Regulatory role of JAK signaling on Keratinocytes and synovial cells (FLS): Novel mechanisms for JAK inhibitors in Psoriatic disease

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Background

- In psoriasis and psoriatic arthritis (PsA), aberrant activation / migration of specific T cell subpopulations (Th17/Th9/MAIT cells) in the skin and joint synovium induces local inflammation and upregulation of cytokines such as IL-9, IL-17A/IL-17F, IL-22 and IL-23.
- IL-23 does not have any biological effect on keratinocytes (KC) and synovial cells (FLS) and IL-17 does not activate the JAK/STAT signaling system.
- IL-9 and IL-22 have regulatory roles on proliferation of KC and FLS, the two key components for plaque and pannus formation; also, JAK-1,3 are activated by IL-9 and JAK1/TYK2 by

Objective

To determine the **functional significance of JAK/STAT signaling system in plaque and pannus formation, we studied the regulatory role of IL-9 induced JAK-1,3 and IL-22 induced JAK1/TYK2 on KC/FLS.** We hypothesized that IL-9/IL-22 induced JAK/STAT signaling regulates the proliferative cascades of KC and FLS in psoriatic disease.

Methods

- Fibroblast Like Synoviocytes from ten Psoriatic arthritis patients and primary human keratinocytes were cultured as per our standardized protocol (Cytokine.2012;60:38-42) with rIL-9 (10ng/ml) and rIL-22 (100ng/ml).
- Proliferation was performed by MTT assay and CFSE dilution assay. IL-6, IL-8 and MMP3 levels were determined by ELISA. Immunoblot studies were done for JAK1/pJAK1, STAT1/pSTAT1, STAT3/pSTAT3.
- Further, to determine the molecular mechanisms of JAK inhibitors(JAKi), these experiments were done in the presence or absence of specific JAKi.

Results

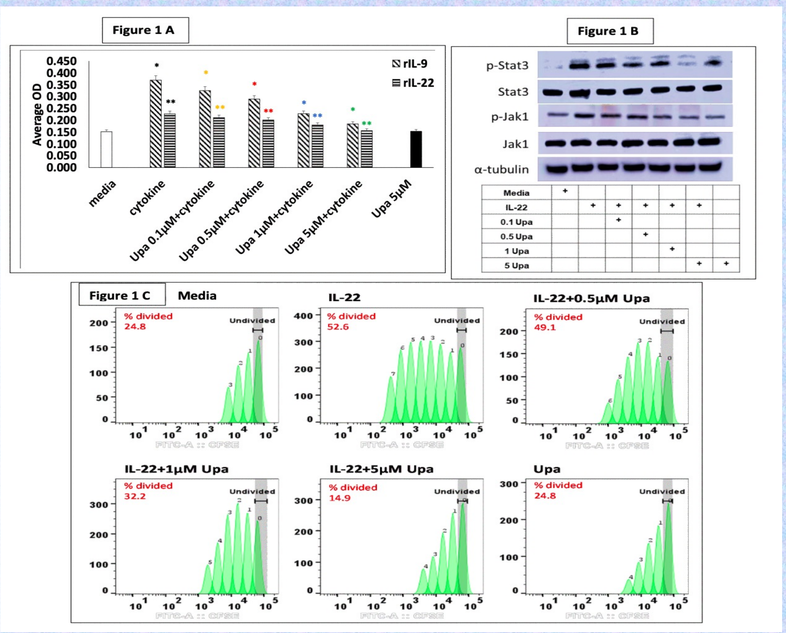

Figure 1A demonstrates results of MTT assay that rIL-9/rIL-22 induced proliferation of FLS is regulated by JAK-1 because rIL-9/rIL-22 induced proliferation could be inhibited by blocking with a specific JAK-1 inhibitor (upa- upadacitinib) [**p values of the FLS MTT assay**:✶/✶✶ proliferation was compared with media (p<.01); inhibitory effect of different doses of upadacitinib was compared with rIL9/rIL22 ✶/✶✶ (p<.05); ✶/✶✶ (p<.05); ✶/✶✶ (p<.01); ✶/✶✶ (p<.001)]. **Figure-1B:** immunoblot study of cultured keratinocytes demonstrates that rIL-22 induced phosphorylation of JAK-1 and STAT-3 were inhibited by specific JAK-1 inhibitor (upadacitinib). **Figure-1C** shows that rIL-22 induced keratinocyte proliferation with increased number cell generations as well as more number of cells in each generation (CFSE dilution assay). These KC proliferation were inhibited by upadacitinib. It is worth noting upadacitinib is effective at <**1μM dose.**

Results

- In cultured FLS and KC, we observed that compared to the media, rIL-22 and rIL- 9 induced increased phosphorylation of JAK1/TYK2 and JAK1/JAK3 respectively (p<0.01).
- rIL9/rIL22 also phosphorylated STAT3/RORγt.
- We observed that the critical events in psoriasis/PsA such as (i) proliferation of KC/FLS (ii) IL-6, IL-8 and MMP-3 production by FLS are induced by rIL-22 and rIL-9 and those were regulated by JAK1/TYK2 and JAK-1,3 respectively.
- Further pan-JAK inhibitors and other specific JAK-1 and TYK2 inhibitors blocked these effects significantly (p<0.01). Figure-1A demonstrates that rIL-9r/rIL-22 induced proliferation of FLS could be inhibited by blocking JAK-1 with JAKi (Upadacitinib) by MTT assay; Figure-1B shows the regulatory role of rIL-22 on JAK/STAT phosphorylation in keratinocytes and Figure-1C shows the effect of JAK-1 on keratinocyte proliferation.

Conclusion

JAK/STAT signaling system of T cells and its regulatory role in the pathogenesis of psoriatic disease is well established. This study provides a novel insight about the role for JAK-STAT signaling on pannus/plaque formation and demonstrates novel mechanisms for JAK inhibitors in psoriatic disease

References

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