Genetic and Functional Associations with Decreased Anti-Inflammatory Tumor Necrosis Factor Alpha Induced Protein 3 in Macrophages from Subjects with Axial Spondyloarthritis

Human Peripheral Blood

CASE STUDY

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Introduction

Tumor necrosis factor alpha-induced protein 3 (TNFAIP3) is an anti-inflammatory protein implicated in multiple autoimmune and rheumatologic conditions. TNFAIP3 is important in controlling systemic inflammation and may play a role in the pathogenesis of spondyloarthritis. It is therefore essential to understand how the levels of TNFAIP3 regulate blood-derived macrophages to alter cytokine production in this complex immunological disease. Lymphocyte Separation Media (LSM) from MP Bio was used to isolate human monocytes from peripheral blood samples. Blood-derived macrophages were analyzed to elucidate the immune signaling and genetic variation regulating TNFAIP3 expression in AxSpA subjects.

Overview

- **Keyword:** Tumor necrosis factor alpha-induced protein 3, spondyloarthritis, cytokine production, macrophages, genetic variants, spondyloarthritis pathogenesis, TNF-α
- **Aim of the study:** To study differential tumor necrosis factor alpha-induced protein 3 (TNFAIP3) regulation in blood-derived macrophages
- **Application:** Human monocytes purification
- **Sample name:** Human peripheral blood
- **Material:** Lymphocyte Separation Medium (LSM™, SKU 0850494) from MP Bio

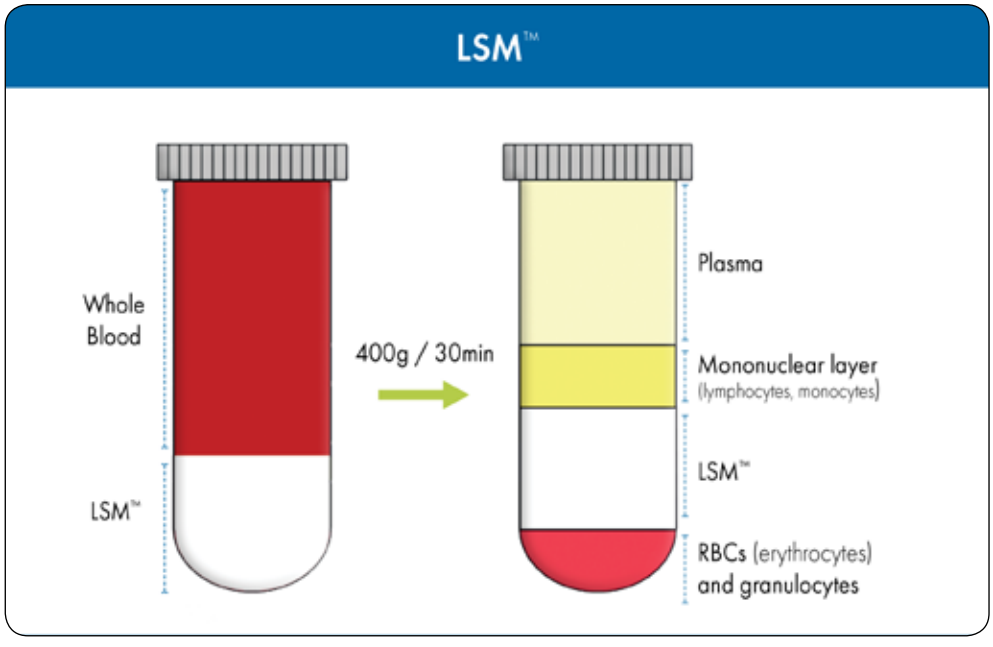

Figure 1.
Isolation of mononuclear cells from whole blood using LSM™ density separation medium.

CASE STUDY

Protocol and Parameters

1. 40mL of blood was collected in yellow top acid citrate dextrose tubes.
2. Blood was centrifuged for 10min at 4°C and plasma removed.
3. An equal amount of PBS was added to the cell layer.
4. The mixture was transferred to polystyrene tubes containing LSM™ Lymphocyte Separation Medium.
5. Tubes were centrifuged 15min at room temperature.
6. PBMCs were collected with a disposable Pasteur pipet washed twice with buffer [PBS with 2% fetal bovine serum (FBS) and 2mM EDTA].
7. Proceed with the human monocyte isolation protocol.

Conclusion

This study demonstrated that monocytes isolated with LSM™ retain their differentiation capabilities and can produce functional M-CSF-derived macrophages for use in immunological studies.

The resulting macrophages from AxSpA patients were found to have decreased levels of the anti-inflammatory protein TNFAIP3, indicating that it is a potential contributor to the cytokine dysregulation observed in this disease.

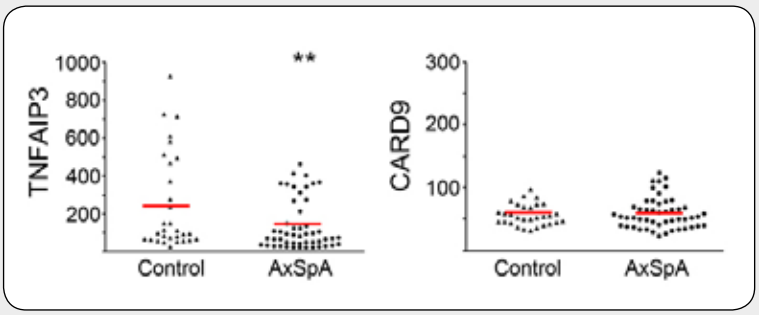

Figure 2.
MP Bio LSM was used to isolate monocytes from AxSpA subjects and healthy controls. ELISA was used to assess TNFAIP3 protein levels from peripheral blood-derived macrophage lysates.

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