HIF1A in Neutrophils: A Potential Crucial Factor for Psoriatic Arthritis through a Positive Feedback Loop with IL-23

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LAY ABSTRACT

Inflammation can create oxygen-deprived conditions in tissues. Our recent discovery found that under low oxygen conditions (called hypoxia), neutrophils significantly increase the production of the protein IL-23, a key player in spondyloarthritis (SpA), including psoriatic arthritis (PsA). **Our preliminary data** from SpA patients show increased neutrophil IL-23 levels under hypoxia compared to healthy individuals. In a pilot study, PX-478 an inhibitor of HIF1A successfully reduced PsA clinical features, including psoriasis and arthritis, by lowering IL-23 levels in neutrophils. Further testing in mice with artificially increased IL-23 levels showed that the level of HIF1A increased in the joint, and PX-478 was successful in alleviating arthritis but not psoriasis, in the model. **This indicates that HIF1A plays a crucial role in sustaining arthritis, but not psoriasis, through a positive feedback loop between HIF1A and IL-23.** Our study aims to investigate HIF1A and IL-23 expression in PsA patients, comparing with controls. Additionally, we'll test the effect of PX-478 on mice with SpA-like conditions induced by curdlan (a bacterial product) or IL-23. Success could reveal a new IL-23-related inflammation pathway, expanding PsA treatment options and insights into managing this inflammatory arthritis.

SCIENTIFIC ABSTRACT

It is well-acknowledged that inflammation induces hypoxic environments in tissues. We recently found that neutrophils in hypoxia substantially increase the expression of IL-23, activating downstream immune cells and leading to the exacerbation of type 3 immunity-mediated inflammation in curdlan-injected SKG mice, a well-established SpA model [***Cell Mol Immunol (Nature)***: Under Revision]. These findings demonstrate that hypoxia-primed neutrophils may be new therapeutic targets for SpA, including PsA. Indeed, our pilot study has shown that PX-478, a specific inhibitor of HIF1A, dramatically suppresses distinct clinical features of PsA, including psoriasis-like dermatitis and arthritis, by suppressing IL-23 in neutrophils. We also tested the efficacy of PX-478 in IL-23 overexpressed SKG mice. Interestingly, our preliminary data showed that PX-478 was effective in attenuating arthritis but not psoriasis, demonstrating that HIF1A is essential in sustaining arthritis, not psoriasis, in SKG mice. This proposed study will first explore the expression of *HIF1A* and *IL23* in PsA patients, comparing them to healthy or non-PsA disease controls, to test if this is specific for SpA, including PsA. Additionally, we will confirm the efficacy of PX-478 in curdlan- or IL-23-induced SKG mice. This study has the tremendous potential to reveal the novel immunoregulatory system of IL-23 and expand treatment options for PsA.

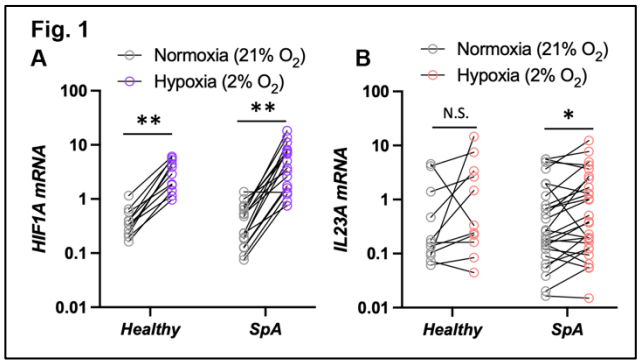
RESEARCH PROPOSAL

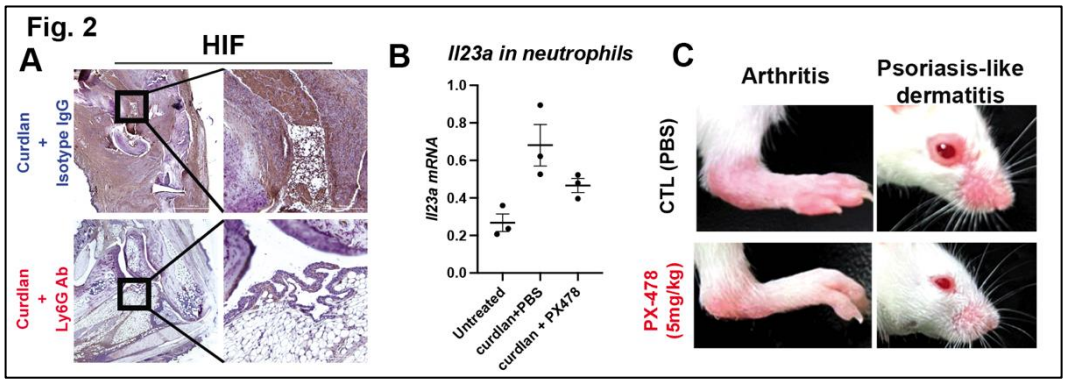
1. BACKGROUND: There is currently no cure for psoriatic arthritis (PsA), one of the most common forms of spondyloarthritis (SpA) characterized by inflammation in the joints, skin, eyes, and gut. IL-23 is a key player in triggering immune responses, including the release of other cytokines such as IL-17A and IL-17F, associated with PsA. Despite existing therapies targeting IL-23 or IL-17A/F, many PsA patients still struggle to manage their symptoms[1–3]. This could be because active inflammatory mediators, either induced by or inducing IL-23, persist in the body.

My group has recently discovered that that neutrophils are one of the primary cells that produce pathogenic cytokines including macrophage migration inhibitor factor (MIF) and IL-23, essential upstream cytokines in activating type 3 immunity (the IL-23/IL-17 axis), in patients with SpA (***Nakamura A, a et al. Sci Trans Med, 2021***)[4]. Furthermore, our recent study has shown that cytoplasmic MIF in neutrophils interact with hypoxia-inducible factor 1 alpha (HIF1A), a molecule stabilized in a hypoxic microenvironment (***Cell Mol Immunol* (*Nature*, Impact Factor: 24.1)** under Revision]. Interestingly, we found that both MIF and HIF1A are indispensable for increasing the secretion of various proinflammatory mediators, especially IL-23, from neutrophils in both humans and mice. **Thus, neutrophils may be an important cell that secretes IL-23 and activates type 3 immunity.** To further support the pathological role of hypoxia in inflammatory arthritis, previous studies also demonstrated that hypoxia is a crucial factor for accelerating arthritis, enthesitis and new bone formation (NBF), by enhancing angiogenesis controlled through upregulated VEGF[5–9]. However, to the best of our knowledge, whether pathogenic neutrophils

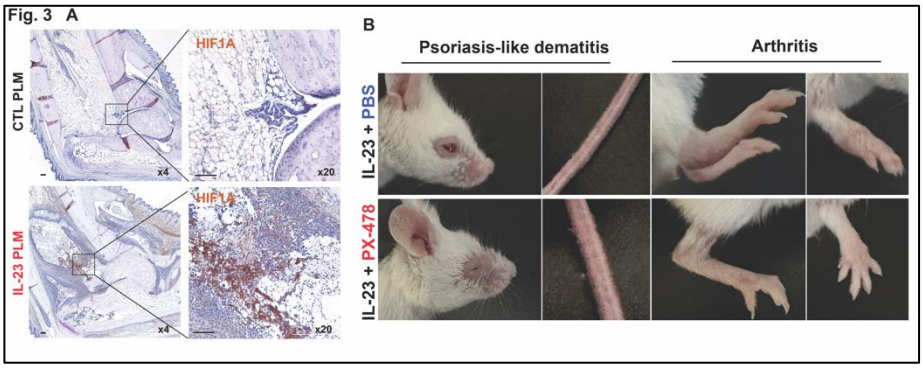
primed by hypoxia are increased and play essential roles in accelerating inflammation in PsA has never been reported. In addition, it is unclear whether PsA patients have enhanced expression of IL-23 in neutrophils in a hypoxic condition, compared to healthy or non-PsA disease controls.

2. PRELIMINARY DATA: In this study, we will first test the gene expression of *HIF1A* and *IL23A* in human peripheral neutrophils isolated from patients with PsA, non-SpA, or healthy controls. The neutrophils will be incubated either in normoxia (21% O_2) or hypoxia (2% O_2). Our preliminary data revealed that the expression of *HIF1A* increased in both groups in hypoxia (**Fig. 1A**). **Notably, the expression of *IL23A* significantly increased only in neutrophils isolated from SpA patients (n=27, including axSpA and PsA), not in healthy controls (n=11), when cultured in hypoxia** (**Fig. 1B**). This suggests that neutrophils in SpA patients, including those with PsA, may be predisposed to express higher levels of IL-23 in hypoxia compared to neutrophils in healthy individuals.





To explore the potential of HIF1A-targeted therapy in PsA, we will assess the efficacy of the HIF1A-specific inhibitor (PX-478) in SKG mice. We first confirmed that curdlan-SKG mice exhibit abundant expression of HIF1A in neutrophils around the inflamed ankle joints, while neutrophil depletion (with anti-Ly6G Ab) dramatically suppressed its expression (**Fig. 2A**). Our preliminary data (n=3 SKG mice/group) showed that pharmacological inhibition of HIF1A (PX-478, 5 mg/kg) decreased the expression of *Il23a* in neutrophils in the joint (**Fig. 2B**) and attenuated PsA pathologies, including psoriasis and arthritis, in curdlan-SKG mice (**Fig. 2C**), suggesting that HIF1A is a pathogenic for arthritis and psoriasis. Additionally, we tested the potential of PX-478 in IL-23-overexpressed SKG mice, where IL-23 plasmid (IL-23 PLM; 5ug/mouse) is injected. We observed that **IL-23 induced both psoriasis and arthritis, along with increased expression of HIF1A in the joints and subcutaneous tissues of IL-23-overexpressed SKG mice (Fig. 3A), suggesting a positive feedback loop between HIF1A and IL-23 in psoriasis and arthritis.**



Interestingly, **IL-23-overexpressed SKG mice treated with PX-478 attenuated only arthritis, not psoriasis** (**Fig. 3B**). These preliminary results suggest that while IL-23 overexpression can induce both psoriasis and arthritis with enhanced expression of HIF1A, **HIF1A is indispensable in sustaining arthritis, but not psoriasis, through the positive feedback between HIF1A and IL-23.**

In this study, we will confirm our preliminary results to reveal a new regulatory system of neutrophil-driven inflammation induced by hypoxia.

2. OVERALL HYPOTHESES: ***1)*** Neutrophils in SpA patients are predisposed to express increased levels of IL-23 under hypoxia and ***2)*** inhibition of HIF1A attenuates skin/joint inflammation in the SpA preclinical model by suppressing type 3 immunity driven by IL-23. Additionally, we will confirm the presence of a positive feedback loop between HIF1A and IL-23 in the joint, but not in the skin.

3. OBJECTIVES and METHODS:

AIM 1: Investigate the expression of *HIF1A* and *IL23A* in neutrophils collected from PsA patients, non-PsA arthritis patients, and healthy controls. To confirm that hypoxia contributes to the expression of IL-23 in neutrophils, we will freshly isolate neutrophils from healthy controls, PsA patients, or osteoarthritis (OA) patients (disease controls, n= 20 samples/group/sex, age: 18 to 80 years). The cells will then be immediately cultured in hypoxia (2% O_2) and compared to normoxia (21% O_2) to assess the expression of *HIF1A* and *IL23A*. We will also include age- and sex-matched healthy individuals (n=20 samples/sex, age: 18 to 80 years) who have never experienced any type of arthritis or major medical conditions. Additionally, we will evaluate the correlation between hypoxia-induced expression of IL-23 in neutrophils and various clinical factors, including age, sex, disease duration, smoking, extra-articular manifestations, and inflammatory markers (CRP/ESR). ***Sample size and feasibility*:** In the absence of prior studies of this nature, we assumed that 55% of SpA patients, compared to 35% of healthy controls, would have increased *IL23A* levels in hypoxia (with an effect size of 2.27). For a power of 80% and a type 1 error rate <5%, we need at least 16 patients in each group per sex. Thus, we decided to include 20 patients/group/sex, expecting higher expression in the group. As I have established the Queen's Research Program for SpA (QURESPA; REB: 6038347), collecting blood, synovial fluids, and spinal tissues from various arthritis patients (n=50 to 60 samples/sex per year), it is highly feasible to perform this experiment.

Aim 2: Blocking the pathogenic neutrophils in *in-vivo* preclinical models: Next, we will test whether blocking hypoxia-induced HIF1A with PX-478 can be a novel therapeutic for PsA. SKG mice will be administered curdlan to induce PsA pathologies. At week 0 or 4, the HIF1A-specific inhibitor (PX-478; 5 or 10 mg/kg, oral gavage, 3 days a week) or PBS (control vehicle) will be administered to the mice (n=8 mice /group/sex) to test the prophylactic or therapeutic effect of PX-478, respectively. Clinical scores, including psoriasis, arthritis, and uveitis, will be performed once a week until week 8. We will also evaluate psoriasis, arthritis, spondylitis, ileitis, uveitis, NBF by histopathology and/or microCT, and immune cells ($CD4^+$ T cells expressing IL-17A, IL-17F, IL-22, and GM-CSF) by flow cytometry (n=8/group/sex), as previously shown[4]. Lastly, we will confirm our preliminary data that PX-478 is effective for IL-23-induced arthritis but not for psoriasis in SKG mice (n=8/group/sex). ***Sample size and feasibility*:** Based on our preliminary data, the difference between PX-478- and PBS (control)-treated SKG mice was evident. Given that the predicted effect size is > 2.5, n=8 mice/group/sex will be sufficient for a power of 80%, and a type 1 error rate <5%. We also have ethical approval for animal usage (project ID# 2023-2420) from Queen's University. **The preliminary data is promising, indicating that the study is highly feasible and can be conducted without any significant limitations.**

4. RESEARCH SIGNIFICANCE and Patient Research Partners

This groundbreaking study aims to uncover a novel immunoregulatory system centered on IL-23. We propose that HIF1A is a key molecule sustaining arthritis after IL-23 stimulation in PsA, and it can further enhance the expression of IL-23 in neutrophils through a positive feedback loop. If successful, we will explore how HIF1A may play a pivotal role in the transition from psoriasis to PsA in our next step. This grant is crucial to advance and confirm our pilot study. We also anticipate that the HIF1A inhibitor (PX-478) identified in our research could offer an innovative therapy for PsA patients.

***Patient Research Partners*:** The unmet need for new therapies were endorsed by patient research partners (PRP) and I will share my research progress with them every 6 months. Please see the attached support letter from Dr. Jennifer Boyle, our PRP advocate.

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