



Exploring Azithromycin's Anti-Inflammatory Mechanisms: Impacts on Immune Cells and Therapeutic Applications

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ABSTRACT

Azithromycin (AZM), a macrolide antibiotic, has attracted considerable attention for its immunomodulatory and anti-inflammatory properties beyond its traditional antimicrobial use. This review examines the effects of AZM on key immune cells, including B cells, T cells, and natural killer (NK) cells, and its potential applications in the treatment of autoimmune and chronic inflammatory diseases. AZM affects the mammalian target of the rapamycin (mTOR) pathway in T cells, inhibiting proliferation and cytokine production, while also modulating B-cell responses through pathways such as NF- κ B and CD27, which affect antibody production. AZM reduces cytotoxicity and cytokine secretion in NK cells without impairing cell viability. The effect of this drug on immune responses to vaccinations, particularly by dampening antibody titers, raises important clinical considerations. While AZM holds promise in managing immune-related conditions such as graft-versus-host disease and asthma, the variability in its effects underscores the need for further research. Understanding these mechanisms will allow for better application of AZM's immunomodulatory potential and mitigation of possible adverse effects on immune function.

Key Words: Azithromycin, Immune modulation, mTOR inhibition, Anti-inflammatory therapy

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INTRODUCTION

Azithromycin (AZM) belongs to a subclass of macrolide antibiotics called azalides and is widely used worldwide to treat bacterial infections [1]. In recent years, it has become the second most commonly used antibiotic in the United States after amoxicillin, with almost 35 million prescriptions written in 2022 [2]. AZM owes its popularity to its exceptional properties, which include broad-spectrum activity against gram-positive, gram-negative, and atypical bacteria, easy tissue penetration, accumulation in macrophages, long half-life in vivo, short treatment duration, and anti-inflammatory activity [1]. The latter is significant as it contributes to its efficacy in the treatment of certain diseases and is currently the subject of much research and debate about new applications for the drug. Areas of interest include oncology, autoimmune, and respiratory diseases, including research into the potential use of AZM in COVID-19 infection [3-7]. However,

research on the anti-inflammatory effects of AZM has yielded mixed results, and many findings remain controversial. The purported benefits of AZM in treating inflammatory conditions are often debated, and there is a need to address prevalent myths and misunderstandings about its efficacy. A comprehensive review of the current evidence from peer-reviewed literature is essential for advancing this field. Such a review could enhance research design, minimize unnecessary suffering among research subjects, and optimize resource allocation by providing a more robust theoretical foundation. Our study aims to clarify and deepen our understanding of the mechanisms underlying AZM's anti-inflammatory effects, contributing valuable insights to the ongoing discussion.

RESULTS AND DISCUSSION

Mammalian target of rapamycin (mTOR) inhibition and impact on T cells

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Rapamycin (sirolimus) is a macrocyclic lacton produced by *Streptomyces hygroscopicus*, isolated in 1964 from soil samples from the Easter Island Rapa Nui. Initial interest in this macrolide antibiotic was directed to antifungal therapy also because of the lack of antibacterial activity [8, 9]. After the observation of inhibition of the growth of eukaryote cells with the following discovery of the protein target of rapamycin (TOR) in yeast responsible for that action, the research on rapamycin turned to its immunosuppressive properties. Rapamycin inhibits its analog in mammals –mTOR via complex formation with the FK506 binding protein 12 (FKBP12). This inhibition is sufficient to completely abrogate T-cell activation including proliferation and cytokine production, which is commonly used e.g., in the prevention and therapy of transplant rejection [9, 10]. The investigations of the impact of other macrolides, in particular AZM on the mTOR pathway and their potential role in its inhibition was the logical step and was highly warranted.

Ratzinger *et al.* [11] were the first who report a decrease in lymphocyte proliferation and cytokine secretion incubated with different doses of AZM in a statistically significant manner and attributed this action to mTOR inhibition, while it is known that the mTOR pathway is critical for the proliferation, apoptosis, autophagy, and differentiation of T cells [10]. The action was less pronounced when using the respective doses of clarithromycin. The research was conducted on an isolated T-cell population from blood specimens derived from 10 healthy volunteers (8 males and 2 females) and recombinant mTOR fragments containing

the kinase domain, providing direct laboratory evidence of mTOR inhibition *in vitro*. It was demonstrated that AZM is a direct inhibitor of mTOR kinase that suppresses its activity on a similar level regardless of the presence or the absence of FKBP12.500 nM of rapamycin that was used as control inhibition acted exclusively in the case of the presence of FKBP12 protein and reduced mTOR activity by 67.3% ($P < 0.001$). In comparison, AZM at a concentration of 1000 mg/L suppressed mTOR activity by 31.5% ($P < 0.001$) in the presence of FKBP12 and by 27% ($P < 0.001$) in the lack of FKBP12 [11]. Ratzinger *et al.* [11] also checked the potential impact of AZM on the phosphoinositide 3-kinase (PI3-K), a major activating factor of mTOR kinase and demonstrated no inhibition ($P = 0.6267$) of PI3-K activity, which excludes the other way of indirect impact on mTOR by AZM. Furtherly, research by Weng *et al.* [12] has shown that the viability of cells exposed to 40 mg/L AZM was strongly reduced by almost 50% compared to the control. The lower doses of AZM and clarithromycin in tested doses did not present any impact on the cell lifespan [12]. **Table 1** presents a summary of studies investigating the inhibition of mTOR by AZM. It is suggested that this effect is primarily due to the suppression of S6RP phosphorylation, a downstream target of mTOR signaling [11-14]. Additionally, a study by Ansari *et al.* [14] provided valuable insights into the molecular mechanisms underlying the suppression of T-cell activation, and Bergström *et al.* [15] compared AZM's anti-inflammatory properties to those of rapamycin.

Table 1. Summary of studies on the effects of AZM on the mTOR pathway.

Research	Conclusions
Ratzinger <i>et al.</i> [11]	AZM inhibited cell proliferation rate and cytokine secretion of CD4 + T cells in a dose-dependent manner
Weng <i>et al.</i> [12]	AZM significantly inhibited T-cell proliferation and promoted apoptosis, while also enhancing the autophagosome formation in T cells
Huang <i>et al.</i> [13]	AZM increased the number of Treg and decreased the number of effector T cells (Teff). Additionally, AZM suppressed the proliferation and activation of CD4 + T cells, inducing apoptosis in CD4 + CD44 + memory T cells and CD4 + CXCR3 + Th1 cells
Ansari <i>et al.</i> [14]	AZM suppresses T-cell proliferation, with a significant reduction in the expression of ICOS and OX40 receptors, crucial costimulatory molecules
Bergström <i>et al.</i> [15]	Both AZM and rapamycin promoted a FoxP3-positive Treg phenotype in Tregs, although Tregs treated with rapamycin were more suppressive.

Natural killer (NK) cells

Little is known about the impact of AZM on NK cell function. A recent study explored this influence, focusing on the activating, apoptotic, and cytotoxic capabilities of NK cells. The study found that AZM, within its therapeutic dose range, did not affect NK cell survival. However, AZM significantly suppressed IL-15-induced CD69 expression in a dose-dependent manner. CD69 is an early activation

marker on NK cells and plays a crucial role in the regulation of NK cell activity and immune responses. Moreover, AZM decreased the cytotoxicity of both resting and IL-15-activated primary NK cells against K562 target cells. This decrease in cytotoxicity is linked to AZM's downregulation of perforin production. Perforin is a critical cytotoxic granule in NK cells that induces necrotic and apoptotic death of target cells and facilitates the entry

of granzymes into these cells. The study noted that CD16 + CD56 + NK cell subsets, which express higher levels of perforin, were more susceptible to AZM-induced suppression compared to CD16–CD56 + subsets. Additionally, AZM inhibited IFN-gamma and TNF-alpha production in NK-92 cells, a cell line derived from a malignant IL-2-dependent NK neoplasm that spontaneously secretes these cytokines. However, AZM did not affect cytokine production in IL-15-activated primary NK cells [16].

These findings suggest that AZM can modulate various aspects of NK cell activity without impacting cell survival. This modulation may offer therapeutic benefits beyond its antimicrobial properties. For example, the reduction in NK cell cytotoxicity and cytokine production could be relevant in treating conditions like asthma and other chronic inflammatory diseases where NK cell hyperactivity is implicated. Further research is needed to fully understand the implications of AZM's effects on NK cells and their potential therapeutic applications.

B cells

AZM has been demonstrated to exert significant effects on B cells by modulating key signaling pathways critical for their activation and function. One major mechanism through which AZM influences B cells is its impact on the NF-κB signaling pathway. NF-κB is a pivotal transcription factor that regulates various aspects of B-cell biology, including activation, survival, and proliferation [17]. AZM

inhibits the activation of NF-κB, which could lead to reduced B-cell activation and proliferation. This inhibition could result in a decreased number of B cells capable of responding to antigens and producing antibodies [18].

In addition to its effects on NF-κB, AZM also impacts CD27, a member of the TNF receptor superfamily that is essential for B-cell activation and differentiation. CD27 interacts with its ligand, CD70, to provide crucial survival and activation signals to B cells [19]. AZM has been shown to alter CD27 signaling, which affects the differentiation of B cells into plasma cells—responsible for antibody production. Specifically, AZM inhibits CD27-mediated signals, which could lead to impaired B-cell differentiation and a subsequent reduction in the number of plasma cells and antibody production [20].

The overall impact of AZM on B cells involves a complex interplay of numerous pathways. By impacting them, AZM can suppress B-cell responses, which may have both beneficial and detrimental effects depending on the clinical context. Further research is needed to elucidate the full extent of AZM's effects on B-cell biology, including how these effects vary with different treatment regimens and patient populations. This knowledge will be vital in harnessing AZM's therapeutic potential while mitigating any adverse impacts on immune function.

IgE antibodies

Table 2 presents an overview of studies investigating the impact of AZM on IgE levels in different research models.

Table 2. Summary of studies on the effects of AZM on IgE levels.

Research	Research model	Effect on IgE
Wang <i>et al.</i> [21]	Mouse	No effect
Tkalčević <i>et al.</i> [22]	Mouse	Decrease in serum level
Hahn <i>et al.</i> [23]	Human	No effect
Borbet <i>et al.</i> [24]	Mouse	Increase in serum level
Smith-Norowitz <i>et al.</i> [25]	Human (obs)	Increase by low concentration, decrease by high concentration
Tiotiu <i>et al.</i> [26]	Human (obs)	No effect
Wan <i>et al.</i> [27]	Rats	No effect
Ni <i>et al.</i> [28]	Human (+ambroxol)	Decrease in serum level
Kang <i>et al.</i> [29]	Mouse	Decrease in serum level

Although the results are inconsistent, it appears that AZM does not significantly affect serum IgE levels. However, worth noting is the fact that studies examining the potential use of AZM in the management of asthma, a condition almost always associated with some form of IgE-related reaction, may help explain the incongruent results of its impact on IgE levels. While some research has shown no impact on the incidence of asthma exacerbations, other studies have suggested that AZM may be beneficial in certain circumstances [30-32]. The current

recommendation from the Global Initiative for Asthma states that low doses of AZM are a therapeutic option for reducing exacerbations in severe asthma [33].

Worth noticing is also another possible impact of AZM on serum IgE level, through the IgE-related hypersensitivity reaction on the drug. A longitudinal study performed on pediatric patients demonstrated that AZM is significantly more allergenic than clarithromycin. In the research, almost 50% of children in the AZM group have a positive result in skin tests or oral provocation tests with this drug.



Moreover, the study also specified the positive predictive value for IgE against AZM at the level of 75%. It is also possible to acquire the allergy through the airborne way, which was suggested by the observation of allergy contact dermatitis in pharmaceutical workers. Nonetheless, it is estimated that the allergy to macrolides including AZM is extremely rare (0.4-3%) indicating a small impact in evoking a hypersensitivity reaction [34]. Interestingly AZM not only could provoke sensitization on itself, but it was proven that it also could prevent the hypersensitive reaction to other substances. A study by Fillaux *et al.* [35] showed that pediatric patients who never received AZM were 1.9 more susceptible to *Aspergillus fumigatus* sensitization than the patients who received AZM. It is important to note that the effect of AZM on serum IgE levels may vary depending on the specific condition being

treated and the characteristics of the patient population studied. Therefore, more research is needed to better understand the potential impact of AZM on IgE levels in different contexts.

Other antibodies

Table 3 summarizes studies examining the impact of AZM on antibody levels, revealing highly inconsistent results. This variability suggests that the effects of AZM on antibody production may depend on the specific condition being treated and the characteristics of the patient population. Additionally, there is a notable gap in research assessing AZM's influence on IgD antibodies and its potential role in modulating the immune system. Overall, the available evidence indicates that AZM does not have a significant impact on overall antibody levels.

Table 3. Summary of studies on the effects of AZM on IgA, IgG, and IgM antibodies.

Research	Antibodies studied	Effect on antibodies
Ni <i>et al.</i> [28]	IgA, IgG, IgM	Decrease in IgA, IgG, and IgM levels
Gabriel <i>et al.</i> [36]	IgA, IgG	No effect
Anderson and Muhlestein [37]	IgA, IgG	No effect
Karlsson <i>et al.</i> [38]	IgA, IgG	No effect, with a slight trend toward a decrease in the IgG level
Xia <i>et al.</i> [39]	IgA, IgG, IgM	Decrease in IgA and IgM levels, increase in IgG level
Bai <i>et al.</i> [40]	IgA, IgM	Increase in IgA and IgM levels

Impact on response to vaccinations

Fernandez *et al.* [41] have shown that the AZM significantly decreases antibody titers in mice vaccinated with 100 µl of 7-valent polysaccharide pneumococcal conjugate vaccine (PCV7) when compared to control. Neither ceftriaxone nor ciprofloxacin significantly affected the total antibodies response to the PCV7 vaccine. This study demonstrates that AZM dampens down the primary humoral response to this vaccine. This effect measured in total antibodies serum level was eventually attributed to decreased serum levels of IgG1 [41]. In a related study, Woo *et al.* [42] observed that mice treated with clarithromycin in response to a pneumococcal polysaccharide vaccine showed reduced total antibody titers, with a decrease in IgM titers but not in IgG1 titers. However, the pneumococcal polysaccharide vaccine they used was a T-cell-independent antigen, whereas the study by Fernandez *et al.* [41] employed a T-cell-dependent vaccine.

In a separate study, Borkner *et al.* [43] reported that AZM effectively clears *Bordetella pertussis* infection in mice. This clearance was associated with a reduction in both local innate and adaptive immune responses in the lungs. Notably, there was a significant decrease in CD4 + T-cell responses and bacterial clearance from the lungs in AZM-treated mice compared to untreated mice immunized with

whole-cell pertussis (wP) vaccine. These findings suggest that AZM treatment may impair the immunological response to vaccines against pathogens [43]. The clinical implications of this are not fully understood. Guidelines indicate that vaccination in humans should not occur during an active infection. Consequently, this implies that humans should not be vaccinated while undergoing any antibiotic treatment, particularly with AZM. Nevertheless, the potential chronic use of AZM in conditions such as chronic obstructive pulmonary disease (COPD) should be carefully considered in the context of vaccination plans for these individuals [44].

Other applications of AZM

Due to its unique properties, AZM has been investigated in various conditions. While its effects are often not significant, this work aims to highlight potential studies exploring AZM's use in different diseases.

Hematology

A study by Ozkan *et al.* [45] demonstrated significant results in using AZM alone or in combination with imatinib for the treatment of chronic myeloid leukemia (CML). AZM was particularly effective in targeting CML stem cells resistant to imatinib, suggesting its potential as an anti-leukemic agent [45].

Other hematological studies have explored AZM as a first-line treatment for mucosa-associated lymphoid tissue (MALT) lymphoma, which is often associated with *Helicobacter pylori* infection. AZM's dual role in treating the neoplasm and eradicating the infection shows promise. In a phase II clinical trial by Lagler *et al.* [46] AZM (administered at 1500 mg) was well-tolerated, though efficacy was limited, with 2 of 16 patients achieving complete remission and 2 others partial remission. Despite some evidence of AZM's anti-cancer potential, further research is needed to better understand its role in lymphoma management.

AZM has also been investigated as an adjunct therapy in graft-versus-host disease GVHD treatment. Some studies suggest that AZM can modulate immune responses by enhancing the activity of T-regulatory cells and altering the balance between Th1 and Th2 cells, thereby reducing inflammation. Iwamoto *et al.* [47] demonstrated that AZM prevented lethal GVHD in mice, likely due to its inhibitory effects on the NF- κ B pathway. A different murine study also confirms this effect, attributing it to a reduction in intestinal T-cell expansion [48]. However, a randomized controlled trial did not show significant efficacy for AZM in GVHD treatment [49].

Autoimmune disorders

AZM's anti-inflammatory properties have also been explored in autoimmune conditions. In the context of SLE, studies reveal that AZM can drive macrophage polarization toward an M2 phenotype, which is associated with reduced inflammation and improved tissue repair. In experimental models of lupus, AZM has been shown to alleviate key disease markers, including reduced serum anti-dsDNA antibodies, lower creatinine levels, and improved kidney pathology [6].

Similarly, in rheumatoid arthritis, AZM's utility extends beyond its antimicrobial action. It has been observed to mitigate pro-inflammatory cytokine production and modulate signaling pathways related to immune response and cellular stress. Specifically, AZM's interaction with glucose-regulated protein 78 (GRP78) has been identified as a crucial mechanism underlying its anti-arthritis effects. This interaction impacts various pathways, including cholesterol and lipid biosynthesis, which are often dysregulated in autoimmune conditions. Furthermore, AZM's capacity to reduce cell migration, invasion, and apoptosis in fibroblast-like synoviocytes demonstrates its potential to alter disease progression effectively [4].

Additionally, emerging evidence supports AZM's potential benefits in other autoimmune-related conditions, such as radiation-induced lung injury (RILI). In this context, its anti-inflammatory and immunomodulatory properties can mitigate the pro-inflammatory responses and tissue damage associated with radiation therapy. AZM's ability to

modulate macrophage function, inhibit neutrophil influx, and regulate autophagy contributes to its therapeutic promise in managing inflammation and fibrosis in RILI [5].

Overall, the repurposing of AZM for autoimmune diseases leverages its well-established safety profile and offers a novel therapeutic approach. By addressing key inflammatory pathways and immune dysregulation, AZM represents a promising addition to the arsenal of treatments for autoimmune conditions, potentially improving patient outcomes through its multifaceted mechanisms of action.

COVID-19

Recent research suggests that AZM may interfere with ligand-CD147 receptor interactions, potentially reducing viral replication in hospitalized patients. Notably, CD147 is one of the receptors, alongside ACE2, that SARS-CoV-2 uses to enter host cells. AZM has also been shown to decrease metalloproteinase expression downstream of CD147, thereby activating antiviral responses in bronchial epithelial cells infected with rhinovirus. Additionally, AZM may help reduce the risk of pulmonary fibrosis in COVID-19 patients by preserving lung progenitor and stem cell reserves, though ongoing clinical trials are needed to further explore these effects [50]. Another review reviewed that AZM may benefit COVID-19 patients by enhancing antiviral responses through increased type I interferon production. It could also play a role in moderating excessive inflammation by reducing pro-inflammatory cytokines and balancing macrophage activity. Additionally, AZM might influence neutrophil activity and T-cell responses, potentially alleviating severe immune activation. Ongoing research is needed to fully elucidate its therapeutic potential and refine its application in COVID-19 treatment [7].

Discussion

AZM exhibits pleiotropic effects on immune cells, highlighting its potential for new clinical applications due to its anti-inflammatory properties, which help alleviate symptoms in patients with severe infections. Our work should serve as a foundation for exploring these new uses and could encourage the development of novel antibiotics with properties similar to AZM, offering comparable therapeutic benefits.

Another advantage of AZM is its favorable safety profile. It is generally considered a safe antimicrobial agent, with only a few patients discontinuing its use due to adverse effects. Additionally, it is regarded as safer and has fewer cardiac side effects compared to other macrolides [1]. Moreover, in the treatment of diffuse panbronchiolitis, AZM is considered superior to erythromycin due to its faster pharmacological activity, fewer drug-drug interactions, longer post-antibiotic effects, and improved

patient compliance. Despite both drugs being indicated for this condition, AZM offers significant advantages in terms of safety and efficacy [12].

Unfortunately, bacterial resistance to AZM, as one of the most effective antibiotic substances, may develop over time. This growing concern underscores the importance of using AZM judiciously and continuing research into resistance mechanisms. Nevertheless, its unique combination of antimicrobial and anti-inflammatory effects, along with a strong safety profile, positions AZM as a valuable therapeutic option. Future studies aimed at mitigating resistance and expanding its clinical applications could further enhance its role in treating both infectious and inflammatory diseases.

CONCLUSION

AZM's dual antimicrobial and anti-inflammatory properties, coupled with its strong safety profile, make it a valuable therapeutic option for treating both infectious and inflammatory diseases. Despite concerns about bacterial resistance, its unique benefits remain significant. Ongoing research is essential to better understand resistance mechanisms and to explore new clinical applications, ensuring AZM continues to play an important role in managing complex diseases.

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