah 51477, Saudi Arabia*

Correspondence should be addressed to Abdullateef A. Alzolibani, azolibani@yahoo.com

Received 21 January 2012; Revised 10 March 2012; Accepted 15 March 2012

Academic Editor: Kazuhito Asano

Long-term therapy with the macrolide antibiotic erythromycin was shown to alter the clinical course of diffuse panbronchiolitis in the late 1980s. Since that time, macrolides have been found to have a large number of anti-inflammatory properties in addition to being antimicrobials. These observations provided the rationale for many studies performed to assess the usefulness of macrolides in other inflammatory diseases including skin and hair disorders, such as rosacea, psoriasis, pityriasis rosea, alopecia areata, bullous pemphigoid, and pityriasis lichenoides. This paper summarizes a collection of clinical studies and case reports dealing with the potential benefits of macrolides antibiotics in the treatment of selected dermatoses which have primarily been classified as noninfectious and demonstrating their potential for being disease-modifying agents.

1. Introduction

The term "macrolide" encompasses a diverse family of unrelated compounds with large macrolactam rings. The macrolide antibiotics consist of 14-, 15-, and 16-member macrolactam ring antimicrobials. Erythromycin A, the prototype macrolide antibiotic was isolated from a Philippine soil sample in the 1940s and was first marketed in 1952 as an alternative therapy to beta lactam agents for the treatment of infections with Gram-positive cocci. During the 1990s clarithromycin, roxithromycin, and azithromycin were introduced. Macrolide antibiotics inhibit RNA-dependent protein synthesis by reversibly binding to the 50S ribosomal subunit of a susceptible microorganism [1].

Macrolides are widely used to treat infections of soft tissues and of the respiratory tract due to their efficacy against Gram-negative and Gram-positive bacteria, including intracellular germs such as Chlamydia and Legionella [2–4]. They are considered safe and easily tolerable. Their main side effects are nausea, vomiting, diarrhea, and abdominal pain, which become more evident when erythromycin is used in place of the other macrolides [5]. Mounting evidence suggests that macrolide antibiotics have both anti-inflammatory and immune-modulatory properties and are thus beneficial to chronic pulmonary diseases such as diffuse panbronchiolitis, cystic fibrosis, asthma, and bronchiectasis. These properties were suspected upon the realization that erythromycin decreased the need for corticosteroids in asthma treatment [6]. It must be pointed out that immune modulation is the suppression of inflammation and immune hyperactivation without causing immune depression (immunosuppression) [7].

Macrolides antibiotics have been shown to modify host functions apart from the antimicrobial potency. They may directly influence phagocyte and lymphocyte function as well as chemotaxis. Effects on the generation and release of various cytokines involved in the inflammatory process have been studied both *in vivo and in vitro* [8].

Interest in the immunomodulatory effects of macrolides began in the 1960s with the observation that the 14-member antibiotic, troleandomycin, was an effective "steroid-sparing" agent when used to treat patients with severe asthma [9]. It has been more than 20 years since the immunomodulatory effects of macrolides were accepted as a standard of care for the treatment of diffuse panbronchiolitis (DPB) in Japan [10]. Erythromycin and clarithromycin are also widely used in Japan for the therapy of sinusitis and chronic obstructive pulmonary disease (COPD) [11]. In

more recent years, azithromycin has been widely adopted as immunomodulatory agents for the treatment of cystic fibrosis (CF) and bronchiectasis.

The anti-inflammatory effects of macrolides are significant. The historical change in the natural course of diffuse panbronchiolitis (DPB), a fatal disorder of the airways, following the introduction of erythromycin in its treatment has focused attention of researchers on the anti-inflammatory properties of macrolides. The clinical impact on diffuse panbronchiolitis (DPB) has improved 10-year survival from 12% to more than 90% for these patients [12]. The immunomodulatory activity of macrolides has been a source of mechanistic research as well as clinical research in non-DPB inflammatory airway disease. Suppression of neutrophilic inflammation of the airways has been demonstrated as the most robust immunomodulatory response from 14- and 15-membered ring macrolides [13].

Macrolide antibiotics are known for their efficacy in treating acute airway infections, but just as importantly, they are also effective anti-inflammatory agents. Their anti-inflammatory properties have been studied most thoroughly in chronic inflammatory airway diseases, particularly diffuse panbronchiolitis (DPB). Erythromycin, azithromycin, clarithromycin, and roxithromycin inhibit chemotaxis and infiltration of neutrophils into the airway and, subsequently, decrease mucus secretion. Mucus formation, a significant cause of morbidity and mortality in patients with chronic airway inflammation, is directly inhibited by macrolides and suppressed by decreased inflammation in the airway. The mechanisms of action for the anti-inflammatory properties of the macrolides are clearly multifactorial. Macrolides inhibit the production of many proinflammatory cytokines, such as interleukin (IL)-1, IL-6, IL-8, and tumor necrosis factor-alpha, perhaps by suppressing the transcription factor nuclear factor-kappa B or activator protein-1. Inhibition of cytokine production has been seen *in vitro* and also in bronchoalveolar lavage fluid, which contains less IL-8 and fewer neutrophils after treatment with macrolides. Macrolides also inhibit formation of leukotriene B4, which attracts neutrophils, and inhibit the release of superoxide anion by neutrophils that may be present in the airway. An important aspect of inflammation is extravasation of neutrophils into the tissues. Macrolides block formation of adhesion molecules necessary for neutrophil migration. Together, these anti-inflammatory effects result in improved pulmonary functions and fewer airway infections. In patients with DPB, the anti-inflammatory effects lead to a significant increase in survival. These effects might be pharmacological functions of the macrolide itself, independent of antibiotic effects. Apart from antibacterial effects, macrolides have effects on neutrophil function (decreased oxidant production, apoptosis) and on the production of cytokines involved in the inflammation cascade (decreased production of IL-1, IL-6, IL-8, and TNF and increased production of IL-10 and, possibly, IL-4). With regard to T lymphocytes, erythromycin (EM) and its derivatives inhibit T-lymphocyte proliferation and induce T-lymphocyte apoptosis [14, 15].

In this paper, we present a collection of clinical studies and case reports dealing with the potential benefits of macrolides antibiotics in the treatment of selected dermatoses which have primarily been classified as noninfectious. A comprehensive search in the PubMed/MEDLINE and Embase databases was performed. We examined the eligible literature. Studies that dealt with the effects of macrolides as anti-inflammatory and immune-modulator in skin and hair disorders were included.

(A) Macrolides and Intractable Rosacea. Rosacea is a common cutaneous disorder which occurs most frequently in light-skinned, middle-aged women. There are variable cutaneous signs of rosacea such as flushing, erythema, telangiectasia, edema, papules, and pustules [16].

Conventional treatment of rosacea is based on a combination of systemic and topical antibiotics. Since the 1950s, tetracycline and erythromycin are the most commonly used oral antibiotics [17]. The therapeutic activity of commonly used antimicrobials including tetracycline, doxycycline has been mainly attributed to their anti-inflammatory activities [18]. However, long-term treatment with antibiotics is not well tolerated due to requiring frequent administration, poor compliances and side effects including gastrointestinal intolerance, photosensitivity, and candidiasis [19].

Azithromycin is effective in treating rosacea. Facial skin biopsies were taken from 17 subjects with papulopustular rosacea and 25 healthy controls. Rosacea patients had greater skin reactive oxygen species levels than healthy controls ($P < 0.001$). Rosacea subjects then received oral azithromycin 500 mg on three days each week for 4 weeks. A statistically significant decrease in chemiluminescence, a measurement of the generation of reactive oxygen species, was demonstrated after treatment with azithromycin [19].

The utility of oral azithromycin was confirmed by several other clinical studies. Fernandez-Obregon [20] reported that all of ten patients who were not tolerated or controlled by conventional treatment of rosacea demonstrated a significant improvement with the oral use of azithromycin. In addition, Modi et al. [21] treated a 67-year-old man who had photosensitivity to the doxycycline and hyperpigmented dyschromia to the minocycline with an oral use of azithromycin in a dose of 250 mg 3 times weekly. Bakar et al. [19] reported that treatment with oral azithromycin led to 75% decreases in the total number of lesions and an 89% decrease in inflammatory lesions compared with basal status. Another open-label study showed that azithromycin is as effective as standard dose of doxycycline and has a positive impact on the quality of life of patients compared with conventional treatment regimens [22].

Kim et al. [23] treated a 52-year-old woman who had intractable rosacea not responding to various conventional treatments including topical benzoyl peroxide and metronidazole as well as oral metronidazole, isotretinoin, and doxycycline, by using oral azithromycin 500 mg per day for 2 weeks. The authors reported that the lesions had mostly disappeared, and no specific side effects related to the azithromycin were noted.

(B) Macrolides and Adult-Onset Still's Disease (AOSD). Adult-onset Still's disease (AOSD), an autoinflammatory syndrome of unknown etiology, typically manifests with spiking fevers, polyarthritis, and characteristic evanescent rash. Thanou-Stavraki et al. [24] described a young woman with AOSD complicated by calf fasciitis that serendipitously responded to clarithromycin administered for another indication. Remarkable improvement followed rechallenges with clarithromycin for subsequent AOSD flares. Although AOSD pathogenesis remains unclear, a role for dysregulation of innate immunity is suggested. Based on this possible innate immune mechanism, the investigators suspected that macrolides may have induced a therapeutic response in this patient with AOSD.

Saviola et al. [25] treated six cases of AOSD with clarithromycin (CM) in combination with low-mild dose of glucocorticoids (GC), and methotrexate (MTX). Four of them were not responsive to high-dose GC added to disease-modifying antirheumatic drugs (DMARDs), while two of them were treated with low-mild dose of GC added to CM from the beginning. CM, 500 mg b.i.d., was added to a mild-low dose of GC and to MTX. The dose of the drugs was reduced (and stopped where possible) following clinical and laboratory parameters. ACR criteria were used to assess clinical improvement. At 6 months, 5 patients reached ACR 70% and could stop any therapy in 6–18 months; 1 continued chronic therapy with low-dose GC added to CM and MTX to maintain ACR 50%. The authors reported that CM can be a useful drug for the treatment of AOSD, even in patients not responsive to high-dose GC and DMARDs.

(C) Macrolides and SAPHO Syndrome. In 1987, synovitis, acne, pustulosis, hyperostosis, and osteitis (SAPHO) syndrome was proposed as an umbrella term for a group of diseases with similar musculoskeletal manifestations, in particular hyperostosis of anterior chest wall, synovitis, and multifocal aseptic osteomyelitis, observed in association with dermatologic conditions such as palmo-plantar pustulosis, severe acne, and hidradenitis suppurativa [26]. Despite recent advances in the understanding of the epidemiologic, pathophysiologic, and immunogenetic mechanisms involved in SAPHO syndrome, etiopathogenesis remains poorly understood. Propionibacterium acnes, the microorganism associated with acne, has been recovered on bone biopsy in some patients, but the possible pathogenetic role of an infectious agent in a genetically predisposed individual, resulting in exaggerated inflammatory response as "reactive osteitis," is a largely unproven hypothesis [27].

Schaeverbeke and colleagues reported one case of successful treatment of a SAPHO patient with azithromycin [28]. Kirchhoff and colleagues presented data for seven patients being treated successfully with azithromycin over 5 months [29]. Assmann et al. [30] reported successful control of the disease with azithromycin over 16 weeks. After antibiotic discontinuation, however, disease relapse was observed.

Matzaroglou et al. [31] reported five patients with SAPHO syndrome (3 women; 2 men), ages 27 to 44 years, showed remarkable response to treatment with macrolide antibiotic (clindamycin) and nonsteroid anti-inflammatory drugs (lornoxicam). All patients did well and remained symptom-free for up to four years, after a 3–8-month course of treatment. The authors concluded that appropriate therapy with antibiotics and NSAIDs can produce rapid symptom resolution, while avoiding unnecessary procedures and long-term antibiotic therapy.

(D) Macrolides and Psoriasis. Psoriasis is a well-known clinical description of an inflammatory skin disorder with other manifestations of what, until now, has been considered as a single disease entity. The characteristic skin lesion is persistent, erythematous, indurated and scaly, reflecting infiltration of inflammatory cells and increased proliferation and turnover of keratinocytes. The infiltrates in the dermis and the deeper layer of the epidermis mostly comprise of macrophages and T cells. Stimulation of dendritic cells and macrophages, which are called antigen-presenting cells, results in the activation of T-helper (Th) cells. These differentiate into IFN-gamma, producing Th 1 cells, and IL-17, producing Th 17 cells. Interaction of these cells with macrophages, mast cells, and neutrophils results in cytokine release and inflammation, leading to keratinocyte proliferation [32].

Psoriasis is characterized by the presence of neutrophil overactivation and overproduction of interleukin (IL)-6 and IL-8 from keratinocytes [33]. Macrolide antibiotics are widely used as antimicrobial agents. It is now clear that macrolide antibiotics inhibit the production of many proinflammatory cytokines, such as IL-6, IL-8, and tumor necrosis factor (TNF)-α, perhaps by suppressing the transcription factors nuclear factor (NF)-κB or activator protein-1, and reduce neutrophil activity [34]. There are conflicting views in the literature regarding the efficacy of macrolides on psoriasis. Although in some studies it has been reported that intervention by antibiotics is not beneficial [35, 36], other studies have shown efficacy of macrolides in psoriasis [33].

A high incidence of streptococcal throat infection as the main trigger for psoriasis exacerbations favors streptococcal antigens as a causative agent, which may induce cross-reactive T-cell responses against skin components [37, 38]. Staphylococcal superantigens have also been associated with psoriatic disease [31].

Ohshima et al. [39] deserves special attention, where ten patients with chronic plaque psoriasis were enrolled and advised to take 150 mg Roxithromycin (a macrolide) orally twice daily for 1 to 7 weeks. Six out of the ten patients exhibited a decrease in psoriasis area and severity index (PASI) score. The mechanism by which macrolides downregulates the host inflammatory response was unclear but certainly multifactorial.

Macrolides, as a class, and azithromycin in particular, have a characteristic immunomodulatory and anti-inflammatory potential, in addition to their main antibacterial action against streptococci. Suppression of secretions of the cytokine-interleukin (IL)-8 may be important. This macrolide probably also suppresses immunological

events in interferon gamma-treated keratinocytes, including expression of MHC class II, secretion of IL-1 alpha, and superantigen presenting ability [40, 41].

Saxena and Dogra [42] tried oral azithromycin in a single blind randomized case-control trial. 50 patients with moderate-to-severe chronic plaque psoriasis were enrolled. Of these, 30 randomly selected patients received azithromycin for 48 weeks as a single oral 500 mg daily dose for 4 days with a gap of 10 days (total 24 such courses). The remaining 20 patients received a vitamin C tablet (nonchewable) in the same dosage schedule. A significant improvement in PASI score was noted from 12 weeks in the majority of patients in the azithromycin group. At the end of 48 weeks, 18 patients (60%) showed excellent improvement, while 6 patients (20%) showed good improvement, and 4 patients (13.33%) showed mild improvement. A significant improvement in the skin lesions was noted at 12 weeks of azithromycin therapy. Based on this study, the authors reported that the results substantiated the hypothesis that chronic ongoing stimulus by the streptococci or its superantigen was indispensable in maintaining the disease.

17 subjects participated in an open trial of macrolides for treatment of psoriasis. Mean PASI scores dropped significantly, and itch was reduced in 11 subjects after therapy. This study showed that macrolide antibiotics may be effective for treatment of psoriatic skin lesion, and that they may have antipruritic effects [33].

Tamaki investigated the antipruritic effects of macrolide antibiotics in several pruritic skin diseases. They found that in most of the patients, the drug was very effective. The reason for the antipruritic effect is not known; however, it is suggested possibly that macrolide antibiotics inhibit production of cytokines or neuropeptides that cause pruritus [43].

Polat et al. [44] studied patients with psoriasis. The patients were divided into two treatment groups: one to receive erythromycin and topical steroids and the other only topical steroids: the first group were treated with erythromycin 1000 mg/day and topical corticosteroids for 4 weeks, while the control group were treated only with topical corticosteroids. The study group comprised 36 patients; the control group comprised 24 patients. There was no significant difference between the baseline mean Psoriasis Area and Severity Index (PASI) of the two groups. They reported that the treatment used for the study group was more effective against pruritus than that used for the control group. Six patients with severe pruritus and six patients with moderate pruritus in the study group found that itch disappeared completely after the treatment. In the control group, none of the patients with severe or moderate pruritus found that itch disappeared completely.

(E) Macrolides and Alopecia Areata, Associated with H. pylori Infection. Campuzano-Maya [45] described a case of a 43-year-old man with patchy alopecia areata and *H. pylori* infection; the patient had hair regrowth after bacterial eradication. The patient was prescribed first-line *H. pylori* eradication with proton pump inhibitor (omeprazole) 20 mg twice daily, amoxicillin 1000 mg twice daily, and clarithromycin 500 mg twice daily for 14 days and was followed photographically every 2 wks. He was instructed not to take or apply any medications for alopecia areata. The patient's condition started to improve within 4 wks of completing *H. pylori* eradication. By week 16, the patient had completely reversed the hair loss, and by week 44, he remained *H. pylori*-negative and completely cured of alopecia areata. The author reported that this is the first documented case of reversed hair loss after *H. pylori* eradication and, if such an association is confirmed by epidemiological studies designed for this purpose, new therapeutic options could be available for these patients, especially in areas where infection with *H. pylori* is highly prevalent.

(F) Macrolides and Chronic Urticaria, Associated with H. pylori Infection. Chronic urticaria is one of the most frequent skin diseases in medical practice. Urticaria is defined as acute if the whealing persists for less than six weeks and as chronic if it persists for longer. Chronic urticaria that lasts from several years to decades significantly impairs the quality of life. There is evidence that *Helicobacter pylori* has a critical role in different extragastric diseases such as chronic urticaria. Ben Mahmoud et al. [46] presented a case of chronic urticaria in an adult patient with *H. pylori* infection and disease regression after triple anti-*H. pylori* therapy. In contrast to the autoimmune mechanisms involved in chronic urticaria against which no specific treatment strategy has been developed, infections with *H. pylori* could be treated with triple therapy. The authors suggested that laboratory tests for the detection of this pathogen should be performed in patients with chronic urticaria.

(G) Macrolides and Pityriasis Rosea. Sharma et al. [47] performed a clinical study to evaluate the efficacy of erythromycin in patients with pityriasis rosea (PR). Ninety patients over a period of 2 years were alternatively assigned to treatment group or placebo group. Patients in the treatment group received erythromycin in divided doses for 14 days. The response was categorized as complete response, partial response, or no response. Complete response was observed in 33 patients (73.33%) in the treatment group and none in the placebo group. The authors concluded that oral erythromycin was effective in treating patients with pityriasis rosea, and that the effect of erythromycin may be related to its anti-inflammatory properties.

Rasi et al. [48] conducted a placebo-controlled study on 184 patients with pityriasis rosea attending the outpatient dermatology department clinic. Adult patients were treated with 200 mg of erythromycin 4 times daily, and children were treated with 20 to 40 mg/kg daily in 4 divided doses. Controls were given a placebo (an emollient cream) that was not identical in appearance. Subjects were seen at follow-up visits 2, 4, 6, and 8 weeks after starting treatment. Both groups were comparable with regard to sex, age, and mean duration of disease at the time of attending the clinic. They found

no significant difference between the 2 treatment groups at weeks 4, 6, and 8 after beginning of treatment.

Other authors believe that the use of macrolides is best considered experimental and should not be adopted into routine clinical practice until further studies are conducted and results are published. Even if macrolides are finally proven to be effective in modifying the course of PR, this does not substantiate that PR is caused by a bacterial rather than a viral infection. Macrolides have anti-inflammatory and immunomodulating effects that might affect the course of PR or other cutaneous eruptions independent of their antibacterial properties [49].

(H) Macrolides and Pityriasis Lichenoides. Pityriasis lichenoides is an uncommon reactive papulosquamous eruption of unknown origin. Truhan et al. [50] performed a study to determine the effects of erythromycin in pityriasis lichenoides. Fifteen of twenty-two children with pityriasis lichenoides were treated with oral erythromycin. Eleven (73%) had a remission, usually within 2 months. Two others showed partial improvement, and two were unimproved. Seven of the children who experienced a remission were off erythromycin and free of lesions after 2 to 5 months of therapy. The authors concluded that a trial of erythromycin should be considered in children with pityriasis lichenoides before other, possibly more toxic, measures are instituted.

Skinner and Levy [51] reported two cases of persistent pityriasis lichenoides et varioliformis acuta (PLEVA) unresponsive to tetracycline and erythromycin that rapidly resolved with bimonthly treatment with azithromycin for 5 days. The first case was a 51-year-old female started on azithromycin 500 mg on day 1 and 250 mg on days 2 through 5, to be taken on the first and third weeks of the month. One week after starting the first course, she reported that no new lesions had formed, and that the current lesions were resolving. After 3 weeks and two courses of azithromycin, the patient was clear of all lesions. She has remained clear for 6 months. The second case was a 5-year-old boy in whom erythromycin taken for 3 months did not improve the rash. He was then started on the same azithromycin regimen stated above. Eight weeks later, the patient had completed four courses of azithromycin. He had marked improvement, with only a few remaining smooth papules. He was continued on azithromycin for one more course and was clear of all lesions on 1-month followup and again 2 months later.

(I) Macrolides and Bullous Pemphigoid. Bullous pemphigoid is the most common autoimmune-mediated bullous disease in men. Mensing and Krausse [52] tested erythromycin combined with a low-dose methylprednisolone in eleven patients in a prospective study. A historical collective of the last 33 patients treated before this study was started served as the control group. The duration of hospitalization as an expression of therapeutic response, but also of lowered side effects dropped down from 43 to 33 days in the erythromycin treated group. Altomare et al. [53] reported that the macrolide antibiotic erythromycin has been effective in bullous pemphigoid in their studied patients. Fox et al. [54] reported two patients with bullous pemphigoid treated with erythromycin demonstrated improvement.

(J) Successful Treatment of Idiopathic Thrombocytopenic Purpura with Macrolides. Ohe and Hashino [55] reported 3 cases of primary immune thrombocytopenia (ITP) patients who were successfully treated with macrolides, irrespective of *Helicobacter pylori* (*H. pylori*) infection status. Case 1, an 88-year-old woman who was an *H. pylori*-positive ITP patient, was treated with clarithromycin (CAM). CAM was effective temporarily. As an alternative to CAM, she was successfully treated with erythromycin (EM) for more than 7 months. Case 2, a 61-year-old man who was an *H. pylori*-negative ITP patient, was unsuccessfully treated with CAM but successfully treated with EM. Case 3, a 75-year-old woman who was a *H. pylori*-negative ITP patient, was treated with CAM. CAM was effective temporarily. After approximately 6 months, she was treated with EM for a common cold, and her platelet count increased rapidly. The authors concluded, based on these findings, that macrolide treatment was effective for ITP. The effectiveness of macrolides might suggest immunomodulatory effects as well as antibacterial effects for *H. pylori*.

In a previous work, the authors have already reported 3 cases of idiopathic thrombocytopenic purpura (ITP), also known as primary immune thrombocytopenia, which show increased platelet counts following clarithromycin treatment, irrespective of *H. pylori* infection status [56].

The authors attributed this therapeutic success of macrolides in treating cases of ITP to the immunomodulatory effects of macrolides. Immunomodulatory effects from macrolide antibiotics might be obtained by the eradication of bacteria or by modulation of the immune system involving the mucosa on which commensal bacteria reside [57].

2. Conclusion

Despite the small number of studies shedding light on the anti-inflammatory and immunomodulatory mechanisms of the macrolides, there is strong evidence providing support to the benefit of using this type of drug for the long term and in low doses to treat some chronic inflammatory skin disorders. The macrolides have some potentially useful immunomodulatory effects. Although additional studies are needed, macrolide therapy in some of chronic dermatoses has the potential of modifying the morbidity and possibly ameliorating the severity of some, but not all, of these conditions. Further well-designed, adequately powered randomized controlled trials are required.

References

[1] J. A. Washington and W. R. Wilson, "Erythromycin: a microbial and clinical perspective after 30 years of clinical use," *Mayo Clinic Proceedings*, vol. 60, no. 3, pp. 189–203, 1985.

[2] Y. S. López-Boado and B. K. Rubin, "Macrolides as immunomodulatory medications for the therapy of chronic lung diseases," *Current Opinion in Pharmacology*, vol. 8, no. 3, pp. 286–291, 2008.

[3] D. P. Healy, "Macrolide immunomodulation of chronic respiratory diseases," *Current Infectious Disease Reports*, vol. 9, no. 1, pp. 7–13, 2007.

[4] D. F. Florescu, P. J. Murphy, and A. C. Kalil, "Effects of prolonged use of azithromycin in patients with cystic fibrosis: a meta-analysis," *Pulmonary Pharmacology and Therapeutics*, vol. 22, no. 6, pp. 467–472, 2009.

[5] M. S. Whitman and A. R. Tunkel, "Azithromycin and clarithromycin: overview and comparison with erythromycin," *Infection Control and Hospital Epidemiology*, vol. 13, no. 6, pp. 357–368, 1992.

[6] I. H. Itkin and M. L. Menzel, "The use of macrolide antibiotic substances in the treatment of asthma," *Journal of Allergy*, vol. 45, no. 3, pp. 146–162, 1970.

[7] M. Shinkai and B. K. Rubin, "Macrolides and airway inflammation in children," *Paediatric Respiratory Reviews*, vol. 6, no. 3, pp. 227–235, 2005.

[8] T. Brinkmeier and P. J. Frosch, "Oral antibiotics with antiinflammatory/immunomodulatory effects in the treatment of various dermatoses," *Hautarzt*, vol. 53, no. 7, pp. 456–465, 2002.

[9] S. L. Spector, F. H. Katz, and R. S. Farr, "Troleandomycin: effectiveness in steroid dependent asthma and bronchitis," *Journal of Allergy and Clinical Immunology*, vol. 54, no. 6, pp. 367–379, 1974.

[10] S. Kudoh, T. Uetake, K. Hagiwara, M. Hirayama, L. H. Hus, H. Kimura et al., "Clinical effects of low-dose long-term erythromycin chemotherapy on diffuse panbronchiolitis," *Nihon Kyobu Shikkan Gakkai Zasshi*, vol. 25, pp. 632–642, 1987.

[11] J. Tamaoki, K. Takeyama, E. Tagaya, and K. Konno, "Effect of clarithromycin on sputum production and its rheological properties in chronic respiratory tract infections," *Antimicrobial Agents and Chemotherapy*, vol. 39, no. 8, pp. 1688–1690, 1995.

[12] S. Kudoh, A. Azuma, M. Yamamoto, T. Izumi, and M. Ando, "Improvement of survival in patients with diffuse panbronchiolitis treated with low-dose erythromycin," *American Journal of Respiratory and Critical Care Medicine*, vol. 157, no. 6, pp. 1829–1832, 1998.

[13] S. Umeki, "Anti-inflammatory action of erythromycin: its inhibitory effect on neutrophil NADPH oxidase activity," *Chest*, vol. 104, no. 4, pp. 1191–1193, 1993.

[14] M. T. Labro, "Anti-inflammatory activity of macrolides: a new therapeutic potential?" *Journal of Antimicrobial Chemotherapy*, vol. 41, pp. 37–46, 1998.

[15] L. Wu, W. Zhang, L. Tian, K. Bao, P. Li, and J. Lin, "Immunomodulatory effects of erythromycin and its derivatives on human T-lymphocyte in vitro," *Immunopharmacology and Immunotoxicology*, vol. 29, no. 3-4, pp. 587–596, 2007.

[16] W. D. James, T. G. Berger, and D. M. Elston, *Andrew's Diseases of the Skin*, WB Saunders, Philadelphia, Pa, USA, 10th edition, 2006.

[17] J. Q. Del Rosso, "Systemic therapy for rosacea: focus on oral antibiotic therapy and safety," *Cutis*, vol. 66, supplement 4, pp. 7–13, 2000.

[18] P. Humbert, P. Treffel, J. F. Chapuis, S. Buchet, C. Derancourt, and P. Agache, "The tetracyclines in dermatology," *Journal of the American Academy of Dermatology*, vol. 25, no. 4, pp. 691–697, 1991.

[19] O. Bakar, Z. Demirçay, M. Yuksel, G. Haklar, and Y. Sanisoglu, "The effect of azithromycin on reactive oxygen species in rosacea," *Clinical and Experimental Dermatology*, vol. 32, no. 2, pp. 197–200, 2007.

[20] A. Fernandez-Obregon, "Oral use of azithromycin for the treatment of acne rosacea," *Archives of Dermatology*, vol. 140, no. 4, pp. 489–490, 2004.

[21] S. Modi, M. Harting, and T. Rosen, "Azithromycin as an alternative rosacea therapy when tetracyclines prove problematic," *Journal of Drugs in Dermatology*, vol. 7, no. 9, pp. 898–899, 2008.

[22] M. Akhyani, A. H. Ehsani, M. Ghiasi, and A. K. Jafari, "Comparison of efficacy of azithromycin vs. doxycycline in the treatment of rosacea: a randomized open clinical trial," *International Journal of Dermatology*, vol. 47, no. 3, pp. 284–288, 2008.

[23] J. H. Kim, Y. S. Oh, and E. H. Choi, "Oral azithromycin for treatment of intractable rosacea," *Journal of Korean Medical Science*, vol. 26, no. 5, pp. 694–696, 2011.

[24] A. Thanou-Stavraki, T. aberle, I. Aksentijevich, B. L. Bane, and J. B. Harley, "Clarithromycin in adult-onset still's disease: a potentially useful therapeutic," *Journal of Clinical Rheumatology*, vol. 17, no. 7, pp. 373–376, 2011.

[25] G. Saviola, M. Benucci, L. Abdi-Ali et al., "Clarithromycin in adult-onset Still's disease: a study of 6 cases," *Rheumatology International*, vol. 30, no. 4, pp. 555–560, 2010.

[26] A. M. Chamot, C. L. Benhamou, M. F. Kahn, L. Beraneck, G. Kaplan, and A. Prost, "Acne- pustulosis- hyperostosis- osteitis syndrome. Results of a national survey: 85 cases," *Revue du Rhumatisme et des Maladies Osteo-Articulaires*, vol. 54, pp. 187–196, 1987.

[27] M. Colina, A. Lo Monaco, M. Khodeir, and F. Trotta, "Propionibacterium acnes and SAPHO syndrome: a case report and literature review," *Clinical and Experimental Rheumatology*, vol. 25, no. 3, pp. 457–460, 2007.

[28] T. Schaeverbeke, L. Lequen, B. de Barbeyrac et al., "Propionibacterium acnes isolated from synovial tissue and fluid in a patient with oligoarthritis associated with acne and pustulosis," *Arthritis & Rheumatism*, vol. 41, pp. 1889–1893, 1998.

[29] T. Kirchhoff, S. Merkesdal, H. Rosenthal et al., "Diagnostic management of patients with SAPHO syndrome: use of MR imaging to guide bone biopsy at CT for microbiological and histological work-up," *European Radiology*, vol. 13, no. 10, pp. 2304–2308, 2003.

[30] G. Assmann, O. Kueck, T. Kirchhoff et al., "Efficacy of antibiotic therapy for SAPHO syndrome is lost after its discontinuation: an interventional study," *Arthritis Research and Therapy*, vol. 11, no. 5, article R140, 2009.

[31] C. Matzaroglou, D. Velissaris, A. Karageorgos, M. Marangos, E. Panagiotopoulos, and M. Karanikolas, "SAPHO syndrome diagnosis and treatment: report of five cases and review of the literature," *The Open Orthopaedics Journal*, vol. 3, pp. 100–106, 2009.

[32] D. D. Balci, N. Duran, B. Ozer, R. Gunessacar, Y. Onlen, and J. Z. Yenin, "High prevalence of Staphylococcus aureus cultivation and super-antigen production in patients with psoriasis," *European Journal of Dermatology*, vol. 19, pp. 238–242, 2009.

[33] M. Komine and K. Tamaki, "An open trial of oral macrolide treatment for psoriasis vulgaris," *Journal of Dermatology*, vol. 27, no. 8, pp. 508–512, 2000.

[34] J. Tamaoki, J. Kadota, and H. Takizawa, "Clinical implications of the immunomodulatory effects of macrolides," *The American Journal of Medicine*, vol. 117, supplement 9, pp. 5s–11s, 2004.

[35] C. M. Owen, R. J. G. Chalmers, T. O'Sullivan, and C. E. M. Griffiths, "A systematic review of antistreptococcal interventions for guttate and chronic plaque psoriasis," *British Journal of Dermatology*, vol. 145, no. 6, pp. 886–890, 2001.

[36] J. K. Wilson, S. N. Al-Suwaidan, D. Krowchuk, and S. R. Feldman, "Treatment of psoriasis in children: is there a role for antibiotic therapy and tonsillectomy?" *Pediatric Dermatology*, vol. 20, no. 1, pp. 11–15, 2003.

[37] H. Valdimarsson, H. Sigmundsdottir, and I. Jonsdottir, "Is psoriasis induced by streptococcal super antigens and maintained by M- protein specific T cells that cross react with keratin?" *Clinical & Experimental Immunology*, vol. 107, pp. 21–24, 1997.

[38] J. C. Prinz, "Psoriasis vulgaris- a sterile anti-bacterial skin reaction mediated by cross reactive T cells? An immunological view of the patho physiology of psoriasis," *Clinical and Experimental Dermatology*, vol. 26, pp. 326–332, 2001.

[39] A. Ohshima, M. Takigawa, and Y. Tokura, "$CD8^+$ cell changes in psoriasis associated with roxithromycin-induced clinical improvement," *European Journal of Dermatology*, vol. 11, no. 5, pp. 410–415, 2001.

[40] S. Konno, M. Adachi, K. Asano, K. Okamoto, and T. Takahashi, "Inhibition of human T-lymphocyte activation by macrolide antibiotic, roxithromycin," *Life Sciences*, vol. 51, no. 24, pp. 231–236, 1992.

[41] H. Wakita, Y. Tokura, F. Furukawa, and M. Takigawa, "The macrolide antibiotic, roxithromycin suppresses IFN-γ-mediated immunological functions of cultured normal human keratinocytes," *Biological and Pharmaceutical Bulletin*, vol. 19, no. 2, pp. 224–227, 1996.

[42] V. N. Saxena and J. Dogra, "Long-term oral azithromycin in chronic plaque psoriasis: a controlled trial," *European Journal of Dermatology*, vol. 20, no. 3, pp. 329–333, 2010.

[43] K. Tamaki, "Antipruritic effect of macrolide antibiotics," *Journal of Dermatology*, vol. 27, no. 1, pp. 66–67, 2000.

[44] M. Polat, N. Lenk, B. Yalcin et al., "Efficacy of erythromycin for psoriasis vulgaris," *Clinical and Experimental Dermatology*, vol. 32, no. 3, pp. 295–297, 2007.

[45] G. Campuzano-Maya, "Cure of alopecia areata after eradication of helicobacter pylori: a new association," *World Journal of Gastroenterology*, vol. 17, no. 26, pp. 3165–3170, 2011.

[46] L. Ben Mahmoud, H. Ghozzi, A. Hakim, Z. Sahnoun, and K. Zeghal, "Helicobacter pylori associated with chronic urticaria," *Journal of Infection in Developing Countries*, vol. 5, no. 8, pp. 596–598, 2011.

[47] P. K. Sharma, T. P. Yadav, R. K. Gautam, N. Taneja, and L. Satyanarayana, "Erythromycin pityriasis rosea: a double-blind, placebo-controlled clinical trial," *Journal of the American Academy of Dermatology*, vol. 42, no. 2, pp. 241–244, 2000.

[48] A. Rasi, L. Tajziehchi, and S. Savabi-Nasab, "Oral erythromycin is ineffective in the treatment of pityriasis rosea," *Journal of Drugs in Dermatology*, vol. 7, no. 1, pp. 35–38, 2008.

[49] A. Chuh, A. Lee, V. Zawar, G. Sciallia, and W. Kempf, "Pityriasis rosea—an update," *Indian Journal of Dermatology, Venereology and Leprology*, vol. 71, no. 5, pp. 311–315, 2005.

[50] A. P. Truhan, A. A. Hebert, and N. B. Esterly, "Pityriasis lichenoides in children: therapeutic response to erythromycin," *Journal of the American Academy of Dermatology*, vol. 15, no. 1, pp. 66–70, 1986.

[51] R. B. Skinner and A. L. Levy, "Rapid resolution of pityriasis lichenoides et varioliformis acuta with azithromycin," *Journal of the American Academy of Dermatology*, vol. 58, no. 3, pp. 524–525, 2008.

[52] H. Mensing and S. Krausse, "Erythromycin treatment for bullous pemphigoid," *Medizinische Klinik*, vol. 85, no. 8, pp. 481–484, 1990.

[53] G. Altomare, G. L. Capella, C. Fracchiolla, and E. Frigerio, "Treatment of bullous pemphigoid with erythromycin: a reappraisal," *European Journal of Dermatology*, vol. 9, no. 7, pp. 583–585, 1999.

[54] B. J. Fox, R. B. Odom, and R. F. Findlay, "Erythromycin therapy in bullous pemphigoid: possible anti-inflammatory effects," *Journal of the American Academy of Dermatology*, vol. 7, no. 4, pp. 504–510, 1982.

[55] M. Ohe and S. Hashino, "Successful treatment with erythromycin for idiopathic thrombocytopenic purpura," *Korean Journal of Hematology*, vol. 46, no. 2, pp. 140–142, 2011.

[56] M. Ohe and M. Kohno, "Three cases of idiopathic thrombocytopenic purpura showing an increase in the platelet count following clarithromycin treatment," *Rinsho Ketsueki*, vol. 44, pp. 1044–1046, 2003.

[57] H. Tlaskalova-Hogenova, R. Stepankova, T. Hudcovic et al., "Commensal bacteria (normal microflora), mucosal immunity and chronic inflammatory and autoimmune diseases," *Immunology Letters*, vol. 93, pp. 97–108, 2004.

Hindawi Publishing Corporation
Mediators of Inflammation
Volume 2012, Article ID 265714, 6 pages
doi:10.1155/2012/265714

Research Article

Azithromycin Inhibits Mucus Hypersecretion from Airway Epithelial Cells

Takeshi Shimizu and Shino Shimizu

Department of Otorhinolaryngology, Shiga University of Medical Science, Seta, Tsukinowa, Otsu, Shiga 520-2192, Japan

Correspondence should be addressed to Takeshi Shimizu, Shimizu@belle.shiga-med.ac.jp

Received 16 January 2012; Revised 27 February 2012; Accepted 2 March 2012

Academic Editor: Kazuhito Asano

To examine the *in vivo* effects of the 15-member macrolide, azithromycin (AZM), on mucus hypersecretion, we induced hypertrophic and metaplastic changes of goblet cells in rat nasal epithelium by intranasal instillation of ovalbumin (OVA) in OVA-sensitized rats, or by intranasal lipopolysaccharides (LPS) instillation. Oral administration of AZM (5–10 mg/kg) or clarithromycin (CAM, 5–10 mg/kg) significantly inhibited OVA- and LPS-induced mucus production, whereas josamycin (JM) or ampicillin (ABPC) showed no effect. *In vitro* effects of AZM on airway epithelial cells were examined using NCI-H292 cells and human nasal epithelial cells cultured in air-liquid interface. Mucus secretion was evaluated by enzyme-linked immunosorbent assay using an anti-MUC5AC monoclonal antibody. AZM or CAM significantly inhibited tumor necrosis factor-α (TNF-α) (20 ng/mL)-induced MUC5AC secretion from NCI-H292 cells at 10^{-6}–10^{-7} M, whereas JM or ABPC showed no effect. AZM significantly inhibited TNF-α (20 ng/mL)-induced MUC5AC secretion from human nasal epithelial cells at 10^{-4} M. MUC5AC mRNA expression was also significantly inhibited. These results indicate that the 15-member macrolide, AZM, exerts direct inhibitory effects on mucus secretion from airway epithelial cells and that it may be useful for the treatment of mucus hypersecretion caused by allergic inflammation and LPS stimulation.

1. Introduction

The 14-member macrolides, clarithromycin (CAM) and erythromycin (EM), and the 15-member macrolide, azithromycin (AZM), are widely used for the treatment of airway inflammation. Low-dose, long-term macrolide therapy has been reported to be very effective for patients with chronic airway diseases, such as diffuse panbronchiolitis [1], chronic bronchitis [2, 3], and chronic rhinosinusitis [4, 5]. It has been suggested that these effects depend on anti-inflammatory and immunomodulatory actions of 14- and 15-member macrolides rather than antibacterial one.

Hypersecretion of mucus is an important characteristic of these airway inflammations. The clinical effectiveness of macrolide therapy was represented by a significant reduction in the amount of secreted mucus. In our previous study, oral administration of CAM or EM significantly inhibited lipopolysaccharides- (LPS-) induced and antigen-induced mucus production in rat nasal epithelium, whereas 16-member macrolide, josamycin (JM), showed no effect. CAM and EM also inhibited mucus secretion from cultured airway epithelial cells, NCI-H292 cells, and human nasal epithelial cells cultured in air-liquid interface [6, 7]. These results indicate that the 14-member macrolides, CAM and EM, exert direct inhibitory effects on mucus secretion from airway epithelial cells. However, the inhibitory effect of 15-member macrolide, AZM, on mucus secretion is less well studied compared with CAM and EM.

In the present study, to demonstrate the effects of AZM on mucus secretion from airway epithelial cells, we evaluated (1) the *in vivo* effects of AZM on antigen-induced and LPS-induced mucus production in rat nasal epithelium, and (2) the *in vitro* effects on tumor necrosis factor-α- (TNF-α-) induced mucus secretion from human mucoepidermoid carcinoma cells (NCI-H292 cells) and from human nasal epithelial cells cultured in air-liquid interface. Mucus

secretion was evaluated by enzyme-linked immunosorbent assay (ELISA) using an anti-MUC5AC monoclonal antibody that recognizes peptide backbones of mucin. The effect on mRNA expression of MUC5AC gene was also examined.

2. Methods

2.1. Mucus Hypersecretion in Rat Nasal Epithelium. All experiments were approved by the Committee for the Care and Use of Laboratory Animals of Mie University School of Medicine. Sensitization and challenge of rats were performed as previously reported [8]. Male Fisher 344 rats (6 weeks old) were immunized with intraperitoneal injection of 200 μg ovalbumin (OVA, grade V; Sigma Chemical Co., St. Louis, MO) and 10 mg of $Al(OH)_3$ at days 1, 2, 3, and 11. At day 19, 0.1 mL saline containing 10 mg of OVA was instilled into nasal cavity for 3 days. For LPS stimulation, rats (9 weeks old) were intranasally instilled with 0.1 mL saline containing 0.1 mg LPS from *Escherichia coli* 0111:B4 (Sigma) for 3 days [9].

AZM (5–10 mg/kg, Pfizer Pharmaceutical, Tokyo), CAM (5–10 mg/kg, Taisho Pharmaceutical, Tokyo), JM (10 mg/kg, Yamanouchi Pharmaceutical, Tokyo), or ampicillin (ABPC, 30 mg/kg, Sigma) in 0.5% carboxymethyl cellulose sodium salt was given orally 1 hour before the intranasal instillation of OVA or LPS for 3 days. Twenty-four hours after the last intranasal instillation of OVA or LPS, rats were sacrificed, and the nasal cavity was transversely sectioned at the level of incisive papilla. Paraffin sections were stained with alcian blue-periodic acid-Schiff and hematoxylin (AB-PAS-H).

2.2. Morphometry. The percentage area of AB-PAS-stained mucosubstance in the surface epithelium was determined with the image analyzer (SP 500, Olympus, Tokyo) [9]. The area of nasal epithelium was outlined, and the image analyzer determined the area of AB-PAS-stained mucosubstance within this reference area. The percentage area of mucosubstance per epithelial area was calculated over 2 mm (1 mm of each side of nasal septum $\times 2$) of the basal lamina at the center of septal cartilage. Since the measured area of mucosubstance changes in the oblique section, the percent area of mucosubstance was used as a parameter of intraepithelial mucus production.

2.3. Cell Cultures. A human mucoepidermoid carcinoma cell line, NCI-H292, was grown on plastic dish in RPMI 1640 medium containing 10% fetal bovine serum, penicillin streptomycin (50 U/mL-50 μg/mL), and Hepes (25 mM).

Human nasal epithelial cells were obtained from nasal polyps from patients with chronic sinusitis. The dissociated epithelial cells were cultured in a serum-free hormone supplement medium according to a technique described previously [10]. An air-liquid interface was created when the cells became confluent, and the cultures were supplemented with medium containing 5×10^{-8} M retinoic acid.

When the NCI-H292 cells become confluent, or at the 14-day culture in the air-liquid interface of nasal epithelial cells, tumor necrosis factor-α (TNF-α), and AZM, CAM, JM, or ABPC was added to the culture medium (pH7.2) for 24 hours, then the culture medium and total RNA were collected.

2.4. ELISA. The culture medium were incubated at 40°C in a 96-well plate, until dry. Plates were blocked with 2% BSA for 1 hour, and then incubated with 50 μL of mouse monoclonal MUC5AC antibody (1 : 100) for 1 hour. The wells were incubated with 100 μL of horseradish peroxidase-goat anti-mouse IgG conjugate (1 : 10,000) for 1 hour. Color reaction was developed using 3,3′,5,5′-tetramethylbenzidine peroxidase solution. Absorbance was read at 450 nm.

2.5. Reverse Transcription-Polymerase Chain Reaction (RT-PCR). Total RNA was extracted from cultured cells, reverse transcribed, then the cDNA was amplified by PCR using the Superscript preamplification system kit (Gibco, Grand Island, NY). The MUC5AC cDNA was amplified using the sense primer 5′-CACCAAATACGCCAACAAGAC-3′ and the antisense primer 5′-CAGGGCCACGCAGCCAGAGAA-3′. The GAPDH cDNA was amplified using the sense primer 5′-CCACCCATGGCAAATTCCATGGCA-3′ and the antisense primer 5′-TCTAGACGGCAGGTCAGGTCCACC-3′.

2.6. Statistics. All data are expressed as mean ± SD. The difference between variables was analyzed by the Mann-Whitney U test. Probability values of $P < 0.05$ were considered significant.

3. Results

3.1. In Vivo Effects on Mucus Production. Intranasal instillation of OVA for 3 consecutive days induced hypertrophic and metaplastic changes of goblet cells in nasal septal epithelium of OVA-sensitized rats. Similar changes of goblet cells occurred after 3 days of LPS instillation. Only a few goblet cells were observed in control groups (untreated control, saline-instilled, and sham-sensitized rats challenged with saline or OVA, and OVA-sensitized rats challenged with saline).

Oral administration of AZM (5–10 mg/kg) or CAM (5–10 mg/kg) significantly inhibited OVA-induced mucus production, whereas treatment with JM (16-member macrolide) or ABPC showed no significant effect (Figure 1). OVA-sensitized rats, challenged with OVA, showed significant infiltration of eosinophils in nasal septal mucosa, however, AZM had no effect on OVA-induced eosinophil infiltration. The number of eosinophils in nasal septal mucosa/8 mm (4 mm in each side $\times 2$) was 2.6 ± 1.8 (saline control), 47.2 ± 17.7 (OVA-induced control), 51.4 ± 18.3 (AZM 5 mg/kg), and 44.4 ± 26,2 (AZM 10 mg/kg). LPS-induced mucus production was also significantly inhibited by the treatment with AZM (10 mg/kg) or CAM (10 mg/kg), whereas JM or ABPC showed no effect (Figure 2).

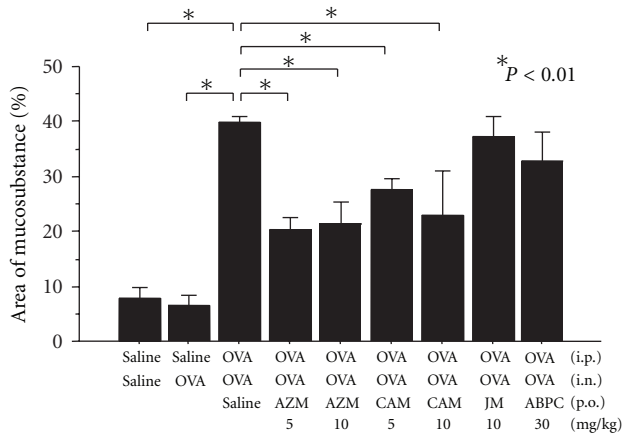


Figure 1: Effects of azithromycin (AZM, 5–10 mg/kg), clarithromycin (CAM, 5–10 mg/kg), josamycin (JM, 10 mg/kg), or ampicillin (ABPC, 30 mg/kg) on OVA-induced mucus production in OVA-sensitized rats ($n = 6$). Significant increase in intraepithelial mucosubstance occurred 24 hours after 3 days of OVA instillation. Oral administration of AZM or CAM significantly inhibited antigen-induced mucus production, whereas JM and ABPC had no effect.

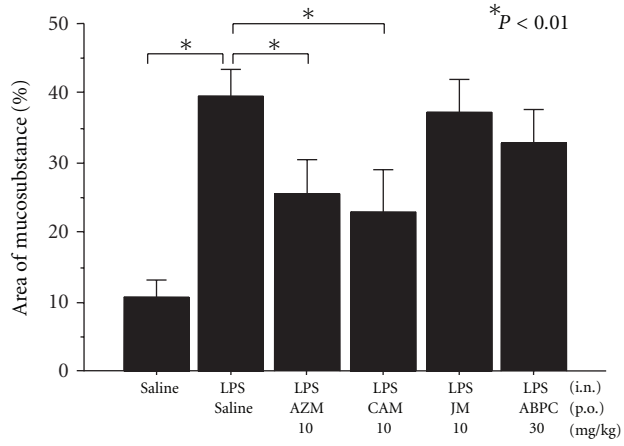


Figure 2: Effects of azithromycin (AZM, 10 mg/kg), clarithromycin (CAM, 10 mg/kg), josamycin (JM, 10 mg/kg), or ampicillin (ABPC, 30 mg/kg) on LPS-induced mucus production in rat nasal epithelium ($n = 6$). Significant increase in intraepithelial mucosubstance occurred 24 hours after 3 days of LPS instillation. Oral administration of AZM or CAM significantly inhibited LPS-induced mucus production, whereas JM and ABPC had no effect.

3.2. In Vitro Effects on Mucin Secretion

3.2.1. NCI-H292 Cells. TNF-α significantly stimulated mucin secretion from NCI-H292 cells. The percentage stimulation of MUC5AC secretion was 44.0% ± 8.6%. AZM showed an inhibitory effect on TNF-α-induced MUC5AC secretion at 10^{-6}–10^{-8} M. CAM (10^{-6}–10^{-7} M) also significantly inhibited TNF-α-induced mucin secretion, whereas JM (16-member macrolide) and ABPC showed no effects (Figure 3).

3.2.2. Human Nasal Epithelial Cells. At the 14-day culture in air-liquid interface condition, secretory cell differentiation was induced in about 25% of cultured cells [10]. The medium in the lower compartment did not react with MUC5AC. Only the samples collected from the apical side contained MUC5AC-reactive mucin, indicating that there was a polarity in mucin secretion. TNF-α (20 ng/mL) significantly stimulated MUC5AC secretion, and AZM significantly inhibited TNF-α-induced mucin secretion at 10^{-4} M from cultured human nasal epithelial cells, whereas ABPC showed no effect. Changes of MUC5AC gene expression were evaluated by RT-PCR, and AZM (10^{-4} M) significantly inhibited MUC5AC mRNA expression of cultured human nasal epithelial cells (Figure 4).

4. Discussion

In the present study, hypertrophic and metaplastic changes of goblet cells were induced in rat nasal epithelium by intranasal challenge with OVA in OVA-sensitized rats or by intranasal LPS instillation. A similar increase of epithelial mucosubstance occurred 24 hours after three days of OVA or LPS instillation. Oral administration of AZM (15-member macrolide) significantly inhibited antigen- or LPS-induced mucus production. These inhibitory effects are similar with CAM (14-member macrolide), whereas JM (16-member macrolide) or ABPC showed no effect. This is the first report showing the *in vivo* effects of AZM on mucus production in upper airways.

Mucus hypersecretion associated with hypertrophy and metaplasia of epithelial secretory cells is a major characteristic of chronic airway diseases, and the clinical effectiveness of low-dose and long-term treatment with 14-member macrolides, CAM and EM, is represented by the significant reduction of the amount of secreted mucus, sputum, and rhinorrhea. Tamaoki and coworkers [11] have reported that erythromycin (EM) significantly inhibited mucus secretion in guinea pig trachea *in vivo*. In our previous studies [6, 7], CAM and EM inhibited antigen- and LPS-induced mucus production in rat nasal epithelium. CAM and EM showed the direct inhibitory effect on mucin secretion from cultured airway epithelial cells [6].

The 15-member macrolide, AZM, also has an anti-inflammatory action, and AZM has been widely used for the treatment of patients with chronic airway inflammation, such as cystic fibrosis [12], chronic obstructive pulmonary disease [13], and bronchiolitis obliterans syndrome [14]. The meta-analysis study revealed that long-term use of AZM in cystic fibrosis patients improved the lung function, especially for *Pseudomonas aeruginosa*-colonized patients [12]. A large randomized placebo-controlled study revealed that long-term use of AZM decreased the risk of acute exacerbations of patients with chronic obstructive pulmonary diseases [13].

Several animal studies demonstrated that AZM attenuated many types of experimental airway inflammation caused by the allergic inflammation [15], by the inhalation of irritant gas, ozone [16], by the lung ischemia reperfusion injury [17], or by bacterial and viral infections [18, 19]

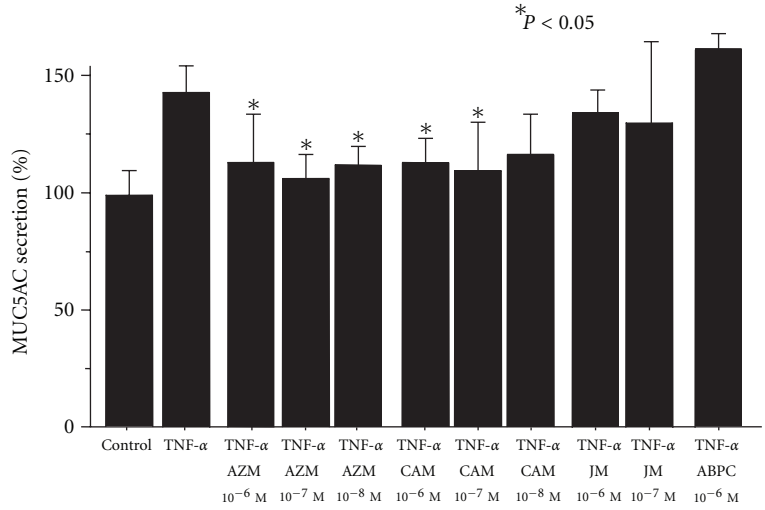


Figure 3: Effects of azithromycin (AZM), clarithromycin (CAM), josamycin (JM), and ampicillin (ABPC) on TNF-α (20 ng/mL)-induced MUC5AC secretion from NCI-H292 cells ($n = 5$). TNF-α stimulated mucin secretion. AZM and CAM significantly inhibited TNF-α-induced MUC5AC secretion, whereas JM and ABPC had no effect.

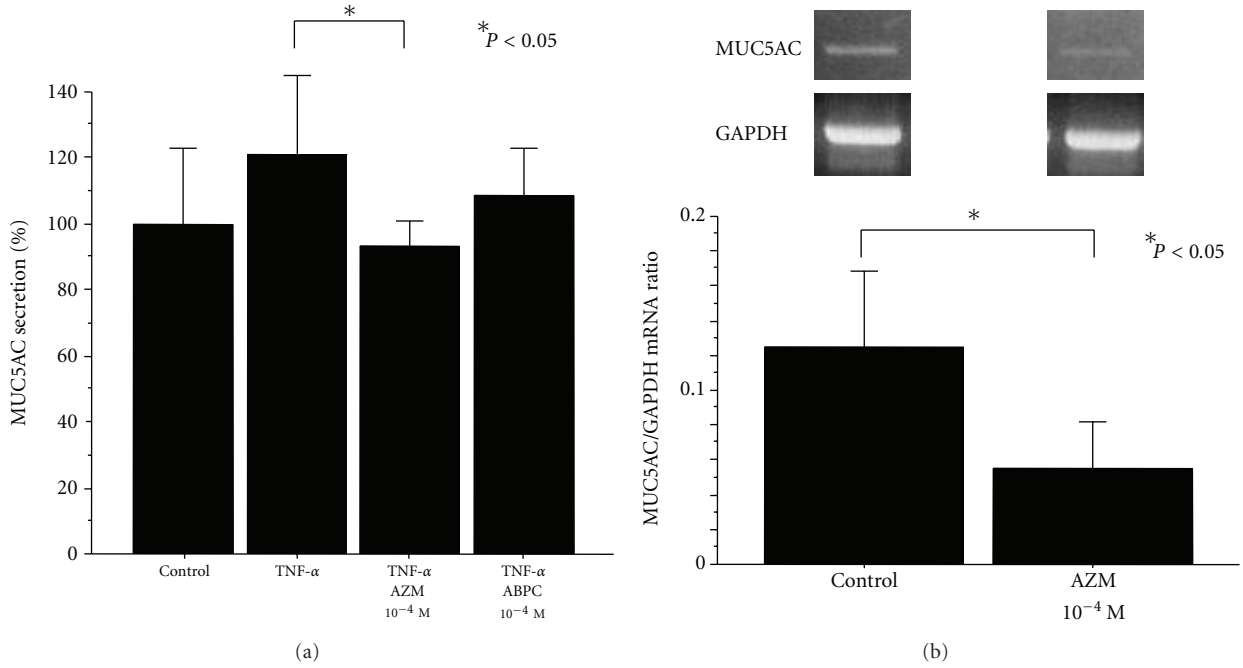


Figure 4: Effects of azithromycin (AZM) and ampicillin (ABPC) on TNF-α (20 ng/mL)-induced mucin secretion (a) and MUC5AC mRNA expression (b) from human nasal epithelial cells cultured at air-liquid interface ($n = 5$). (a) TNF-α stimulated MUC5AC secretion, and AZM significantly inhibited TNF-α-induced mucin secretion at 10^{-4} M, whereas ABPC showed no effect. (b) Total RNA was isolated and analyzed for MUC5AC and GAPDH mRNA expression by RT-PCR ($n = 5$). AZM significantly inhibited MUC5AC mRNA expression at 10^{-4} M as demonstrated by the MUC5AC/GAPDH ratio.

in lower airways. In the present study, AZM also attenuated antigen- or LPS-induced mucus production in rat nasal epithelium. Many investigators demonstrated the anti-inflammatory action of AZM, which includes the immunomodulatory effects on inflammatory cells [19, 20], the modulation of cytokine production [21], and the inhibition of bacterial function and biofilm formation [22].

Recently, several *in vitro* studies have demonstrated the inhibitory effects of AZM on mucus secretion from airway epithelium. AZM inhibited MUC5AC expression and secretion from NCI-H292 cells, induced by human neutrophil peptide-1 and LPS [23], by *Pseudomonas aeruginosa*-derived N-(3-Oxododecanoyl) homoserine lactone [24], or by nontypable *Haemophilus influenza* and *Chlamydophilia*

pneumoniae [25, 26]. AZM inhibited acetylcholine-induced MUC5AC release from swine airway submucosal gland cells [27]. In the present study, we examined the TNF-α-induced MUC5AC secretion from airway epithelial cells. TNF-α has been implicated in LPS-induced airway inflammation. LPS stimulation enhanced the TNF-α/β generation in rat lung [28], and TNF-α antagonist inhibited the LPS-induced mucus hypersecretion in rat nasal epithelium [29]. We found that AZM and CAM significantly inhibited TNF-α-induced MUC5AC secretion from NCI-H292 cells. AZM also inhibited mucin secretion from human nasal epithelial cells cultured in air-liquid interface, and MUC5AC mRNA expression was significantly inhibited. This is the first report showing the inhibitory effects of AZM on mucus secretion from normal human airway epithelial cells. These inhibitory actions appeared to be unique for 14- and 15-member macrolides because other antibiotics, JM (16-member macrolide) and ABPC, did not show any effect.

In our previous study, the active concentrations of CAM and EM for the inhibition of mucin secretion are 10^{-6} to 10^{-7} M for NCI-H292 cells and 10^{-4} to 10^{-5} M for human nasal epithelial cells [6]. The different results may be caused by the different responses between mucoepidermoid carcinoma cells and normal nasal epithelial cells. In the present study, AZM showed the similar inhibitory effect on MUC5AC secretion from NCI-H292 cells and from human nasal epithelial cells. It is well known that the macrolide antibiotics achieve higher concentration in airway tissues, and the therapeutic concentrations are 10^{-5} to 10^{-6} M in tissues. In our *in vivo* study, oral administration of 5–10 mg/kg AZM or CAM significantly inhibited epithelial mucus production, and a previous study demonstrated that this is comparable with tissue concentration of 10^{-5} to 10^{-6} M in rats [30]. These results indicate that the *in vivo* effect of AZM or CAM is caused in some parts by the direct inhibitory effect on mucus secretion from the epithelial cells.

5. Conclusion

We have induced hypertrophic and metaplastic changes of goblet cells in rat nasal epithelium by intranasal challenge with OVA in OVA-sensitized rat and by LPS instillation, and we have demonstrated in this model that AZM inhibits epithelial mucus production produced by allergic inflammation and by LPS stimulation. We have also demonstrated that AZM directly inhibits MUC5AC secretion from NCI-H292 cells and human nasal epithelial cells. These novel findings may explain the clinical efficacy of AZM in patients with chronic airway inflammation.

References

[1] S. Kudoh, A. Azuma, M. Yamamoto, T. Izumi, and M. Ando, "Improvement of survival in patients with diffuse panbronchiolitis treated with low-dose erythromycin," *American Journal of Respiratory and Critical Care Medicine*, vol. 157, no. 6, pp. 1829–1832, 1998.

[2] T. Shirai, A. Sato, and K. Chida, "Effect of 14-membered ring macrolide therapy on chronic respiratory tract infections and polymorphonuclear leukocyte activity," *Internal Medicine*, vol. 34, no. 6, pp. 469–474, 1995.

[3] K. Oishi, F. Sonoda, S. Kobayashi et al., "Role of interleukin-8 (IL-8) and an inhibitory effect of erythromycin on IL-8 release in the airways of patients with chronic airway diseases," *Infection and Immunity*, vol. 62, no. 10, pp. 4145–4152, 1994.

[4] K. Fujita, T. Shimizu, Y. Majima, and Y. Sakakura, "Effects of macrolides on interleukin-8 secretion from human nasal epithelial cells," *European Archives of Oto-Rhino-Laryngology*, vol. 257, no. 4, pp. 199–204, 2000.

[5] T. Yamada, S. Fujieda, S. Mori, H. Yamamoto, and H. Saito, "Macrolide treatment decreased the size of nasal polyps and IL-8 levels in nasal lavage," *American Journal of Rhinology*, vol. 14, no. 3, pp. 143–148, 2000.

[6] T. Shimizu, S. Shimizu, R. Hattori, E. C. Gabazza, and Y. Majima, "*In vivo* and *in vitro* effects of macrolide antibiotics on mucus secretion in airway epithelial cells," *American Journal of Respiratory and Critical Care Medicine*, vol. 168, no. 5, pp. 581–587, 2003.

[7] Y. Takahashi, T. Shimizu, and Y. Sakakura, "Effects of indomethacin, dexamethasone, and erythromycin on endotoxin-induced intraepithelial mucus production of rat nasal epithelium," *Annals of Otology, Rhinology and Laryngology*, vol. 106, no. 8, pp. 683–687, 1997.

[8] T. Shimizu, H. Hirano, Y. Majima, and Y. Sakakura, "A mechanism of antigen-induced mucus production in nasal epithelium sensitized rats: a comparison with lipopolysaccharide-induced mucus production," *American Journal of Respiratory and Critical Care Medicine*, vol. 161, no. 5, pp. 1648–1654, 2000.

[9] T. Shimizu, Y. Takahashi, S. Kawaguchi, and Y. Sakakura, "Hypertrophic and metaplastic changes of goblet cells in rat nasal epithelium induced by endotoxin," *American Journal of Respiratory and Critical Care Medicine*, vol. 153, no. 4, pp. 1412–1418, 1996.

[10] S. Usui, T. Shimizu, K. Fujita, C. Kishioka, and Y. Sakakura, "Secretory cell differentiation and mucus secretion in cultures of human nasal epithelial cells: use of a monoclonal antibody to study human nasal mucin," *Annals of Otology, Rhinology and Laryngology*, vol. 109, no. 3, pp. 271–277, 2000.

[11] J. Tamaoki, K. Takeyama, I. Yamawaki, M. Kondo, and K. Konno, "Lipopolysaccharide-induced goblet cell hypersecretion in the guinea pig trachea: inhibition by macrolides," *American Journal of Physiology*, vol. 272, no. 1, pp. L15–L19, 1997.

[12] Y. Cai, D. Chai, R. Wang, N. Bai, B. B. Liang, and Y. Liu, "Effectiveness and safety of macrolides in cystic fibrosis patients: a meta-analysis and systemic review," *Journal of Antimicrobial Chemotherapy*, vol. 66, pp. 968–978, 2011.

[13] R. K. Albert, J. Connett, W. C. Bailey et al., "Azithromycin for prevention of exacerbations of COPD," *The New England Journal of Medicine*, vol. 365, pp. 689–698, 2011.

[14] S. G. Gerhardt, J. F. McDyer, R. E. Girgis, J. V. Conte, S. C. Yang, and J. B. Orens, "Maintenance azithromycin therapy for bronchiolitis obliterans syndrome: results of a pilot study," *American Journal of Respiratory and Critical Care Medicine*, vol. 168, no. 1, pp. 121–125, 2003.

[15] A. Beigelman, S. Gunsten, C. L. Mikols et al., "Azithromycin attenuates airway inflammation in a noninfectious mouse model of allergic asthma," *Chest*, vol. 136, no. 2, pp. 498–506, 2009.

[16] G. I. Criqui, C. Solomon, B. S. Welch, R. E. Ferrando, H. A. Boushey, and J. R. Balmes, "Effects of azithromycin on ozone-

induced airway neutrophilia and cytokine release," *European Respiratory Journal*, vol. 15, no. 5, pp. 856–862, 2000.

[17] N. Geudens, L. Timmermans, H. Vanhooren et al., "Azithromycin reduces airway inflammation in a murine model of lung ischaemia reperfusion injury," *Transplant International*, vol. 21, no. 7, pp. 688–695, 2008.

[18] A. Beigelman, C. L. Mikols, S. P. Gunsten, C. L. Cannon, S. L. Brody, and M. J. Walter, "Azithromycin attenuates airway inflammation in a mouse model of viral bronchiolitis," *Respiratory Research*, vol. 11, article 90, 2010.

[19] W. C. Tsai, M. L. Rodriguez, K. S. Young et al., "Azithromycin blocks neutrophil recruitment in *Pseudomonas* endobronchial infection," *American Journal of Respiratory and Critical Care Medicine*, vol. 170, no. 12, pp. 1331–1339, 2004.

[20] D. J. Feola, B. A. Garvy, T. J. Cory et al., "Azithromycin alters macrophage phenotype and pulmonary compartmentalization during lung infection with *Pseudomonas*," *Antimicrobial Agents and Chemotherapy*, vol. 54, no. 6, pp. 2437–2447, 2010.

[21] H. Yamasawa, K. Oshikawa, S. Ohno, and Y. Sugiyama, "Macrolides inhibit epithelial cell-mediated neutrophil survival by modulating granulocyte macrophage colony-stimulating factor release," *American Journal of Respiratory Cell and Molecular Biology*, vol. 30, no. 4, pp. 569–575, 2004.

[22] I. Perez-Martinez and D. Haas, "Azithromycin inhibits expression of the GacA-dependent small RNAs RsmY and Rsm Z in *Pseudomonas aeruginosa*," *Antimicrobial Agents and Chemotherapy*, vol. 55, pp. 3399–3405, 2011.

[23] H. Ishimoto, H. Mukae, N. Sakamoto et al., "Different effects of telithromycin on MUC5AC production induced by human neutrophil peptide-1 or lipopolysaccharide in NCI-H292 cells compared with azithromycin and clarithromycin," *Journal of Antimicrobial Chemotherapy*, vol. 63, no. 1, pp. 109–114, 2009.

[24] Y. Imamura, K. Yanagihara, Y. Mizuta et al., "Azithromycin inhibits MUC5AC production induced by the *Pseudomonas aeruginosa* autoinducer N-(3-oxododecanoyl) homoserine lactone in NCI-H292 cells," *Antimicrobial Agents and Chemotherapy*, vol. 48, no. 9, pp. 3457–3461, 2004.

[25] N. Araki, K. Yanagihara, Y. Morinaga et al., "Azithromycin inhibits nontypeable *Haemophilus influenzae*-induced MUC5AC expression and secretion via inhibition of activator protein-1 in human airway epithelial cells," *European Journal of Pharmacology*, vol. 644, no. 1–3, pp. 209–214, 2010.

[26] Y. Morinaga, K. Yanagihara, N. Miyashita et al., "Azithromycin, clarithromycin and telithromycin inhibit MUC5AC induction by *Chlamydophila pneumoniae* in airway epithelial cells," *Pulmonary Pharmacology and Therapeutics*, vol. 22, no. 6, pp. 580–586, 2009.

[27] S. Lu, H. Liu, and J. M. Farley, "Macrolide antibiotics inhibit mucus secretion and calcium entry in swine airway submucosal mucous gland cells," *Journal of Pharmacology and Experimental Therapeutics*, vol. 336, no. 1, pp. 178–187, 2011.

[28] M. Ermert, C. Pantazis, H. R. Duncker, F. Grimminger, W. Seeger, and L. Ermert, "In situ localization of TNFα/β, TACE AND TNF receptors TNF-R1 and TNF-R2 in control and LPS-treated lung tissue," *Cytokine*, vol. 22, no. 3-4, pp. 89–100, 2003.

[29] D. H. Kim, E. J. Jeon, S. N. Park, K. H. Park, Y. S. Park, and S. W. Yeo, "Effects of a tumor necrosis factor-α antagonist on experimentally induced rhinosinusitis," *Journal of Biomedicine and Biotechnology*, vol. 2011, Article ID 360457, 9 pages, 2011.

[30] H. Yoshida and T. Furuta, "Tissue penetration properties of macrolide antibiotics-comparative tissue distribution of erythromycin-stearate, clarithromycin, roxythromycin and azithromycin in rats," *Japanese Journal of Antibiotics*, vol. 52, pp. 497–503, 1999.

Hindawi Publishing Corporation
Mediators of Inflammation
Volume 2012, Article ID 134605, 7 pages
doi:10.1155/2012/134605

Review Article

The Role of Macrolides in Childhood Non-Cystic Fibrosis-Related Bronchiectasis

R. Masekela and R. J. Green

Paediatric Pulmonology Division, Department of Paediatrics and Child Health, Steve Biko Academic Hospital, University of Pretoria, Pretoria 0001, South Africa

Correspondence should be addressed to R. Masekela, refiloe.masekela@up.ac.za

Received 19 January 2012; Accepted 31 January 2012

Academic Editor: Kazuhito Asano

Non-cystic fibrosis-related bronchiectasis is a chronic inflammatory lung disease, which is regarded as an "orphan" lung disease, with little research devoted to the study of this condition. Bronchiectasis results in impaired quality of life and mortality if left untreated. The tools available in the armamentarium for the management of bronchiectasis entail antibiotic therapy traditionally used to treat exacerbations, stratagems to improve mucociliary clearance, and avoidance of toxins. Macrolides have been known for the last two decades to have not only anti-bacterial effects but immunomodulatory properties as well. In cystic fibrosis, the use of macrolides is well documented in subjects colonized with *Pseudomonas aeruginosa*, to improve quality of life and lung function. There is currently emerging evidence to suggest the benefit of macrolides in subjects not colonized with *Pseudomonas aeruginosa*. This beneficial effect has been less explored in the context of bronchiectasis from other causes. The purpose of this paper is to review the current literature on the use of macrolides in non-cystic fibrosis related bronchiectasis in paediatrics.

1. Bronchiectasis

The term bronchiectasis is derived from the Greek words *bronkia* (bronchial tubes), *ek* (out), and *tasis* (stretching). The earliest description of bronchiectasis was by Laennac in 1819 [1]. There are two anatomical classification systems used for the diagnosis of bronchiectasis, namely, the Reid and Whitwell classifications [2, 3]. In the past few years, the diagnostic criteria for bronchiectasis have changed, with the diagnosis being based on the less invasive high-resolution computerized tomography (HRCT). HRCT scanning has led to easier diagnosis and follow up of bronchiectasis [4].

The exact pathophysiological mechanisms for bronchiectasis are unknown, with the currently accepted concept being the "vicious cycle" theory proposed by Cole in the mid-eighties (Figure 1) [5]. Cole's theory evolves around an initial "hit" or trigger that results in airway inflammation. The inflammatory process is established such that, with subsequent lung infections, persistent airway inflammation occurs. This is associated with release of proinflammatory cytokines interleukin-(IL-) 6, IL-8, and neutrophil elastases [6–8]. These cytokines recruit inflammatory mediators, whose end-product is mucous gland hypertrophy and mucus hyperproduction. Excess mucus compromises the mucociliary escalator, which further perpetuates microbial invasion of the airway. Mucus performs an innate immune function property in the lungs by acting as the first barrier in the airways. Mucus is made up of mucin proteins, water, surfactant phospholipids, peptides, and defence proteins. There are many changes that occur to the mucus properties of patients with chronic inflammatory lung disease [9]. There is goblet cell hyperplasia, which contributes to excessive mucus production. In the presence of infection epithelial cells modulate the recruitment of inflammatory cells by the production of chemokines, cytokines, adhesion molecules, and modulation of expression of receptors. The presence of persistent infection, impairment of the protective mucociliary escalator, and the presence of enzymes such as elastases cause damage to the airway and lung tissue [10].

Risk factors associated with bronchiectasis are overcrowding, poverty, damp housing, macro- and micromalnutrition, indoor pollution with biomass fuels, and

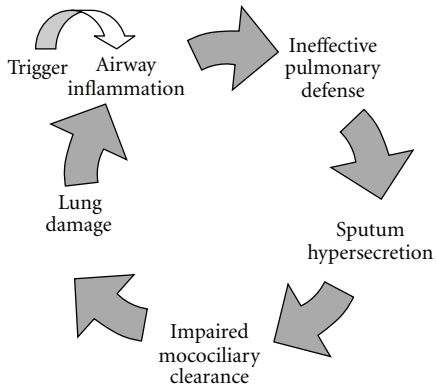


Figure 1: The pathophysiology of bronchiectasis the inflammatory cycle as proposed by Cole.

environmental tobacco smoke. These risks factors have been largely diminished in developing countries with rates of bronchiectasis as low as 0.49 per 100 000 population in Finnish children [11–13]. Certain groups in developed countries, such as the Alaskan natives of the Yokun Kuskokwim Delta, the New Zealand Maori, and the Aborigines of Australia, have inordinately high bronchiectasis rates, ranging from 3.5 to 16 per 10 000 [14–16]. This is in contradistinction to developing countries where there is a high infectious disease burden and consequently high bronchiectasis rates [17]. There is, however, no accurate prevalence data available to quantify the problem in developing countries.

2. Immunology of Bronchiectasis

The innate immune system is activated by pathogen-associated molecular patterns (PAMPs), which are recognized by pattern recognition receptors such as toll-like receptors (TLRs) [18, 19]. TLR activation triggers a cascade resulting in the activation and nuclear translocation of nuclear factor $\kappa\beta$ (NF$\kappa\beta$) with subsequent release of proinflammatory cytokines IL-1β, IL-8, and TNF-α [20]. IL-8 is a potent chemoattractant for neutrophils [21]. Neutrophils are integral to the innate immune mechanisms in the lung, with neutrophillic inflammation being central in the pathogenesis of bronchiectasis. Elevated levels of neutrophil derived products IL-6, IL-8 and TNF-α have been found in the sputum of adults with stable bronchiectasis [22]. Transepithelial migration of neutrophils from the intravascular compartment occurs in a coordinated fashion with interplay of various adhesion molecules. Three families of adhesion molecules mediate this; the selectins, the integrins CD11/CD18, and the immunoglobulin superfamily that is, intravascular adhesion molecule 1 (ICAM-1) and vascular adhesion molecule 1 (VCAM-1) [23]. These adhesion molecules are upregulated in the presence of IL-1, TNF-α, and IL-8. Both VCAM-1 and ICAM-1 have been found to be elevated in bronchiectasis subjects [10]. Adherent neutrophils migrate to the inflammatory site under the direction of the neutrophil chemoattractant IL-8. Once activated, neutrophils produce neutrophil elastase (NE) and matrix metalloproteinases: MMP-8 and MMP-9. NE has three main mechanisms of action. Firstly, it has proteolytic effects from toxic products that digest the airway elastin, basement membrane collagen, and proteoglycan [23]. Secondly, it induces the release of cytokines IL-6, IL-8, and GM-CSF [23]. Finally, it is a powerful secretagogue inducing expression of mucin gene MUC5AC via the generation of reactive oxygen species [23]. In CF, the free elastase is associated with reduced opsonization of pathogens, thus acting as a potent stimulator for IL-8 production [24].

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a potent chemokine that allows prolonged survival of neutrophils in the airway. The intensity of the proinflammatory cytokines was also found to be elevated in subjects with colonization of the airways by microorganisms. This elevation in the cytokines, coupled with the elevated proteases released from neutrophils, namely, neutrophil elastase, MMP-2, MMP-6, and MMP-9, overwhelms the antiprotease defence mechanisms rendering the lung vulnerable to destruction [25–27]. The use of antibiotics has been shown to result in a reduction of these proinflammatory cytokines [28].

3. Management of Bronchiectasis

Interventions in the management of bronchiectasis include medical as well as adjunctive therapies. The therapeutic goals of management include the following: treatment of the underlying disease, aggressive treatment of infections, promotion of mucociliary clearance, promotion of normal growth, avoidance of toxins, identification and management of complications, and treatment of the chronic inflammation to retard disease progression [29].

Although airway clearance with chest physiotherapy is universally recommended the evidence for benefit is limited. A Cochrane review demonstrated no improvement in lung function in patients who had regular multimodality airway clearance techniques [30]. The benefit to individuals seems to lie in the reduction of cough frequency and improvement in quality of life. The technique used does not appear to have any impact on the outcome, although in patients with gastroesophageal reflux, care should be taken when instituting techniques that use the head down position. This is particularly important in young children. There have been no favourable outcomes, in terms of lung function parameters, with the use of physiotherapy [31].

In bronchiectasis, the rheological properties of mucus are abnormal with variation in the rheology depending on the cause of bronchiectasis. In childhood, postinfective bronchiectasis mucus is less viscous and more transportable than that of children with CF [32]. The agents used for airway clearance are either airway hydrators or mucolytics. Mucolytic agents reduce mucus viscosity and promote clearance of secretions. They do this via several mechanisms, which include disruption of disulphide bonds and liquefying proteins that degrade DNA filaments and actin. This modality of treatment is an attractive option in a condition where increased mucus tenacity and viscosity is

a problem. Recombinant DNAse (rhDNAse) has been used with excellent results in CF. However, in non-CF bronchiectasis such results are not obtained. In a large multicentre trial by O'Donnell et al., rhDNAse was found to have detrimental effects in participants with worsening decline in lung function [33]. Forced vital capacity (FVC) was reduced by 3.1% compared to placebo. Patients also suffered an increase in the number of exacerbations in the intervention group. This finding is in contradistinction to the benefits documented in CF. This may have several explanations: firstly, there are differences in rheological properties of mucus in the CF airway when compared to the non-CF bronchiectatic airway [32]. Secondly, in CF, the pathology is mostly in the upper lobes, and the use of mucolytics may facilitate clearance with gravity, whilst in non-CF bronchiectasis the lower lobes are affected and this may hamper their effective clearance of thin secretions against gravity [33, 34]. Due to the harm demonstrated in this study, there have been no paediatric studies conducted in the use of rhDNAse. Therefore, the use of this drug is strongly discouraged in patients with non-CF bronchiectasis. The use of mucus hydrators like hypertonic saline and mannitol have been studied. Hypertonic saline has shown benefit in one small adult study when used in conjunction with chest physiotherapy [35]. A Cochrane review and a recent trial of the use of mannitol also have shown benefit in changing the physical properties of mucus in fourteen adults with bronchiectasis [36, 37].

Antibiotic therapy forms the cornerstone of bronchiectasis treatment. The use of antibiotics can prevent airway damage by treating infections, maintain and improve lung functions, and improve quality of life. Pseudomonas infection is rare in children with non-CF bronchiectasis [38]. Inhaled antibiotics have been extensively studied in the context of CF. The use of this strategy has the benefit of targeted drug delivery, limitation of systemic drug absorption, and reduction of side effects. The drug doses required for oral and intravenous antibiotics, to achieve bactericidal levels in airway secretions, need to be between 10 and 25 times above the mean inhibitory concentration. This, therefore, renders inhaled therapies a more attractive option in bronchiectasis. In order to have optimal use of inhaled drugs, they need to be at a pH above 4.0 and have an osmolarity between 100–1100 mOsmol. Several antibiotics, including tobramycin, ceftazidime, and gentamycin, have been studied especially in the context of CF in subjects colonized with *Pseudomonas aeruginosa* [39–41]. There is currently insufficient evidence for the recommendation of the use of inhaled antibiotics, especially since pseudomonas colonization is a rare event in non-CF bronchiectasis in children, although small studies with inhaled tobramycin, colistin, and aztreonam have suggested benefit [39].

Anti-inflammatory drugs like corticosteroids are a natural candidate in the management of bronchiectasis as they can play a pivotal role in breaking the cycle of inflammation. The anti-inflammatory effects are mediated by a reduction of inflammatory cytokines, inhibition of prostaglandins, reduction in adhesion molecules, and the inhibition of nitric oxide in the airway. Regrettably, systemic corticosteroids cannot be used long term due to their unfavourable side-effect profile. Inhaled corticosteroids have been shown in randomized trials to reduce the number of exacerbations, reduce sputum volume, and improve quality of life in bronchiectasis [22, 42, 43]. One randomized trial of eighty-six adults showed that subjects colonized with *Pseudomonas aeruginosa* derived the most benefit from the use of inhaled corticosteroids [22].

Table 1: Types of macrolide antibiotics.

14-member ring macrolides	Erythromycin Troleandomycin Clarithromycin Roxithromycin
15-member ring macrolides	Azithromycin
16-member ring macrolides	Josamycin Spiramycin Midecamycin

4. Macrolides and Bronchiectasis

Macrolide antibiotics are a group of antibiotics that contain a macrocytic lactone ring with a number of sugar moieties attached to these rings. Macrolides are further subclassified according to the number of lactone rings into the 14-, 15-, and 16-member ring macrolides (Table 1). The oldest of these drugs is erythromycin. Erythromycin is a 14-member macrolide, which was first isolated by McGiure and colleagues in 1952 from *Streptomyces erythreus* found in soil samples in the Philippines. The other macrolides are semisynthetic agents.

Azithromycin is an azalides with an added methyl-substituted nitrogen atom onto the lactone ring to form the 15-member ring. Clarithromycin is formed by the methylation of the hydroxyl group at position 6 of the lactone ring. These structural modifications confer azithromycin and clarithromycin a slightly better side effect profile when compared to erythromycin. These modifications reduce the interaction of these drugs with drugs metabolized by the cytochrome P450 system. There are also significantly fewer gastrointestinal side effects. Azithromycin and clarithromycin also have a far superior tissue penetration in vitro and a longer elimination half life and, thus, need once daily dosing. The drawback of the use of these agents is their significantly higher cost when compared to erythromycin, which is a relatively cheap and effective drug. Macrolide concentrations are at least 10-fold higher in epithelial lung fluid than in serum [44].

The mode of action of macrolides is by reversible binding to the 50 s subunit of the ribosome in prokaryocytes. This results in prevention of ribosomal translation and thus prevention of bacterial replication. Macrolides are bacteriostatic for *Staphylococci*, *Streptococci*, and *Haemophilus*, but they may exert bactericidal effects at very high concentrations. Macrolides do not have bactericidal effects against *Pseudomonas aeruginosa* but do result in inhibition of biofilm formation and also inhibit the organism's ability to produce

toxins [45]. Macrolides are commonly used as a first-line therapy for treatment of acute bacterial infections such as community-acquired pneumonia in adults. The potential use of macrolides for their immune modifying effects was first discovered in patients with severe steroid dependent asthma [46]. The concomitant use of troleandomycin was found to result in significant improvement in asthma control in patients and also led to dose reduction of steroids without loss of asthma control. These immunomodulatory effects of macrolides are limited to the 14- and 15-membered ring macrolides.

The use of low-dose macrolides in the management of chronic inflammatory lung disease was initially found in Japanese patients with diffuse panbronchiolitis (DPB) [47–50]. DPB, a common condition in Japan and South East Asia, is a progressive inflammatory disorder whose sufferers present with chronic productive cough, wheezing, exertional dyspnoea, chronic sinusitis, mucoid *Pseudomonas aeruginosa* colonization, mixed restrictive and obstructive pulmonary functions, and diffuse chronic inflammation involving the bronchiolar and centrilobular regions of the airway. Untreated, DPB has a very poor prognosis; in 1984, the five-year survival rate was 26%. With the use of low dose erythromycin, the mortality of these patients was dramatically reduced with 10-year survival rates increasing to 92% [50]. This was coupled with an improvement in lung function and quality of life of sufferers. The immunomodulatory effects of macrolides are thought to result in reduction in sputum volume, inhibition of virulence factor production by bacteria, diminished neutrophil influx and downregulation of IL-8 production, inhibition of NF-$\kappa\beta$ production, and reduction in both ICAM-1 and neutrophil elastase [51–54]. These immunomodulatory effects result in a reduction in pulmonary exacerbations, improved lung function, and improved quality of life [28, 55–61]. The clinical improvement of subjects may take up to three months to show an effect.

The use of macrolides is not only limited to DPB. In the late 1990s, there was rekindled interest in the use of macrolides in the treatment of other chronic inflammatory lung disorder including CF. CF is a genetic disorder caused by a defect on chromosome 7, resulting in an abnormal CF transmembrane regulator gene, which results in an abnormal chloride secretion by the apical epithelial cells. The accumulation of aberrant CFTR in the endoplasmic reticulum is thought to result in calcium release and stimulation of NF$\kappa\beta$. NF$\kappa\beta$ causes the release of IL-8 and inflammation of the airway. As the inflammatory process becomes chronic, there is histotoxic inflammation with an increase of lymphocytes and monocytes; this process occurs in the CF airway with continued predominance of neutrophils [62, 63]. It is thought that the chronic infections that occur in CF cause an increase in granulocyte colony stimulating factor (GCSF) and GM-CSF with signalling of reduction in cellular apoptosis causing this persistence of neutrophillic airway inflammation. In the setting of CF, azithromycin has been consistently found to result in a reduction in the number of pulmonary exacerbations, time to first exacerbation, and improvement in nutritional parameters [64–67]. In CF, macrolides form part of the cornerstone of therapy in subjects colonized with *Pseudomonas aeruginosa*, with emerging evidence of their benefit in CF subjects without *Pseudomonas aeruginosa* [68]. With initiation of macrolides, there is a modest initial improvement in lung functions.

There are a few studies looking at the immunomodulatory role of macrolides in the management of patients with non-CF bronchiectasis (Table 2). One adult study by Tsang et al. studied the effect of erythromycin in patients with severe idiopathic bronchiectasis. They found a significant improvement in FEV1, FVC, and sputum volume over a period of 8 weeks in 11 patients when compared to 10 controls [58]. In this study, there was no change in the proinflammatory mediators (IL-8, TNF-α, IL-1$\alpha\beta$, and leukotriene B4). Only one study in children showed an improvement on the small airways (maximal mid-expiratory flow) and a reduction in IL-8 [59]. The trials conducted on macrolides in bronchiectasis are limited in patient numbers and length of treatment but universally all have shown a consistent reduction in the frequency of exacerbations and sputum volumes [28, 57, 59, 60].

5. Macrolide Resistance and Safety

Long-term use of macrolides results in resistance particularly to *Streptococci*, *Haemophilus*, and *Staphylococci.* There are three mechanisms by which resistance occurs [69]. Firstly, this may be due to ribosomal target modification mediated by methylases encoded by the *erm*(B) gene. The second mechanism is due to mutation of the 23S rRNA or ribosomal proteins L4 and L22. This leads to conformational changes in the binding site of macrolides. Finally, active drug efflux occurs due to the membrane bound efflux protein *mef*(A) gene. Phaff et al. found increasing resistance of *S. aureus* to macrolides in CF patients, with an in resistance of 17.2% in those on macrolides versus 3.6% in CF subjects not on macrolides [70]. Tramper-Stranders et al. also found an exponential increase in *Staphylococcal* resistance to macrolides with increases from 83% in the first year of therapy to 100% in the third year of macrolide use [71].

There are safety concerns on the long-term use of macrolides. There is concern of cardiac side-effects (torsades de pointes) when using macrolides, particularly erythromycin, in conjunction with drugs that inhibit the CYP3A pathway. Postmarketing surveillance of the long-term use of erythromycin in Japan indicate this to be extremely rare [69]. The biggest concern with the use of macrolides is the development of resistant organisms, particularly the nontuberculous mycobacteria (NTM), which are commonly found in bronchiectasis patients. The newer macrolides azithromycin and clarithromycin form the backbone therapy for NTM management. It is known that carriage of NTM is high in bronchiectasis patients. A multicentre trial of CF subjects recovered NTM in 13% of over 900 subjects studied [72]. There is, therefore, a need for the development of novel macrolides that have no antimicrobial activity and only immunomodulatory properties.

Table 2: A summary of clinical trials of the use of macrolide therapy in bronchiectasis.

Author	Year	Study drug	Study design	Age group	Benefit
Tsang et al. [58]	1999	Erythromycin	RDBPCT	Adult	↑ FEV1, ↑ FVC ↓ sputum volume
Yalcin et al. [28]	2006	Clarithromycin	RPCT	Paediatric	↓ sputum volume, ↓ sputum cytokines
Koh et al. [59]	1997	Roxithromycin	RDBPCT	Adult	↓ airway reactivity to methacholine
Davies and Wilson [60]	2004	Azithromycin	Prospective open-label	Adult	↓ symptoms and ↑ D_{LCO}
Cymbala et al. [57]	2005	Clarithromycin	Randomised open-label, crossover	Adult	↓ sputum volume
Serisier and Martin [55]	2011	Erythromycin	Retrospective RCT	Adult	↓ exacerbations ↓ antibiotic use
Coeman et al. [61]	2011	Erythromycin	Retrospective observational	Adult	Improved symptom score
Anwar et al. [56]	2008	Azithromycin	Retrospective observational	Adult	↑ FEV1 ↓ exacerbations

Abbreviations: ↑, increased, ↓, decreased; D_{LCO}, pulmonary diffusion capacity for carbon monoxide; FVC, forced vital capacity; FEV1, forced expiratory volume in one second; RCT, randomised controlled trial; RDBCT, randomised double-blind controlled trial; RDBPCT, randomised double-blind placebo-controlled trial.

6. Conclusion

Macrolides have immunomodulatory properties in addition to their anti-bacterial effects. The use of macrolides in non-CF-related bronchiectasis holds great promise as a therapeutic intervention that will not only affect the quality of life of sufferers but also act on the pathopysiological mechanism of bronchiectasis. More studies on the use of macrolides in this condition are needed to further ascertain their efficacy.

References

[1] R. T. H. Laennac, "A treatise on the disease of the chest," in *Trans New York: Library of the New York Academy of Medicine*, J. Forbes, Ed., p. 78, Hafner, 1962.

[2] L. M. Reid, "Reduction in bronchial subdivision in bronchiectasis," *Thorax*, vol. 5, no. 3, pp. 233–247, 1950.

[3] D. Whitwell, "A study of the pathology and pathogenesis of bronchiectasis ," *Thorax*, vol. 7, pp. 213–239, 1952.

[4] N. A. Kothari and S. S. Kramer, "Bronchial diseases and lung aeration in children," *Journal of Thoracic Imaging*, vol. 16, no. 4, pp. 207–223, 2001.

[5] P. J. Cole, "Inflammation: a two-edged sword—the model of bronchiectasis," *European Journal of Respiratory Diseases*, vol. 69, no. 147, pp. 6–15, 1986.

[6] K. W. Tsang, K. N. Chan, P. L. Ho et al., "Sputum elastase in steady-state bronchiectasis," *Chest*, vol. 117, no. 2, pp. 420–426, 2000.

[7] J. B. Y. Richman-Eisenstat, P. G. Jorens, C. A. Hebert, I. Ueki, and J. A. Nadel, "Interleukin-8: an important chemoattractant in sputum of patients with chronic inflammatory airway diseases," *American Journal of Physiology*, vol. 264, no. 4, pp. L413–L418, 1993.

[8] N. Aldallal, E. E. McNaughton, L. J. Manzel et al., "Inflammatory response in airway epithelial cells isolated from patients with cystic fibrosis," *American Journal of Respiratory and Critical Care Medicine*, vol. 166, no. 9, pp. 1248–1256, 2002.

[9] B. K. Rubin, "Mucus structure and properties in cystic fibrosis," *Paediatric Respiratory Reviews*, vol. 8, no. 1, pp. 4–7, 2007.

[10] L. Zheng, W. K. Lam, G. L. Tipoe et al., "Over expression of matrix metalloproteinases-8 and -9 in bronchiectasis airways in vivo," *European Respiratory Journal*, vol. 20, pp. 170–176, 2002.

[11] O. Säynäjäkangas, T. Keistinen, T. Tuuponen, and S.-L. Klvelä, "Bronchiectasis in Finland: trends in hospital treatment," *Respiratory Medicine*, vol. 91, no. 7, pp. 395–398, 1997.

[12] P. Goeminne and L. Dupont, "Non-cystic fibrosis bronchiectasis: diagnosis and management in 21st century," *Postgraduate Medical Journal*, vol. 86, no. 1018, pp. 493–501, 2010.

[13] C. E. Field, "Bronchiectasis. Third report on a follow-up study of medical and surgical cases from childhood," *Archives of Disease in Childhood*, vol. 44, no. 237, pp. 551–561, 1969.

[14] R. Singleton, A. Morris, G. Redding et al., "Bronchiectasis in Alaska Native children: causes and clinical courses," *Pediatric Pulmonology*, vol. 29, no. 3, pp. 182–187, 2000.

[15] J. Twiss, R. Metcalfe, E. Edwards, and C. Byrnes, "New Zealand national incidence of bronchiectasis "too high" for a developed country," *Archives of Disease in Childhood*, vol. 90, no. 7, pp. 737–740, 2005.

[16] A. B. Chang, K. Grimwood, E. K. Mulholland, and P. J. Torzillo, "Bronchiectasis in Indigenous children in remote Australian communities," *Medical Journal of Australia*, vol. 177, no. 4, pp. 200–204, 2002.

[17] D. Doğru, A. Nik-Ain, N. Kiper et al., "Bronchiectasis: the consequence of late diagnosis in chronic respiratory symptoms," *Journal of Tropical Pediatrics*, vol. 51, no. 6, pp. 362–365, 2005.

[18] F. Hayashi, T. K. Means, and A. D. Luster, "Toll-like receptors stimulate human neutrophil function," *Blood*, vol. 102, no. 7, pp. 2660–2669, 2003.

[19] S. Akira, "Toll-like receptors and innate immunity," *Advances in Immunology*, vol. 78, pp. 1–56, 2001.

[20] J. L. Simpson, T. V. Grissell, J. Douwes, R. J. Scott, M. J. Boyle, and P. G. Gibson, "Innate immune activation in neutrophilic asthma and bronchiectasis," *Thorax*, vol. 62, no. 3, pp. 211–218, 2007.

[21] M. Mikami, C. G. Llewellyn-Jones, D. Bayley, S. L. Hill, and R. A. Stockley, "The chemotactic activity of sputum from patients with bronchiectasis," *American Journal of Respiratory and Critical Care Medicine*, vol. 157, no. 3, pp. 723–728, 1998.

[22] K. W. Tsang, K. C. Tan, P. L. Ho et al., "Inhaled fluticasone in bronchiectasis: a 12 month study," *Thorax*, vol. 60, no. 3, pp. 239–243, 2005.

[23] S. Fuschillo, A. De Felice, and G. Balzano, "Mucosal inflammation in idiopathic bronchiectasis: cellular and molecular mechanisms," *European Respiratory Journal*, vol. 31, no. 2, pp. 396–406, 2008.

[24] A. B. Chang and G. J. Redding, "Bronchiectasis," in *Kendig's Disorders of the Respiratory Tract in Children*, V. Chernick, T. F. Boat, R. W. Wilmott, and A. Bush, Eds., p. 460, Saunders Elsevier, Philadelphia, Pa, USA, 7th edition, 2006.

[25] W. C. Parks, C. L. Wilson, and Y. S. López-Boado, "Matrix metalloproteinases as modulators of inflammation and innate immunity," *Nature Reviews Immunology*, vol. 4, no. 8, pp. 617–629, 2004.

[26] S. Lanone, T. Zheng, Z. Zhu et al., "Overlapping and enzyme-specific contributions of matrix metalloproteinases-9 and -12 in IL-13-induced inflammation and remodeling," *Journal of Clinical Investigation*, vol. 110, no. 4, pp. 463–474, 2002.

[27] C. C. Taggart, C. M. Greene, T. P. Carroll, S. J. O'Neill, and N. G. McElvaney, "Elastolytic proteases: inflammation resolution and dysregulation in chronic infective lung disease," *American Journal of Respiratory and Critical Care Medicine*, vol. 171, no. 10, pp. 1070–1076, 2005.

[28] E. Yalçin, N. Kiper, U. Özçelik et al., "Effects of claritromycin on inflammatory parameters and clinical conditions in children with bronchiectasis," *Journal of Clinical Pharmacy and Therapeutics*, vol. 31, no. 1, pp. 49–55, 2006.

[29] C. Feldman, "Bronchiectasis: new approaches to diagnosis and management," *Clinics in Chest Medicine*, vol. 32, no. 3, pp. 535–546, 2011.

[30] M. R. Elkins, A. Jones, and C. van der Schans, "Positive expiratory pressure physiotherapy for airway clearance in people with cystic fibrosis," *Cochrane Database of Systematic Reviews*, no. 2, Article ID CD003147, 2006.

[31] M. P. Murray, J. L. Pentland, and A. T. Hill, "A randomised crossover trial of chest physiotherapy in non-cystic fibrosis bronchiectasis," *European Respiratory Journal*, vol. 34, no. 5, pp. 1086–1092, 2009.

[32] A. Bush, D. Payne, S. Pike, G. Jenkins, M. O. Henke, and B. K. Rubin, "Mucus properties in children with primary ciliary dyskinesia: comparison with cystic fibrosis," *Chest*, vol. 129, no. 1, pp. 118–123, 2006.

[33] A. E. O'Donnell, A. F. Barker, J. S. Ilowite, and R. B. Fick, "Treatment of idiopathic bronchiectasis with aerosolized recombinant human DNase I," *Chest*, vol. 113, no. 5, pp. 1329–1334, 1998.

[34] B. Karadag, F. Karakoc, R. Ersu, A. Kut, S. Bakac, and E. Dagli, "Non-cystic-fibrosis bronchiectasis in children: a persisting problem in developing countries," *Respiration*, vol. 72, no. 3, pp. 233–238, 2005.

[35] F. Kellett, J. Redfern, and R. McL Niven, "Evaluation of nebulised hypertonic saline (7%) as an adjunct to physiotherapy in patients with stable bronchiectasis," *Respiratory Medicine*, vol. 99, no. 1, pp. 27–31, 2005.

[36] E. Daviskas, S. D. Anderson, and I. H. Young, "Effect of mannitol and repetitive coughing on the sputum properties in bronchiectasis," *Respiratory Medicine*, vol. 104, no. 3, pp. 371–377, 2010.

[37] P. Wills and M. Greenstone, "Inhaled hyperosmolar agents for bronchiectasis," *Cochrane Database of Systematic Reviews*, no. 2, Article ID CD002996, 2006.

[38] N. Kapur, I. B. Masters, and A. B. Chang, "Exacerbations in noncystic fibrosis bronchiectasis: clinical features and investigations," *Respiratory Medicine*, vol. 103, no. 11, pp. 1681–1687, 2009.

[39] B. K. Rubin, "Aerosolized antibiotics for non-cystic fibrosis bronchiectasis," *Journal of Aerosol Medicine and Pulmonary Drug Delivery*, vol. 21, no. 1, pp. 71–76, 2008.

[40] R. Orriols, J. Roig, J. Ferrer et al., "Inhaled antibiotic therapy in non-cystic fibrosis patients with bronchiectasis and chronic bronchial infection by *Pseudomonas aeruginosa*," *Respiratory Medicine*, vol. 93, no. 7, pp. 476–480, 1999.

[41] P. Scheinberg and E. Shore, "A pilot study of the safety and efficacy of tobramycin solution for inhalation in patients with severe bronchiectasis," *Chest*, vol. 127, no. 4, pp. 1420–1426, 2005.

[42] M. A. Martínez-García, M. Perpiñá-Tordera, P. Román-Sánchez, and J. J. Soler-Cataluña, "Inhaled steroids improve quality of life in patients with steady-state bronchiectasis," *Respiratory Medicine*, vol. 100, no. 9, pp. 1623–1632, 2006.

[43] N. Kapur, S. Bell, J. Kolbe, and A. B. Chang, "Inhaled steroids for bronchiectasis," *Cochrane Database of Systematic Reviews*, no. 1, Article ID CD000996, 2009.

[44] K. Togami, S. Chono, and K. Morimoto, "Distribution characteristics of clarithromycin and azithromycin, macrolide antimicrobial agents used for treatment of respiratory infections, in lung epithelial lining fluid and alveolar macrophages," *Biopharmaceutics & Drug Disposition*, vol. 32, pp. 389–397, 2011.

[45] M. Shinkai, M. O. Henke, and B. K. Rubin, "Macrolide antibiotics as immunomodulatory medications: proposed mechanisms of action," *Pharmacology and Therapeutics*, vol. 117, no. 3, pp. 393–405, 2008.

[46] S. L. Spector, F. H. Katz, and R. S. Farr, "Troleandomycin: effectiveness in steroid dependent asthma and bronchitis," *Journal of Allergy and Clinical Immunology*, vol. 54, no. 6, pp. 367–379, 1974.

[47] H. Nagai, H. Shishido, R. Yoneda, E. Yamaguchi, A. Tamura, and A. Kurashima, "Long-term low-dose administration of erythromycin to patients with diffuse panbronchiolitis," *Respiration*, vol. 58, no. 3-4, pp. 145–149, 1991.

[48] J. Tredaniel, G. Zalcman, F. Gerber et al., "Diffuse panbronchiolitis: efficacy of low-dose erythromycin," *Respiratory Medicine*, vol. 87, no. 3, pp. 229–230, 1993.

[49] N. Hoiby, "Diffuse panbronchiolitis and cystic fibrosis: east meets West," *Thorax*, vol. 49, no. 6, pp. 531–532, 1994.

[50] S. Kudoh, A. Azuma, M. Yamamoto, T. Izumi, and M. Ando, "Improvement of survival in patients with diffuse panbronchiolitis treated with low-dose erythromycin," *American Journal of Respiratory and Critical Care Medicine*, vol. 157, no. 6, pp. 1829–1832, 1998.

[51] H. Takizawa, M. Desaki, T. Ohtoshi et al., "Erythromycin modulates IL-8 expression in normal and inflamed human bronchial epithelial cells," *American Journal of Respiratory and Critical Care Medicine*, vol. 156, no. 1, pp. 266–271, 1997.

[52] O. A. Khair, J. L. Devalia, M. M. Abdelaziz, R. J. Sapsford, and R. J. Davies, "Effect of erythromycin on *Haemophilus influenzae* endotoxin-induced release of IL-6, IL-8 and sICAM-1 by cultured human bronchial epithelial cells," *European Respiratory Journal*, vol. 8, no. 9, pp. 1451–1457, 1995.

[53] M. Gorrini, A. Lupi, S. Viglio et al., "Inhibition of human neutrophil elastase by erythromycin and flurythromycin, two macrolide antibiotics," *American Journal of Respiratory Cell and Molecular Biology*, vol. 25, no. 4, pp. 492–499, 2001.

[54] C. Taggart, R. J. Coakley, P. Greally, G. Canny, S. J. O'Neill, and N. G. McElvaney, "Increased elastase release by CF neutrophils is mediated by tumor necrosis factor-α and interleukin-8," *American Journal of Physiology*, vol. 278, no. 1, pp. L33–L41, 2000.

[55] D. J. Serisier and M. L. Martin, "Long-term, low-dose erythromycin in bronchiectasis subjects with frequent infective exacerbations," *Respiratory Medicine*, vol. 105, no. 6, pp. 946–949, 2011.

[56] G. A. Anwar, S. C. Bourke, G. Afolabi, P. Middleton, C. Ward, and R. M. Rutherford, "Effects of long-term low-dose azithromycin in patients with non-CF bronchiectasis," *Respiratory Medicine*, vol. 102, no. 10, pp. 1494–1496, 2008.

[57] A. A. Cymbala, L. C. Edmonds, M. A. Bauer et al., "The disease-modifying effects of twice-weekly oral azithromycin in patients with bronchiectasis," *Treatments in Respiratory Medicine*, vol. 4, no. 2, pp. 117–122, 2005.

[58] K. W. T. Tsang, P. I. Ho, K. N. Chan et al., "A pilot study of low-dose erythromycin in bronchiectasis," *European Respiratory Journal*, vol. 13, no. 2, pp. 361–364, 1999.

[59] Y. Y. Koh, M. H. Lee, Y. H. Sun, K. W. Sung, and J. H. Chae, "Effect of roxithromycin on airway responsiveness in children with bronchiectasis: a double-blind, placebo-controlled study," *European Respiratory Journal*, vol. 10, no. 5, pp. 994–999, 1997.

[60] G. Davies and R. Wilson, "Prophylactic antibiotic treatment of bronchiectasis with azithromycin," *Thorax*, vol. 59, no. 6, pp. 540–541, 2004.

[61] M. Coeman, Y. Van Durme, F. Bauters et al., "Neomacrolides in the treatment of patients with severe asthma and/or bronchiectasis: a retrospective observational study," *Therapeutic Advances in Respiratory Disease*, vol. 5, no. 6, pp. 377–386, 2011.

[62] J. Eller, J. R. Lapa e Silva, L. W. Poulter, H. Lode, and P. J. Cole, "Cells and cytokines in chronic bronchial infection," *Annals of the New York Academy of Sciences*, vol. 725, pp. 331–345, 1994.

[63] S. Loukides, D. Bouros, G. Papatheodorou, S. Lachanis, P. Panagou, and N. M. Siafakas, "Exhaled H_2O_2 in steady-state bronchiectasis: relationship with cellular composition in induced sputum, spirometry, and extent and severity of disease," *Chest*, vol. 121, no. 1, pp. 81–87, 2002.

[64] J. McCormack, "Effect of long term treatment with azithromycin on disease parameters in cystic fibrosis: a randomised trial," *Thorax*, vol. 57, no. 3, pp. 212–216, 2002.

[65] A. Equi, I. M. Balfour-Lynn, A. Bush, and M. Rosenthal, "Long term azithromycin in children with cystic fibrosis: a randomised, placebo-controlled crossover trial," *Lancet*, vol. 360, no. 9338, pp. 978–984, 2002.

[66] L. Saiman, B. C. Marshall, N. Mayer-Hamblett et al., "Azithromycin in patients with cystic fibrosis chronically infected with *Pseudomonas aeruginosa*. A randomized controlled trial," *Journal of the American Medical Association*, vol. 290, no. 13, pp. 1749–1756, 2003.

[67] A. Clement, A. Tamalet, E. Leroux, S. Ravilly, B. Fauroux, and J. P. Jais, "Long term effects of azithromycin in patients with cystic fibrosis: a double blind, placebo controlled trial," *Thorax*, vol. 61, no. 10, pp. 895–902, 2006.

[68] L. Saiman, M. Anstead, N. Mayer-Hamblett et al., "Effect of azithromycin on pulmonary function in patients with cystic fibrosis uninfected with *Pseudomonas aeruginosa*: a randomized controlled trial," *Journal of the American Medical Association*, vol. 303, no. 17, pp. 1707–1715, 2010.

[69] S. Kanoh and B. K. Rubin, "Mechanisms of action and clinical application of macrolides as immunomodulatory medications," *Clinical Microbiology Reviews*, vol. 23, no. 3, pp. 590–615, 2010.

[70] S. J. Phaff, H. A. W. M. Tiddens, H. A. Verbrugh, and A. Ott, "Macrolide resistance of Staphylococcus aureus and Haemophilus species associated with long-term azithromycin use in cystic fibrosis," *Journal of Antimicrobial Chemotherapy*, vol. 57, no. 4, pp. 741–746, 2006.

[71] G. A. Tramper-Stranders, T. F. W. Wolfs, A. Fleer, J. L. L. Kimpen, and C. K. Van Der Ent, "Maintenance azithromycin treatment in pediatric patients with cystic fibrosis: long-term outcomes related to macrolide resistance and pulmonary function," *Pediatric Infectious Disease Journal*, vol. 26, no. 1, pp. 8–12, 2007.

[72] K. N. Olivier, D. J. Weber, R. J. Wallace et al., "Nontuberculous mycobacteria—I: multicenter prevalence study in cystic fibrosis," *American Journal of Respiratory and Critical Care Medicine*, vol. 167, no. 6, pp. 828–834, 2003.