Investigation of a novel pan-PIM kinase inhibitor, IBL-101, as a potential treatment for psoriasis

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INTRODUCTION

Psoriasis is an autoimmune disease of the skin that affects 3% of the population. This disease impacts both the physical and psychological well-being of patients (1). Psoriasis pathogenesis implies the infiltration of immune cells in the skin that maintain inflammation and promote abnormal growth of keratinocytes. The pathogenesis is driven by the dysregulation of T helper cells (Th1, 2, 17, 22) and T regulatory cells that secrete inflammatory cytokines like IFNγ and IL-17, among others, promoting persistent inflammatory signaling in the skin (2). The current therapies for mild psoriasis include topical treatments, but most severe forms require the use of inhibitors against TNF-α (*etanercept*), p40 subunit of IL-12/23 (*ustekinumab*), IL-17 (*secukinumab*) and p19 subunit of IL-23 (*guselkumab*) (3) and some patients are non responders to the available treatments.

The serine/threonine-specific proviral integration site for Moloney murine leukemia virus (PIM) kinases is a family of serine/threonine kinases consisting of three isoforms: PIM-1, PIM-2, and PIM-3. They play a central role in cell cycle regulation and survival, proliferation, and migration, have anti-apoptotic activity. These proteins also are implicated in the amplification and differentiation of T cell responses. PIM kinases are over-expressed in different types of cancers (4), early rheumatoid arthritis (RA) (5), inflammatory bowel disease (IBD) (6,7) and lupus nephritis (LN) (8).

PIM kinase inhibitors have been under clinical investigation as novel therapeutics for oncology (4) and recently in preclinical investigation for autoimmune/inflammatory diseases. In this study, we examined the potential role of PIM kinases in psoriasis pathogenesis and the effects of IBL-101, a novel potent and selective pan-PIM (PIM-1/2/3) inhibitor, in modulating proinflammatory CD4⁺ T helper (Th) cell responses implicated in disease. The results are useful for other autoimmune diseases were T cells play a role in pathogenesis such as RA, IBD and LN.

MATERIAL AND METHODS

Gene Expression analysis. Patient gene expression data were collected from Gene Expression Omnibus database GDS4602. Gene expression profiles of PIM isoforms and downstream target genes were analysed using Gene Expression Omnibus datasets from healthy individuals and lesional and non-lesional skin from psoriasis patients.

Cell culture and flow cytometry. The immunomodulatory effects of the PIM kinase inhibitor IBL-101 (obtained from Inflection Biosciences) were analysed on splenic naïve CD4⁺ T cells isolated from C57Bl/6 WT mice, unstimulated or stimulated with anti-CD3 and anti-CD28 mAb (Th0), without or adding IBL-101 at different concentrations (1.25 nM, 12.5 nM or 125 nM) and differentiated *in vitro* towards Th1 (IL-12 & anti-IL-4 mAb for 72 hours), Th17 (TGF-β, IL-6, anti-IFNγ & anti-IL-4 mAbs for 120 hours) and induced regulatory T cell (iTreg) phenotype (TGF-β for 120 hours). Polarised cells treated with cyclosporin A (100 ng/mL) were positive controls.

For flow cytometry, cultured cells were pooled and stimulated 4h (Th1) or 6h (Th17) with PMA, ionomycin and brefeldin A. After fixation and permeabilization, Th1, Th17 or iTreg were stained for anti-IFNγ-mAb and anti-IL-17—mAb and FoxP3 respectively, as well as anti-CD4, anti-CD69 and Live/Dead Fixable Aqua Dead Cell stain.

ELISA. Differentiation of CD4⁺ T cell subsets was determined ELISA for IFNγ, IL-2 and IL-17A. In addition, early T cell activation responses in the presence/absence of IBL-101 were analysed through surface CD69 expression and secretion of IL-2 after 24hrs.

Statistical Analysis. Normality and homoscedasticity were assessed in the data. In order to determine statistically significant differences among the groups, T-test or Mann-Whitney test was used with parametric or non-parametric data, respectively.

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RESULTS

A gene expression analyses from psoriatic patients' skin (Fig. 1) revealed that expression levels of PIM1 and PIM2, and their positive regulator EIF4E, are upregulated in lesional skin compared to healthy control, indicating increased PIM kinase activity in psoriatic skin. Also, PPP2CA, a negative regulator of PIM kinases, is significantly down-regulated in the lesional skin. Interestingly, lesional skin from psoriatic patients showed elevated expression of Th1 (IFNG, TNFa) and Th17 cell cytokines (IL17A) compared to non-lesional or controls. Also, FOXP3, a transcription factor of iTregs, showed an elevated expression in both lesional and non-lesional skin compared to healthy skin. We observed up-regulation of Pim1 and Pim2 genes in a mouse model of psoriasiform inflammation after 7 days of application of Aldara cream (5% imiquimod) in mice ears (Fig. 2).

Furthermore, by we analysed the effects of IBL-101 on Th1, Th17 and iTreg differentiated cells. We show that IBL-101 acts as a potent inhibitor of Th1 and Th17 differentiation while enhancing iTreg generation, without affecting T cell viability (Fig. 3). Similar results were observed by ELISA (Fig. 4). Similarly, IBL-101 inhibited IL-2 production and expression of CD69 early upon T cell activation (Fig. 5). These results indicate that PIM-1/2 is functioning upstream of the IL-2 signaling pathway and affects the expression of the early activation marker CD69.

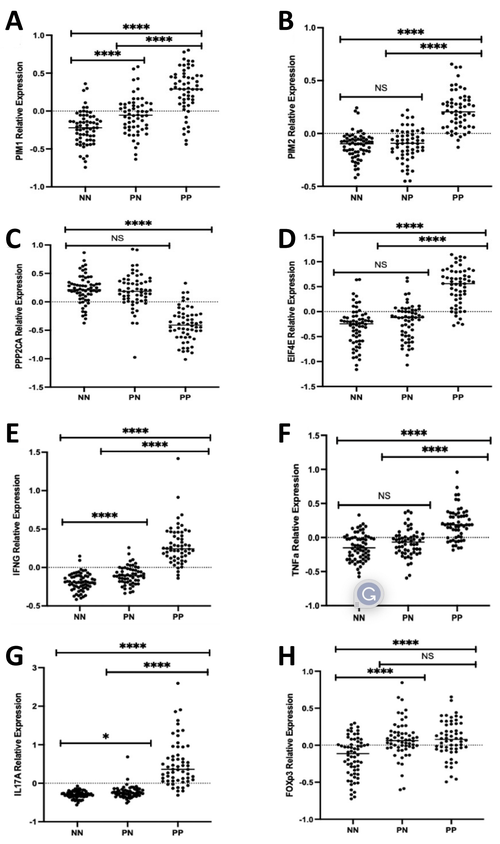

Figure 1.- Gene expression profile in psoriatic patients. Relative levels of expression of **(A)** PIM-1, **(B)** PIM-2, **(C)** PPP2A and **(D)** EIF4E, **(E)** IFNG, **(F)** TNFa, **(G)** IL17A, **(H)** FOXP3 in lesional (PP) and non-lesional psoriatic (PN) skin (n=58) compared to control healthy skin (NN) (n=64). Significant differences were obtained by Mann-Whitney test: *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, NS: Not Significant. Data are publicly available in the GEO database (GDS4602).

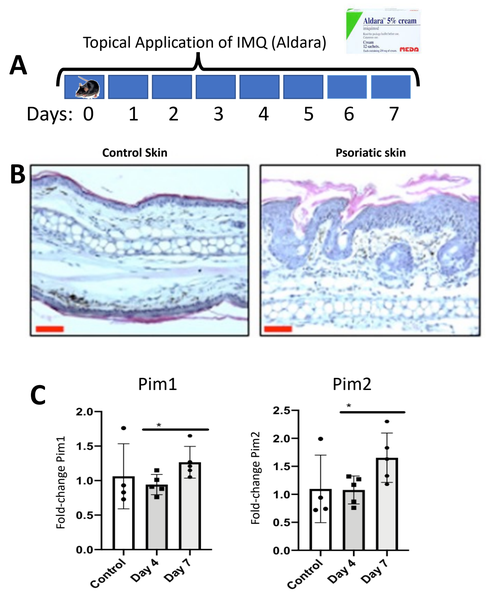

Figure 2.- Pim1/2 are over-express in mice psoriatic skin upon topical application of imiquimod for 7 days. (A) Scheme of topical application of Aldara cream (5% imiquimod, IMQ) in the ear of mice for 6 days. **(B)** Representative micrographs were obtained after haematoxylin and eosin staining of ear sections of control (vehicle-treated) and after 6-d Aldara cream topical administration. *Scale bar = 1 μm*. **(C)** Relative gene expression levels of Pim1 and Pim2 in cDNA from ear extracts of uninflamed controls and mice topically administered Aldara cream for 4/7 days (*n* = 4). Data show means ± SEM. Significant differences were obtained by t-test: *p<0.05.

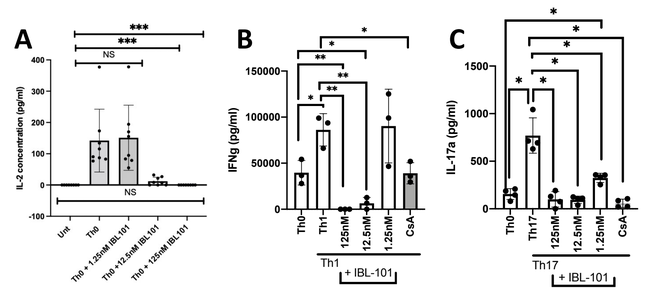

Figure 4.- Level of IL-2, IFNγ, IL-17A after IBL-10 treatment. Cytokines were detected and measured by ELISA in supernatants from **(A)** Th0, **(B)** Th1 and **(C)** Th17 cultured cells in presence of IBL-101 (125, 12.5, 1.25 nM), Cyclosporin A (100 ng/mL) or media. IL-2 was detected after 24 hours post-TCR stimulation. IFNγ and IL17a levels were detected after 72 hours under Th1/17 conditions. Significance analysed by Mann-Whitney: *p<0.05, **p<0.01, ***p<0.001, NS: Not Significant.

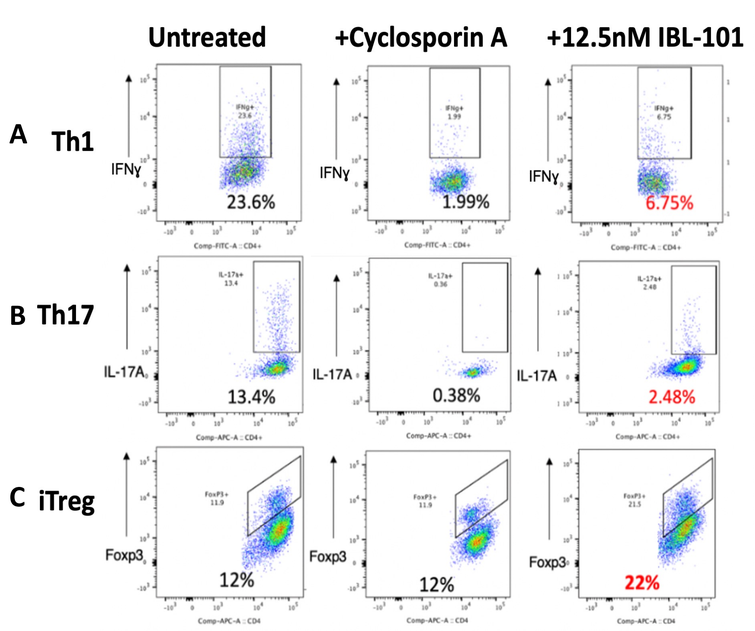

Figure 3.- Changes in Th differentiation due to inhibition of PIM-1/2/3 with IBL-101 treatment. Percentage of Th1 cells secreting IFNγ **(A)**, Th17 cells secreting IL-17A **(B)** and iTreg cells expressing FoxP3 **(C)** in presence of IBL-101 (12.5 nM), Cyclosporin A (100 ng/ml) or media determined by flow cytometry.

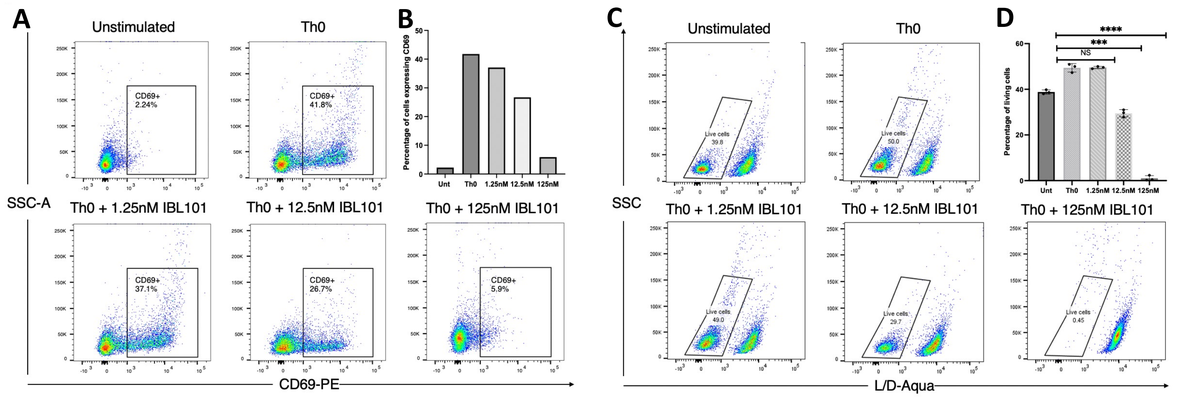

Figure 5.- IBL-101-mediated inhibition of PIM-1/2/3 signaling decreased expression of CD69 after TCR stimulation. Naïve CD4⁺ T cells unstimulated or stimulated with anti-CD3 and anti-CD28 mAb (Th0) with or without IBL-101 at 1.25 nM, 12.5 nM or 125 nM incubated at 37ºC for 24 hours. **(A)** Percentage of CD69⁺ cells. **(B)** Comparison of percentages of cells expressing CD69 for unstimulated CD4⁺ T cells (Unt), stimulated (Th0) with or without IBL-101 at different concentrations. **(C)** Live/Dead viability marker (L/D-Aqua) was used to stain cells and determine viability. The percentage of live cells present in the sample by gating L/D⁻ cells and further analysed for significance (**D**). Data is representative of triplicate experiments. Bars represent average ± SEM (n=3). Significance analysed by Unpaired Student t-test: ***p<0.001, ****p<0.0001, NS: Not significant.

CONCLUSIONS

IBL-101 is a pan-PIM kinase inhibitor able to suppress inflammatory cytokine production (e.g. IFNγ, IL-2 and IL-17A) from CD4⁺ T cells, and skew T helper cells towards an iTreg cell phenotype. IBL-101 may exert these effects by modulating early T cell activation responses.

These data provide Proof-of-Concept (PoC) for further evaluation of IBL-101 in pre-clinical and clinical settings for psoriasis and also other autoimmune diseases affected by T cell dysregulation, such as RA, IBD and LN.