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PI3K-γ inhibitors in the therapeutic intervention of diseases caused by obligate intracellular pathogens

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Our increased understanding of host pathogen interactions shows that pathogens could capitalize on host cell pathways to favor entry and disease establishment. One such pathway used by *Leishmania mexicana* to enter into neutrophils and macrophages is the PI3Kγ signaling pathway. We recently showed that the use of the PI3Kγ inhibitor AS-605240 for the treatment of experimental *L. mexicana* infection in mice resulted in significantly lower parasite burdens and lesion sizes than WT untreated mice. Further, AS-605240 was found to be as effective as Sodium Stibogluconate, the drug of choice for treatment of *L. mexicana* infection, in reducing parasite burdens in mice. Here, we provide potential mechanisms of PI3Kγ blockade in promoting resistance to *L. mexicana* infection in mice. As a proof of principle, we propose that targeting host cell signaling pathways used in the establishment of infection could be a possible therapeutic option in the management of obligate intracellular pathogens.

Keywords: PI3Kγ, AS-605240, *Leishmania*, neutrophils, macrophages, phagocytosis, cell migration

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PI3Kinase belongs to a large family of enzymes that phosphorylate phosphoinositol containing lipids at the 3' hydroxyl of the inositol head group, generating 3' phosphoinositides which serve as second messengers involved in signal transduction.[1] These lipid kinases are involved in a host of cellular homeostatic processes including growth, cell survival, cell cycle regulation and apoptosis. PI3Kγ, a class IB isoform, has received much attention because it is predominantly expressed in immune cells and inactivation of this enzyme genetically or pharmacologically protects against various mouse models of inflammatory diseases such as SLE, rheumatoid arthritis, COPD and atherosclerosis.[2,3] In these cases, inhibiting PI3Kγ activity attenuates innate and adaptive immune responses thereby significantly reducing the severity of disease.

While such studies are very exciting and offer a therapeutic alternative to the use of corticosteroids in the treatment of autoimmune diseases, the application of PI3Kγ inhibitors to the treatment of infectious diseases have not been fully exploited. Our increased understanding of host-pathogen interactions reveal that pathogens could capitalize on host cell signaling pathways aimed at eliminating the pathogen to favor its establishment.[4] This is especially true in the case of the intracellular parasite *Leishmania mexicana*.

L. mexicana belongs to a group of intracellular parasites of the genus *Leishmania* which cause diseases that have long been considered a major public health concern in many regions of the world.[5] These obligate intracellular parasites are transmitted by a phlebotomine sand fly vector and cause cutaneous, mucocutaneous or visceral leishmaniasis in patients. According to the World Health Organization, it is estimated that about 350 million people currently suffer from leishmaniasis in about 88 countries (www.who.int). *L. mexicana* causes chronic localized infections on the skin of mice and humans.

Current approaches to the treatment of *Leishmania* involve the use of drugs that target the pathogen itself or metabolic

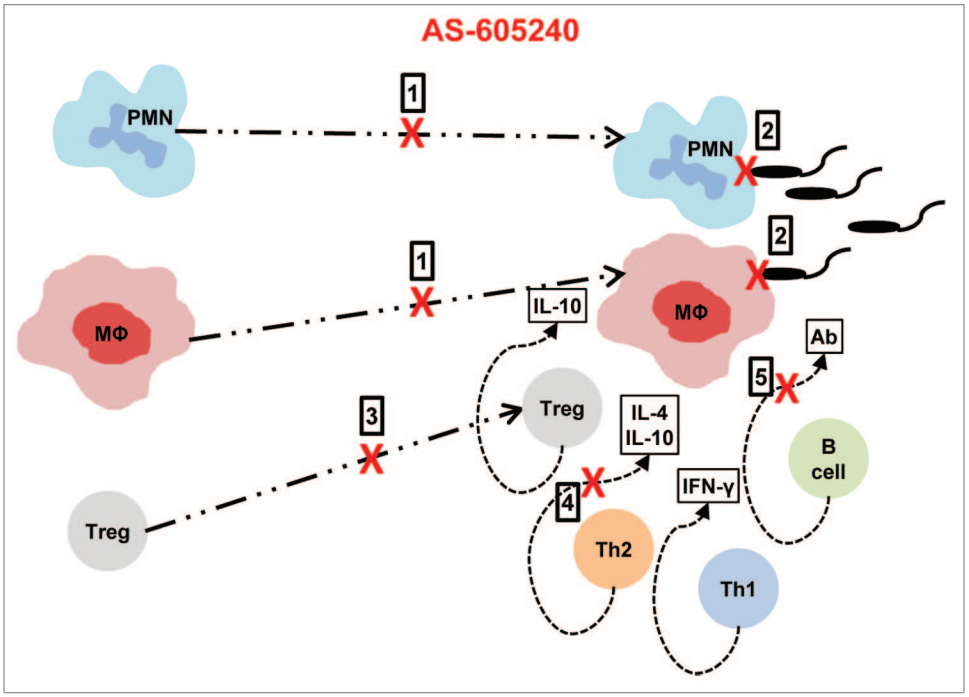

Figure 1. Possible mechanisms of action of PI3Kγ inhibitor AS-605240 in mediating resistance to *L. mexicana* infection: (**1**) prevent migration of neutrophils and macrophages to *L. mexicana* infected sites; (**2**) block phagocytosis of *L. mexicana* promastigotes by neutrophils and macrophages; (**3**) inhibit migration of IL-10 producing regulatory T cells to infected areas which would otherwise dampen the immune response; (**4**) indirectly prevent production of Th-2 associated cytokines IL-4 and IL-10; and (**5**) directly or indirectly suppress the production of serum antibodies which could otherwise contribute to Fc receptor mediated internalization of *L. mexicana* parasites.

pathways employed by the parasite to establish infection. However, increasing reports of multidrug resistant strains as well as problems associated with toxicity and patient compliance of antileishmanial drugs make alternative approaches to the management of *Leishmania* and other intracellular pathogens a viable, attractive and even necessary option.

In a recent study, we demonstrated that PI3Kγ mediates the entry of *L. mexicana* into phagocytic host cells and that blockade of this enzyme significantly lowers parasite entry into macrophages and neutrophils in vitro and in vivo.[6] Neutrophils not only serve as 'Trojan Horses' for the establishment of *Leishmania*, but may also protect the parasite from extracellular destruction.[7-10] And although macrophages are the principal immune cells required for the eradication of *Leishmania*, these cells are targeted by the parasite for its growth and propagation. Since PI3Kγ is involved in cellular trafficking and phagocytosis of immune cells, we investigated the effects of PI3Kγ blockade in *L. mexicana* infection. We showed that genetic deletion of PI3Kγ or selective inhibition using the PI3Kγ inhibitor AS-605240 significantly reduced parasite entry into neutrophils and macrophages. Further, lesion growths and parasite burdens were lower in PI3Kγ-/- mice and in mice treated with AS-605240 than in WT C57BL/6 mice. AS-605240 was also found to be as effective as Sodium Stibogluconate (the drug of choice for treatment of *L. mexicana* infection) in reducing parasite burdens in mice. We therefore showed PI3Kγ as a possible drug target for the management of *L. mexicana* and potentially other obligate intracellular pathogens. We also demonstrate that targeting host cell signaling pathways exploited by pathogens provide a viable alternative to conventional therapeutic approaches which tend to focus on the pathogen alone. This expanded approach to the management of infectious diseases is beginning to gain wide attention in the scientific community owing to an increased understanding of host-pathogen interactions, and a combination of therapeutic approaches could very well be the future of effective disease control and eradication.

Mechanisms involved in PI3Kγ mediated susceptibility to intracellular pathogens are not fully understood, but seem to primarily involve phagocytosis and phagocytic cell recruitment to infected tissues. PI3Kγ has been shown to initiate F-actin polymerization and cytoskeletal rearrangement, mechanisms involved in migration and phagocytosis.[1] PI3Kγ is also a mediator in G-protein coupled receptor signaling and therefore is involved in the directed migration of neutrophils and macrophages in response to chemokines.[11,12] This suggests that interfering with PI3Kγ signaling prevents pathogen entry and establishment by selective inhibition of macrophage recruitment and phagocytic mechanisms.

PI3Kγ is part of a complex signaling network associated with a wide range of immune receptors in a variety of cells.[13] Inhibition of PI3Kγ therefore affects other immune cells and these effects in the context of intracellular infections are still being investigated. In *L. mexicana* infection, we observed significantly decreased levels of serum IgG1 and IgG2a in PI3Kγ-/- mice compared with WT

mice (unpublished data) which suggests possible B cell defects. Interestingly, some researchers have shown that in the absence of circulating antibody, Fc receptor mediated internalization of *L. mexicana* in mouse phagocytes is compromised, leading to increased host protection.[14] Other possible mechanisms of PI3Kγ blockade based resistance of *L. mexicana* include suppression of migration of IL-10 producing regulatory T cells (Tregs) to the site of infection as well as impaired production of Th2 associated cytokines, IL-4 and IL-10.[6] However, suppression of Th2 cytokine production in PI3Kγ inhibitor treated mice infected with *L. mexicana* seems to be an indirect effect. A summary of the effects of PI3Kγ inhibition in the resistance to *L. mexicana* infection in mice is presented in **Figure 1**.

Studies that define mechanisms of *Leishmania* pathogenesis, host immune evasion and exploitation have been particularly useful in designing efficient therapeutic strategies. Successful approaches do not always directly target the pathogen, but could capitalize on host immune response pathways exploited by the pathogen to facilitate entry and establishment of disease. Although the use of PI3Kγ inhibitor AS-605240 for the management of intracellular pathogens like *Leishmania* in humans still requires additional research, it does show a lot of promise. When used in combination with current treatment options for cutaneous *Leishmania* infections, PI3Kγ inhibitors like AS-605240 present a viable alternative.

Disclosure of Conflicts of Interest

There were no potential conflicts of interest to disclose.

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