Expression of PI3K Signaling Associated with T Cells in Psoriasis Is Inhibited by Seletalisib, a PI3Kδ Inhibitor, and Is Required for Functional Activity

TO THE EDITOR

The phosphoinositide 3-kinase (PI3K) pathway plays a key role in many cellular processes, including cell proliferation, survival, and protein synthesis, with the PI3K isoform, PI3Kδ, involved in normal T-cell development and function (Jarmin et al., 2008; Lucas et al., 2016; Vanhaesebroeck et al., 2012). Evidence to suggest that PI3K signaling might play a role in psoriasis comes from reports of increased factor receptor signaling in keratinocytes triggers interleukin-24-dependent psoriasis-like skin inflammation in mice. Immunity 2013;39: 899–911.


knockout mice are greatly protected from imiquimod-induced psoriasis-like skin inflammation compared with wild-type mice (Roller et al., 2012).

As psoriasis is a T-cell-mediated skin disease, the recent development of the selective PI3Kδ inhibitor, seletalisib (Allen et al., 2017), led us to investigate the effects of PI3Kδ inhibition in T cells of psoriatic subjects. All investigations were conducted under ethics committee approval, with written, informed patient consent. Immunofluorescence staining and confocal microscopy, used to characterize the expression of the PI3K pathway in psoriatic skin and its colocalization with infiltrating T lymphocytes (see Supplementary Materials and Methods online), identified pS6-positive T cells present in the dermis of lesional psoriatic skin.

**Figure 1. PI3Kδ inhibition of pS6 in psoriatic T cells.** (a) Identification of pS6+ T cells in psoriatic lesional skin determined by immunofluorescence confocal analysis. Arrows point to CD3+ pS6+ cells. Scale bar = 20 μm. (b) Western blot showing phosphorylation of the ribosomal protein S6 in human CD4+ T cells after stimulation with anti-CD3 and with anti-CD3/anti-CD28. (c) Representative histogram of pS6 during a phosflow assay, gated on CD3+ cells in a lymphocyte population determined by FSC and SSC. (d–g) Combined psoriatic subject data shown as % of (d) CD3+ pS6+ cells from psoriatic lesional dermis, (e) CD3+ pS6+ cells from psoriatic PBMCs, (f) CD4+CD45RO+pS6+ cells from psoriatic lesional dermis, and (g) CD8+CD45RO+pS6+ cells from psoriatic lesional dermis. In d–g, each color represents a single psoriatic subject. *P < 0.05, **P < 0.01. FSC, forward scatter; PBMC, peripheral blood mononuclear cell; PI3K, phosphoinositide 3-kinase; SSC, side scatter.
Figures S1 and S2 online). Western phosphorylation of S6 protein in CD4 blotting demonstrated enhanced phosflow assay, stimulation with anti-CD3/CD28 of T lymphocytes isolated from lesional skin biopsies and peripheral blood mononuclear cells (PBMCs) of subjects with psoriasis led to an increase in pS6, which was dose-dependently inhibited by seletalisib (Figures 1c−g). Inhibition of PI3Kδ by seletalisib at 10 μM reduced the median percentage of pS6-positive dermal lesional T cells and peripheral blood T cells by 74% and 49%, respectively (P = 0.0081 and P = 0.017, respectively, Kruskal-Wallis test with Dunn’s post-test, Figure 1d and e). A comparison of the proportion of lesional CD3+ T cells positive for pS6 in the phosflow assay (Figure 1d) with immunofluorescence microscopy of lesional psoriasis biopsies (Supplementary Figures S1 and S2) suggests that immunofluorescence microscopy gives an underrepresentation of the total number of psoriatic CD3+ T cells with PI3Kδ activity. Although phosphorylated protein kinase B proved more difficult to measure, a
reduction in phosphorylated protein vkinase B in CD3+ T cells from PBMCs of psoriatic subjects was seen after inhibition of PI3Kδ in each of three cases where the phosflow assay was conducted (Supplementary Figure S3 online).

Flow cytometry of T cells from lesional psoriatic dermis showed that they were largely effector/memory (CD45RO+ CD3+ CD4+ cells, whereas those from PBMCs were a mixture of both naïve (CD45RO−) and effector/ memory CD3+CD4+ and CD3+CD4− cells (Supplementary Figure S4 online). The analysis of pS6-positive psoriatic dermal memory T cells indicated that 10 μM seletalisib reduced the median percentage of pS6 in CD4+45RO+ cells by 68% and CD8+45RO+ cells by 82% (P = 0.0062 and P = 0.014, respectively, Kruskal-Wallis test with Dunn’s post-test, Figure 1f and g).

We next wished to determine whether inhibition of PI3K signaling in activated psoriatic T lymphocytes by seletalisib was sufficient to elicit a functional effect. Using a carboxyfluorescein diacacetate succinimidyl ester (CFSE) assay, PBMCs from subjects with psoriasis were stimulated with anti-CD3 and incubated either in vehicle or seletalisib for 72 hours. As the dose of seletalisib increased, there was a corresponding decrease in the number of cell divisions, and inhibition of the percentage of CD4+ and CD8+ cells that had divided, as demonstrated by a reduction in the peaks of CD4+CFSE− and CD8+CFSE− populations (Figure 2a). After the addition of 1 and 10 μM seletalisib, the mean percentage of lymphocytes that had proliferated (i.e., % divided) was reduced in CD4+ cells by 54% and 93% (P = 0.0072 and P = 0.0002, one-way analysis of variance with Dunnett’s post-test) and in CD8+ cells by 54% and 91% (P = 0.0002 and P < 0.0001, one-way analysis of variance with Dunnett’s post-test), respectively (Figure 2b and c). Furthermore, at 10 μM seletalisib, in five psoriatic subjects, <1% of CD4+ cells had proliferated and in four psoriatic subjects, <3% of CD8+ cells had divided.

Next, we evaluated the effect of seletalisib on IFNγ, tumor necrosis factor-α, and IL-17 release. PBMCs from psoriatic subjects were stimulated with anti-CD3 and incubated either in vehicle or seletalisib for 48 hours, and supernatants were then removed and IFNγ, tumor necrosis factor-α, and IL-17 measured by ELISA. A significant decrease in IFNγ and tumor necrosis factor-α release was seen after exposure to seletalisib, with doses of 1 and 10 μM seletalisib causing reductions of 79% and 98% for IFNγ (P = 0.0254 and P = 0.0126, one-way analysis of variance with Dunnett’s post-test) and 59% and 93% for tumor necrosis factor-α (P = 0.0017 and P = 0.0005, one-way analysis of variance with Dunnett’s post-test), respectively (Figure 2d and e). Mean IL-17 release was reduced by 68% and 79% at doses of 1 and 10 μM seletalisib, respectively; this was not statistically significant, probably as a result of the undetectable levels of IL-17 in three of the subjects’ T cells when stimulated in vehicle (Figure 2f).

This study suggests that PI3K δ signaling is altered in lesional psoriatic T cells, as seen by the presence of pS6 in psoriatic skin on immunofluorescence staining, and that the use of the PI3Kδ-selective inhibitor, seletalisib, to block PI3K δ signaling in human psoriatic T cells significantly inhibits S6 phosphorylation, lymphocyte proliferation, and cytokine release. Although a previous study reported that another PI3Kδ inhibitor, IC87114, reduced IFNγ and IL-17 production by PBMCs from donors with psoriasis (and provided representative data from two of five individuals) (Roller et al., 2012), a recent first-in-human study of oral seletalisib demonstrated acceptable safety and pharmacological profiles, as well as preliminary evidence of target engagement in psoriatic skin disease (Helmer et al., 2017). Whereas recent psoriatic drug development has mainly centered on injected biological therapies, there is a great need for effective systemic treatments that can be administered orally, and the results of this study suggest that the PI3Kδ pathway would make an excellent target for the treatment of psoriasis and that further studies are warranted on seletalisib as an oral drug in psoriatic subjects.

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CONFLICT OF INTEREST
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SUPPLEMENTARY MATERIAL
Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2017.12.028.

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Regnase-1, an Immunomodulator, Limits the IL-36/IL-36R Autostimulatory Loop in Keratinocytes to Suppress Skin Inflammation


TO THE EDITOR

Regnase-1 (Reg1), also known as Zc3h12a or MCPIP-1, is a member of a family that includes Zc3h12b, Zc3h12c, and Zc3h12d, all of which harbor two conserved domains, a ribonuclease domain and a CCCH-type zinc-finger domain (Liang et al., 2008). Reg1 destabilizes translationally active mRNAs, such as Il6, Il12b, and Il1b in macrophages (Iwasaki et al., 2011; Matsushita et al., 2009) and Il2, Rel, and OX40 (Tnfrsf4) in T cells (Li et al., 2012; Uehata et al., 2013), as well as other mRNAs, such as Nkbi, Nkbid, Cxcl1, and Reg1 itself (Mino et al., 2015). In response to toll-like receptor ligands or IL-1β (Iwasaki et al., 2011; Liang et al., 2008; Sparna et al., 2010), Reg1 is rapidly degraded by ubiquitination and degradation via the proteasome machinery (Iwasaki et al., 2011). Indeed, the degradation of Reg1 leads to release of the “brake” on target mRNA expression if needed. Thus, Reg1 represents a modulator of inflammation in a cross-regulatory fashion with proinflammatory signals. Previous studies have reported that, in keratinocytes (KCs), Reg1 expression is induced by stimulation with IL-17A (Ruiz-Romeu et al., 2016) and IL-36s (Mahil et al., 2017). Here, we further explored the role of Reg1 in epidermis.

Reg1 protein in cultured KCs was rapidly decreased upon treatment with IL-1β, IL-17A, or IL-36α within 30 minutes, but it reappeared at 180 minutes (Figure 1a). In contrast, Reg1 was not affected by IL-6, IFN-γ, TNF-α, or either of the toll-like receptor ligands (see Supplementary Figure S1a online). Also, the Reg1 protein treated with IL-1β, IL-17A or IL-36α migrated

Abbreviations: CH, contact hypersensitivity; IL-36R, IL-36 receptor; KC, keratinocyte; KD, knock-down; KO, knock-out; IMQ, imiquimod; Reg1, regnase-1

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