The PARIS Study - Immune cell profile in patient with Psoriatic Arthritis

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INTRODUCTION

Psoriatic Arthritis (PsA) is chronic immune-mediated disease characterized by peripheral and spinal arthritis associated with psoriasis.
Cells of innate and adaptive immune systems are playing important role in the pathogenesis of PsA and contribute to inflammation of the joints. (Figure 1). Monocytes and B and T cells are Peripheral Blood Mononuclear Cells (PBMCs) can be isolated from blood samples of PsA patients for detailed analysis.

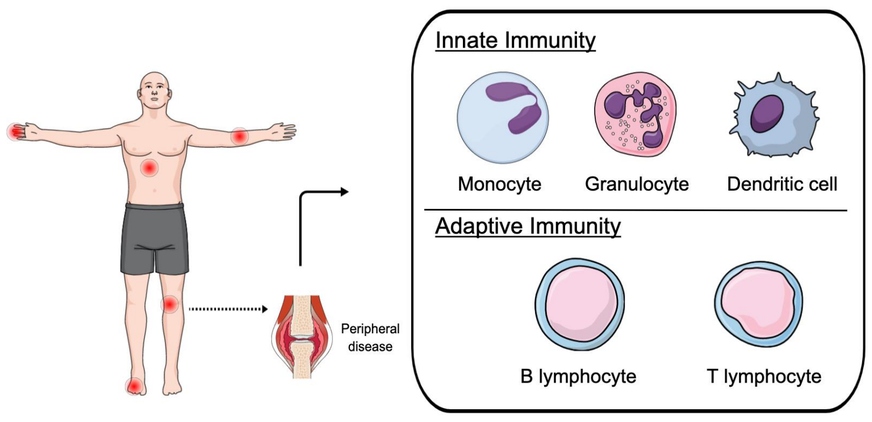


Figure 1: Patients with PsA can be diagnosed with axial and peripheral arthritis. Immune cells such as monocytes/macrophages, granulocytes, dendritic cells, and B and T cells infiltrate the damaged joint and contribute to inflammation leading to pain, stiffness and swelling.

Biologic treatments are designed to target some of these immune cells and the pro-inflammatory cytokines they secrete. First-line biologic treatments (TNF inhibitors) target the pro-inflammatory cytokine TNFα secreted by T cells, and T cells themselves consist of numerous subsets of lymphocytes. Each subset of T cell secretes a different type of pro-inflammatory cytokines, and analysing the immune cell profile of an individual may predict if the individual will respond or not to specific biologic treatment.

METHODS

Healthy donors and patients with PsA who have not yet started TNF inhibitor treatment were recruited under informed consent, and 20ml of blood were collected. PBMCs were isolated from whole blood using density gradient centrifugation and frozen in liquid nitrogen for future experiments.

The immune cell profile of healthy individuals and patients with PsA are compared using a Hematology analyzer ABX Micros ES 60 (Horiba) (Figure 2A) for the healthy donors, and clinical data from Electronic Patients Records (EPR) for the PsA patients. Flow cytometry (Figure 2B) is also used (with a 7 antibodies panel) to perform a more detailed immunophenotyping of T helper (Th) cells, more particularly Th1, Th2 and Th17 cells.

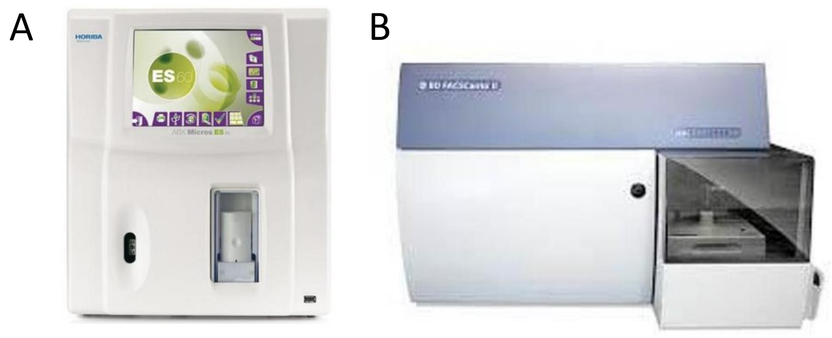
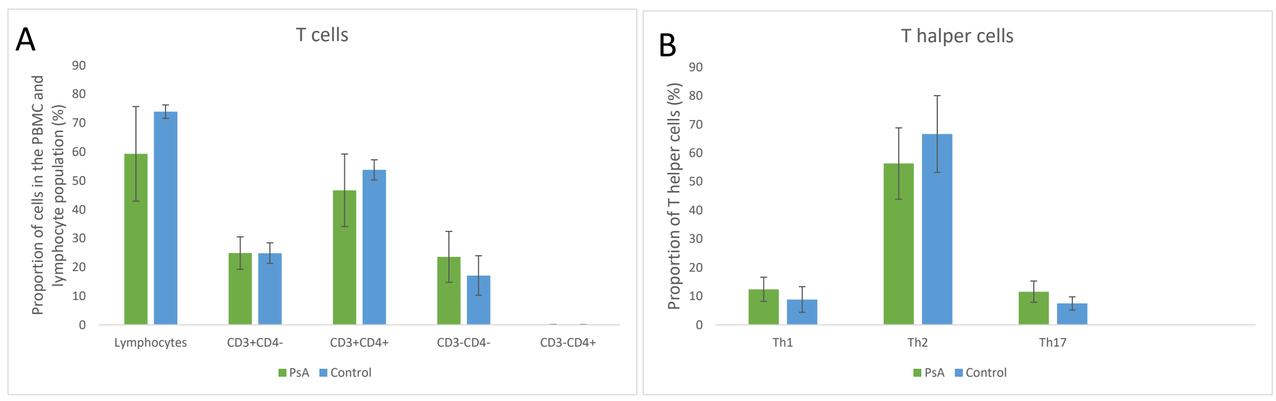


Figure 2: Instruments used to perform the immune cell profile. A) The Hematology analyzer ABX Micros ES 60 (Horiba) was used to know the number of monocytes and granulocytes of healthy donors; B) The flow cytometer BD FACSCanto II (BD) was used to perform the T cell phenotyping for both healthy donors and PsA patients

Statistics were performed using SPSS software after verification of data normality and variance homogeneity.

RESULTS



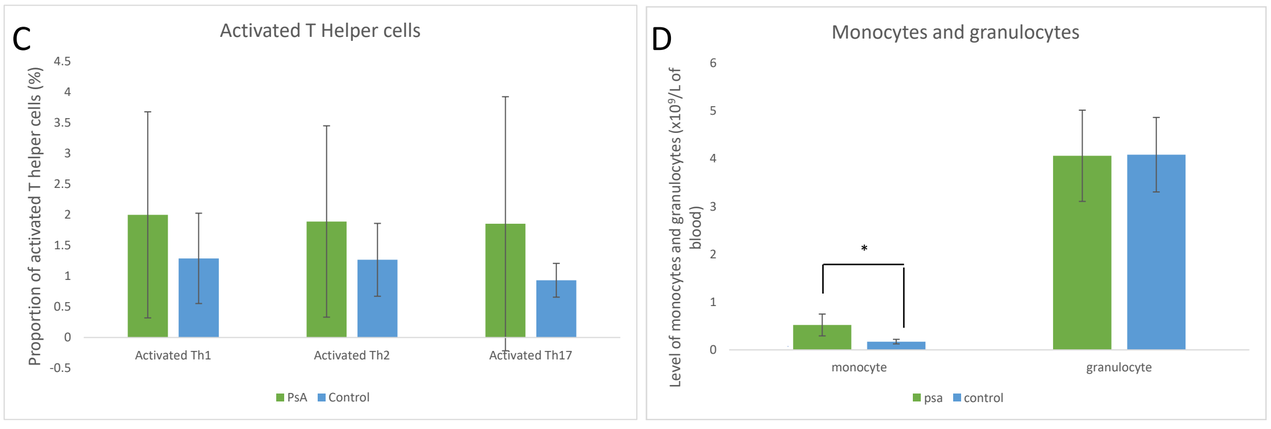


Figure 3: T cell profiling: A) Proportion of total lymphocytes and each lymphocyte subset (CD3+CD4-, CD3+CD4+ = T helper cells, CD3-CD4- and CD3-CD4+); B) Proportion of Th1, Th2 and Th17 cells (3 cell types include in the T helper subset); C) Proportion of activated Th1, Th2 and Th17 cells; and D) Proportion of monocytes and granulocytes.
T cells (healthy donor: n = 3, PsA patient: n = 3); Monocytes and Granulocytes (healthy donor: n = 15, PsA patient: n = 5). *p<0,05, **p<0,01, ***p<0,001, mean ± SD.

We performed an immune cell profiling of healthy donors and patients with PsA, from isolated PBMCs:

- T cells represent 59.2% of PsA patients' PBMCs and 68.1% of healthy donors' PBMCs. We used the surface markers CD3 (T cell marker) and CD4 (T helper marker) to isolate different subsets of T cells. Each subset is a proportion of total lymphocytes.
  - $CD3^+CD4^+$ = T helper cells (PsA = 46.68% and control = 53.77%)
  - $CD3^+CD4^-$ = Mainly cytotoxic T cells (PsA = 24.9% and control = 24.88%)
  - $CD3^-CD4^+$ = Innate-like T cells (PsA = 0.13% and control = 0.12%)
  - $CD3^-CD4^-$ = Include B and NK cells (PsA = 23.58 and control = 17.13)
- T helper cells were analysed using the surface markers CXCR3 (Th1 marker) and CCR6 (Th17 marker). Each subset is a proportion of total lymphocytes.
  - $CXCR3^+CCR6^-$ = Th1 cells (PsA = 12.42 and control = 8.85)
  - $CXCR3^-CCR6^-$ = Th2 cells (PsA = 56.33 and control = 66.63)
  - $CXCR3^-CCR6^+$ = Th17 cells (PsA = 11.59 and control = 7.48)
- Activated T helper were analysed using the surface markers HLA-DR and CD38 (activation markers). Each subset is a proportion of total lymphocytes.
  - $HLA\text{-}DR^+CD38^+$ = Activated Th1 (PsA = 1.99 and control = 1.29)
  - $HLA\text{-}DR^+CD38^+$ = Activated Th2 (PsA = 1.89 and control = 1.27)
  - $HLA\text{-}DR^+CD38^+$ = Activated Th17 (PsA = 1.85 and control = 0.93)

The T cell analysis didn't give any significant results. The proportion of each T cell subset was equivalent in PsA patients and healthy donors, but SEM was high in some case (Th17 and activated Th17).

- Level of monocytes was significantly higher in PsA patients ($0.52x10^9$/L) than in healthy donors ($0.17x10^9$/L) with a p-value = 0.027. Level of granulocytes were equivalent (PsA = $4.06x10^9$ and control = $4.03x10^9$).

DISCUSSION

→The immune profile is equivalent to the profiles we can find in the literature (1). However, the level of innate-like T cells is lower than expected. They are increased in rheumatoid Arthritis (RA) and are a source of TNFα (2), we were expecting equivalent levels in PsA.

→Level of monocytes is higher in PsA patients, they play an important role in inflammation development and secrete pro-inflammatory cytokines such as TNF, IL-6 and IL-1β (3).

→The level of activated Th cells is low, this is what Miyagawa and colleagues (4) show using the same antibodies panel and the same gating strategy. To confirm that we have a normal activation level, we will repeat the flow cytometry experiment with fresh blood to know if freezing has an impact on T cell activation state.

→The level of Th17 cells is higher than the level of Th1 cells in PsA patients (not significative). A high level of Th17 cells might indicate that patients will better respond to anti-IL17 therapy.

CONCLUSION

The aim of the PARIS study (Psoriatic Arthritis – Resistance to TNF Inhibitor Study) is to analyse the blood of patients with PsA, before and after treatment with TNF inhibitor, to find potential biomarkers able to predict if the patient will respond or not to treatment. One of our approaches is to study the immune cell profile. We can see that it is equivalent to healthy donors, except for monocytes which level is higher in PsA patients.

Our results were only from baseline samples (before treatment) and will be compared to 3-months and 6-months follow-up samples. Proportion of cells will also be correlated to specific potential biomarkers (proteins in plasma and serum of patients).

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