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

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ABSTRACT

Azithromycin (AZM), a macrolide antibiotic, has garnered significant interest for its immunomodulatory and anti-inflammatory properties beyond its traditional antimicrobial use. This review delves into AZM's effects on key immune cells, including B, T, and natural killer (NK) cells, and its potential applications in treating autoimmune and chronic inflammatory diseases. AZM impacts the mammalian target of rapamycin (mTOR) pathway in T cells, inhibiting proliferation and cytokine production, while also modulating B-cell responses through pathways such as NF-κB and CD27, which affect antibody production. AZM reduces cytotoxicity and cytokine secretion in NK cells without impairing cell viability. The drug's influence 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 in relation to 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.

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

Rapamycin (sirolimus) is a macrocyclic lacton produced by *Streptomyces hygroscopicus*, isolated in 1964 from soil

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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 – mammalian target of rapamycin (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 azithromycin on the mTOR pathway and their potential role in its inhibition was the logical step and was highly warranted.

Ratzinger *et al*. were the first who report the 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 [11], 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 FKB12 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*. 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*. has shown that the viability of cells exposed to 40 mg/L AZM was strongly reduced by almost 50% compared to 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*. provided valuable insights into the molecular mechanisms underlying the suppression of T-cell activation [14], and Bergström *et al*. compared AZM's anti-inflammatory properties to those of rapamycin [15].

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

Research	Conclusions
Ratzinger *et al*. (2014) [11]	AZM inhibited cell proliferation rate and cytokine secretion of CD4+ T cells in a dose-dependent manner
Weng *et al*. (2019) [12]	AZM significantly inhibited T-cell proliferation and promoted apoptosis, while also enhancing the autophagosome formation in T cells
Huang *et al*. (2021) [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 *et al*. (2023) [14]	AZM suppresses T-cell proliferation, with a significant reduction in the expression of ICOS and OX40 receptors, crucial costimulatory molecules
Bergström *et al*. (2019) [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 natural killer (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 *et al*. (2022) [21]	Mouse	No effect
Tkalčević *et al*. (2012) [22]	Mouse	Decrease in serum level.
Hahn *et al*. (2012) [23]	Human	No effect
Borbet *et al*. (2022) [24]	Mouse	Increase in serum level.
Smith-Norowitz *et al*. (2020) [25]	Human (obs)	Increase by low concentration, decrease by high concentration
Tiotiu *et al*. (2020) [26]	Human (obs)	No effect
Wan *et al*. (2013) [27]	Rats	No effect
Ni *et al*. (2023) [28]	Human (+ambroxol)	Decrease in serum level
Kang *et al*. (2016) [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 azithromycin 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 to 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*. showed that pediatric patients who never received AZM were 1.9 more susceptible to *Aspergillus fumigatus* sensitization than the patients who received AZM [35].

It is important to note that the effect of azithromycin 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 azithromycin 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 *et al*. (2023) [28]	IgA, IgG, IgM	Decrease in IgA, IgG, and IgM levels.
Gabriel *et al*. (2003) [36]	IgA, IgG	No effect
Anderson and Muhlestein (2000) [37]	IgA, IgG	No effect
Karlsson *et al*. (2009) [38]	IgA, IgG	No effect, with a slight trend toward a decrease in the IgG level
Xia *et al*. (2016) [39]	IgA, IgG, IgM	Decrease in IgA and IgM levels, increase in IgG level.
Bai *et al*. (2019) [40]	IgA, IgM	Increase in IgA and IgM levels.

Impact on response to vaccinations

Fernandez *et al*. have shown that the AZM significantly decreases antibody titers in mice vaccinated with 100µl 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 azithromycin 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*. 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 [42]. However, the pneumococcal polysaccharide vaccine they used was a T-cell–independent antigen, whereas the study by Fernandez *et al*. employed a T-cell–dependent vaccine [41].

In a separate study, Borkner *et al*. 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*. 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*. 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 [46]. 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*. demonstrated that AZM prevented lethal GVHD in mice, likely due to its inhibitory effects on the NF-κB pathway [47]. 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-arthritic 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].

RESULTS AND 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

Azithromycin'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 azithromycin continues to play an important role in managing complex diseases.

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REFERENCES

[1] Sandman Z, Iqbal OA. Azithromycin [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 [cited 2024 Aug 30]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK557766/

[2] Centers for Disease Control and Prevention. Outpatient antibiotic prescriptions — United States, 2022 [Internet]. Atlanta: Centers for Disease Control and Prevention; 2022 [updated 2023 Nov 15; cited 2024 Aug 31]. Available from: https://www.cdc.gov/antibiotic-use/data/report-2022.html

[3] Takano N, Hiramoto M, Yamada Y, Kokuba H, Tokuhisa M, Hino H, et al. Azithromycin, a potent autophagy inhibitor for cancer therapy, perturbs cytoskeletal protein dynamics. Br J Cancer. 2023;128(10):1838-49.

[4] Zhang Y, Ge L, Song G, Zhang R, Li S, Shi H, et al. Azithromycin alleviates the severity of rheumatoid arthritis by targeting the unfolded protein response component of glucose-regulated protein 78 (GRP78). Br J Pharmacol. 2022;179(6):1201-19.

[5] Yan Y, Wu L, Li X, Zhao L, Xu Y. Immunomodulatory role of azithromycin: Potential applications to radiation-induced lung injury. Front Oncol. 2023;13:966060.

[6] Wang J, Chen Q, Zhang Z, Wang S, Wang Y, Xiang M, et al. Azithromycin alleviate systemic lupus erythematosus via the promotion of M2 polarisation in lupus mice. Cell Death Discov. 2021;7(1):82.

[7] Venditto VJ, Haydar D, Abdel-Latif A, Gensel JC, Anstead MI, Pitts MG, et al. Immunomodulatory effects of azithromycin revisited: Potential applications to COVID-19. Front Immunol. 2021;12:574425.

[8] Liu GY, Sabatini DM. mTOR at the nexus of nutrition, growth, aging, and disease. Nat Rev Mol Cell Biol. 2020;21(4):183-203.

[9] Pollizzi KN, Powell JD. Regulation of T cells by mTOR: The known knowns and the known unknowns. Trends Immunol. 2015;36(1):13-20.

[10] Wang D, Eisen HJ. Mechanistic target of rapamycin (mTOR) inhibitors. Handb Exp Pharmacol. 2022;272:53-72.

[11] Ratzinger F, Haslacher H, Poeppl W, Hoermann G, Kovarik JJ, Jutz S, et al. Azithromycin suppresses CD4(+) T-cell activation by direct modulation of mTOR activity. Sci Rep. 2014;4(1):7438.

[12] Weng D, Wu Q, Chen XQ, Du YK, Chen T, Li H, et al. Azithromycin treats diffuse panbronchiolitis by targeting T cells via inhibition of the mTOR pathway. Biomed Pharmacother. 2019;110:440-8.

[13] Huang J, Li Z, Hu Y, Chen G, Li Z, Xie Y, et al. Azithromycin modulates Teff/Treg balance in retinal inflammation via the mTOR signaling pathway. Biochem Pharmacol. 2021;193:114793.

[14] Ansari AW, Ahmad F, Raheed T, Jochebeth A, Mateo JMP, Abdulrahman N, et al. Azithromycin downregulates ICOS (CD278) and OX40 (CD134) expression and mTOR activity of TCR-activated T cells to inhibit proliferation. Int Immunopharmacol. 2023;124(Pt A):110831.

[15] Bergström M, Müller M, Karlsson M, Scholz H, Vethe NT, Korsgren O. Comparing the effects of the

mTOR inhibitors azithromycin and rapamycin on in vitro expanded regulatory T cells. Cell Transplant. 2019;28(12):1603-13.

[16] Lin SJ, Yan DC, Lee WI, Kuo ML, Hsiao HS, Lee PY. Effect of azithromycin on natural killer cell function. Int Immunopharmacol. 2012;13(1):8-14.

[17] Sasaki Y, Iwai K. Roles of the NF-κB pathway in B-lymphocyte biology. Curr Top Microbiol Immunol. 2016;393:177-209.

[18] Cheung PS, Si EC, Hosseini K. Anti-inflammatory activity of azithromycin as measured by its NF-kappaB, inhibitory activity. Ocul Immunol Inflamm. 2010;18(1):32-7.

[19] Grimsholm O. CD27 on human memory B cells-more than just a surface marker. Clin Exp Immunol. 2023;213(2):164-72.

[20] Ansari AW, Jayakumar MN, Ahmad F, Venkatachalam T, Salameh L, Unnikannan H, et al. Azithromycin targets the CD27 pathway to modulate CD27hi T-lymphocyte expansion and type-1 effector phenotype. Front Immunol. 2024;15:1447625.

[21] Wang Z, Xin L, Zhang W. Study effect of azithromycin and doxycycline in mucus producing and inflammatory signaling pathways of allergic asthma. Iran J Allergy Asthma Immunol. 2022;21(2):119-27.

[22] Ivetić Tkalčević V, Cužić S, Kramarić MD, Parnham MJ, Eraković Haber V. Topical azithromycin and clarithromycin inhibit acute and chronic skin inflammation in sensitized mice, with apparent selectivity for Th2-mediated processes in delayed-type hypersensitivity. Inflammation. 2012;35(1):192-205.

[23] Hahn DL, Schure A, Patel K, Childs T, Drizik E, Webley W. Chlamydia pneumoniae-specific IgE is prevalent in asthma and is associated with disease severity. PLoS One. 2012;7(4):e35945.

[24] Borbet TC, Pawline MB, Zhang X, Wipperman MF, Reuter S, Maher T, et al. Influence of the early-life gut microbiota on the immune responses to an inhaled allergen. Mucosal Immunol. 2022;15(5):1000-11.

[25] Smith-Norowitz TA, Huang Y, Loeffler J, Klein E, Norowitz YM, Hammerschlag MR, et al. Azithromycin decreases Chlamydia pneumoniae-mediated Interleukin-4 responses but not Immunoglobulin E responses. PLoS One. 2020;15(6):e0234413.

[26] Tiotiu A, Salvator H, Jaussaud R, Jankowski R, Couderc LJ, Catherinot E, et al. Efficacy of immunoglobulin replacement therapy and azithromycin in severe asthma with antibody deficiency. Allergol Int. 2020;69(2):215-22.

[27] Wan L, Liu L, Zhang Z, Zhou Y, Xiong Y, Li D, et al. Low-dose azithromycin attenuates OVA-induced airway remodeling and inflammation via down-regulating TGF-βl expression in RAT. Eur J Inflamm. 2013;11(1):133-43.

[28] Ni Y, Fang M, Jiang J, Lou Q, Wu L, Liu Z. Effect of azithromycin combined with ambroxol in children with Mycoplasma pneumoniae pneumonia. Am J Transl Res. 2023;15(1):202-12.

[29] Kang JY, Jo MR, Kang HH, Kim SK, Kim MS, Kim YH, et al. Long-term azithromycin ameliorates not only airway inflammation but also remodeling in a murine model of chronic asthma. Pulm Pharmacol Ther. 2016;36:37-45.

[30] Douglas LC, Choi J, Esteban-Cruciani N. Azithromycin treatment in children hospitalized with asthma: A retrospective cohort study. J Asthma. 2020;57(5):525-31.

[31] Ghimire JJ, Jat KR, Sankar J, Lodha R, Iyer VK, Gautam H, et al. Azithromycin for poorly controlled asthma in children: A randomized controlled trial. Chest. 2022;161(6):1456-64.

[32] Gibson PG, Yang IA, Upham JW, Reynolds PN, Hodge S, James AL, et al. Efficacy of azithromycin in severe asthma from the AMAZES randomized trial. ERJ Open Res. 2019;5(4):00056-2019.

[33] Reddel HK, Bacharier LB, Bateman ED, Brightling CE, Brusselle GG, Buhl R, et al. Global initiative for asthma strategy 2021: Executive summary and rationale for key changes. Am J Respir Crit Care Med. 2022;205(1):17-35.

[34] Araújo L, Demoly P. Macrolides allergy. Curr Pharm Des. 2008;14(27):2840-62.

[35] Fillaux J, Brémont F, Murris M, Cassaing S, Tétu L, Segonds C, et al. Aspergillus sensitization or carriage in cystic fibrosis patients. Pediatr Infect Dis J. 2014;33(7):680-6.

[36] Gabriel AS, Ahnve S, Gnarpe H, Gnarpe J, Martinsson A. Azithromycin therapy in patients with chronic Chlamydia pneumoniae infection and coronary heart disease: Immediate and long-term effects on inflammation, coagulation, and lipid status in a double-blind, placebo-controlled study. Eur J Intern Med. 2003;14(8):470-8.

[37] Anderson JL, Muhlestein JB. The ACADEMIC study in perspective (Azithromycin in coronary artery disease: Elimination of myocardial infection with Chlamydia). J Infect Dis. 2000;181 Suppl 3:S569-71.

[38] Karlsson L, Gnarpe J, Bergqvist D, Lindbäck J, Pärsson H. The effect of azithromycin and Chlamydophilia pneumonia infection on the expansion of small abdominal aortic aneurysms-A prospective randomized double-blind trial. J Vasc Surg. 2009;50(1):23-9.

[39] Xia YP, Fangu Y. Effect on immune function and pulmonary function of montelukast sodium combined

with azithromycin in children with mycoplasma pneumonia. J Hainan Med Univ. 2016;22(18):48-51.

[40] Bai XR, Gao L, Li L. Effects of Qingfei Xiaoyan pill combined with ceftriaxone and azithromycin on immune index, oxidative stress and inflammatory index in senile CAP patients. J Hainan Med Univ. 2019;25(10):36-9.

[41] Fernandez AD, Elmore MK, Metzger DW. Azithromycin modulates murine immune responses to pneumococcal conjugate vaccine and inhibits nasal clearance of bacteria. J Infect Dis. 2004;190(10):1762-6.

[42] Woo PC, Tsoi HW, Wong LP, Leung HC, Yuen KY. Antibiotics modulate vaccine-induced humoral immune response. Clin Diagn Lab Immunol. 1999;6(6):832-7.

[43] Borkner L, Misiak A, Wilk MM, Mills KHG. Azithromycin clears bordetella pertussis infection in mice but also modulates innate and adaptive immune responses and T cell memory. Front Immunol. 2018;9:1764.

[44] Cuevas E, Huertas D, Montón C, Marin A, Carrera-Salinas A, Pomares X, et al. Systemic and functional effects of continuous azithromycin treatment in patients with severe chronic obstructive pulmonary disease and frequent exacerbations. Front Med. 2023;10:1229463.

[45] Ozkan T, Hekmatshoar Y, Karabay AZ, Koc A, Altinok Gunes B, Karadag Gurel A, et al. Assessment of azithromycin as an anticancer agent for treatment of imatinib sensitive and resistant CML cells. Leuk Res. 2021;102:106523.

[46] Lagler H, Kiesewetter B, Dolak W, Obermueller M, Simonitsch-Klupp I, Lukas J, et al. Treatment of mucosa-associated lymphoid tissue lymphoma with a long-term once-weekly regimen of oral azithromycin: Results from the phase II MALT-A trial. Hematol Oncol. 2019;37(1):22-6.

[47] Iwamoto S, Azuma E, Kumamoto T, Hirayama M, Yoshida T, Ito M, et al. Efficacy of azithromycin in preventing lethal graft-versus-host disease. Clin Exp Immunol. 2013;171(3):338-45.

[48] Radhakrishnan SV, Palaniyandi S, Mueller G, Miklos S, Hager M, Spacenko E, et al. Preventive azithromycin treatment reduces noninfectious lung injury and acute graft-versus-host disease in a murine model of allogeneic hematopoietic cell transplantation. Biol Blood Marrow Transplant. 2015;21(1):30-8.

[49] Barkhordar M, Mohammadi M, Hadjibabaie M, Ghavamzadeh A. Suppl azithromycin for prevention of graft-versus-host disease: A randomized placebo-controlled trial. Int J Hematol Oncol Stem Cell Res. 2018;12(2):77-83.

[50] Ulrich H, Pillat MM. CD147 as a target for COVID-19 treatment: Suggested effects of azithromycin and stem cell engagement. Stem Cell Rev Rep. 2020;16(3):434-40.