AB0022  IL-23 POSITIVE NEUTROPHIL IS THE MAIN CELL SUBTYPE IDENTIFIED FROM SYNOVIAL FLUID OF PSORIATIC ARTHRITIS WITH THE POTENTIATION OF NEUTROPHIL EXTRACELLULAR TRAPS FORMATION

Keywords: Psoriatic arthritis

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Background: Psoriatic arthritis (PsA) is a systemic autoimmune disease. Interleukin-23 (IL-23) and IL-17 are two major cytokines involving in the pathogenesis of psoriasis (PsO) and PsA. The origin and maintenance of IL-23 is the stakeholder of disease progression in PsA and should be carefully evaluated.

Objectives: To validate the role of neutrophils in maintaining the inflammation in PsA joint.

Methods: Synovial fluid from PsA was obtained from swelling knee joints, and the immune cells were classified by flowcytometry. The IL-23 positive cells were evaluated with marker of CD66b, CD14, and CD16. Neutrophils from healthy donor were collected and treated with interferon-α (IFN-α) and proteoglycan (PGN) for mimicking the neutrophils activation in PsO/PsA patients. Subsequent evaluation neutrophil extracellular traps (NETs) formation was conducted.

Results: 10 PsA joints fluid was collected. Among active and chronic PsA synovial fluid, neutrophil is the most abundant cell type identified among IL-23 positive cells (ranging from 65-90%). The t-SNE plot for mapping the repertoire of IL-23 containing cells confirmed the majority cell subtype by CD66b+ positive neutrophils. After stimulating neutrophils from healthy donor with IFN-α and PGN revealed increasing IL-23p19 expression after 16 hours stimulation. The NETs formation is profound and IL-23p19 containing NETs material could be identified.

Conclusion: IL-23 containing CD66b+ neutrophil is the main cell type in synovial fluid of PsA, which might be activated by inflammatory factors and release IL-23 with NETs material to promote type 17 T cell differentiation in joints.

REFERENCES:

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AB0023  GLUCOCORTICOIDS AND TNF INHIBITOR EXERT NOVEL REGULATORY MECHANISM THROUGH LAG-3 MODULATION IN SYNOVIAL CELLS

Keywords: Disease-modifying drugs (DMARDs), TNF, Spondyloarthritis

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Background: Intra-articular (IA) injections of glucocorticoids (GCs) are clinically effective for the treatment of synovial inflammation in patients with persistent arthritides. TNF inhibitors (TNFi) utilization for IA injections was also reported with a varying efficacy [1, 2]. The mechanism of action of GCs and TNFi on regulatory mediators in the synovium is not fully understood. LAG-3 is a regulatory molecule that promotes suppressive immune response. LAG-3 expression could be regulated through multiple mechanisms, including modulation by different drugs.

Objectives: To investigate whether the regulatory mechanism of GCs and other anti-rheumatic drugs is mediated through change in LAG-3 expression in synovial cells derived from psoriatic arthritis (PsA) patients ex-vivo.

Methods: Synovial fluid mononuclear cells (SFMCs) derived from nine PsA patients were cultured ex-vivo with GCs ((betamethasone (BET) and methylprednisolone acetate (MPA)), Methotrextate (MTX), TNF, IL-17A, IL-12/23, and IL-1 receptor inhibitors namely: (Infliximab (IFX), Secukinumab, (SEC), Ustekinumab, (UST), and Anakinra (ANX), respectively) or with medium alone. After 5 incubation days, %CD45+LAG-3+ cell numbers in culture was accompanied with LAG-3 modulation. LAG-3 distribution on CD45, CD3 and CD14 cells was analyzed by flow cytometry (FCM).

Results: SFMCs treated with GCs showed a significant increase in %CD45+LAG-3+ cells (BET 1µg/ml, 6.8±1.3; BET 10µg/ml, 7.1±1.4; MPA 1µg/
ml, 6.7±1.3; and MPA 10µg/ml, 9.4±2.0, p<0.002, respectively), while IFX showed only a small increase of these cells' population (2.0±0.3, p<0.08) compared to the medium (1.0±0.3). Other treatments, including SEC, UST, ANK and MTX had no effect on %CD45+LAG-3+ cells compared to the medium (1.0±0.3). Other treatments, including SEC, UST, ANK and MTX had no effect compared to the medium (49±4.8X10^5/well and 51±5.1X10^5/well, respectively) (Figure 1A). After 5 days in culture, GCs (MPA) and IFX reduced SFMCs cell counts but this change was statistically significant only for GCs (21±5.1X10^5/well, p<0.01 and 41±5.1X10^5/well, respectively), while MTX had no effect compared to the medium (49±4.8X10^5/well and 51±5.1X10^5/well, respectively) (Figure 1A). In this culture setting, GCs significantly increased the %LAG-3+CD14+ cells (12.8±2.1, p<0.001) compared to the medium (0.9±0.4) but not the %LAG-3+CD3+ cells (11.5±1.7, p<0.005) compared to the medium (3.9±1.7) and MTX had no effect (9.4±3.5, p=0.08) compared to the medium (9.0±3.0) (Figure 1B). In this culture setting, GCs increased %LAG-3+CD14+ cells (12.8±2.1, p<0.001) compared to the medium (0.9±0.4) but not the %LAG-3+CD3+ cells (Figure 1C and D).

**Figure 1.** GCs and to a lesser extent infliximab up-regulate LAG-3 in SFMCs (A) PsA patients (n=9) SFMCs were cultured with BET, MPA, IFX, SEC, UST, ANK and MTX and analyzed by FACS for %CD45+LAG-3+ cells (B) SFMCs cell count analysis was performed after 5 days in culture with: MPA, IFX, MTX or medium (C) Cultures were then analyzed for %CD3+LAG-3+ and %CD14+LAG-3+ cells by FACS (D) Representative PsA patient SFMCs FACS plots showing CD14 and LAG-3 expression after 5 days in culture with medium or the drugs indicated. Positive staining is presented in each plot right upper quadrants with the % indicated, *p<0.01, **p<0.002, ***p<0.0001.

**Conclusion:** Our data shows that GCs immunosuppressive activity is mediated through LAG-3 up-regulation in SFMCs. Within the drugs tested, this activity was exclusively mediated by GCs and to a lesser extent by a TNF inhibitor. GCs reduced the SFMCs cell numbers and concomitantly up-regulated LAG-3 expressing cells, mainly in monocytes. This emphasizes that monocytes seem to be the main mediators of the GCs immunosuppressive effect.

**REFERENCES:**


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**Figure 1.**