Developing a high parameter flow cytometry panel to identify specific immune cell phenotypes key to the pathogenesis of Systemic Juvenile Idiopathic Arthritis

Systemic Juvenile Idiopathic Arthritis (SJIA) is a rare and severe subtype of arthritis that affects 10% to 15% of children with juvenile idiopathic arthritis. SJIA is often more severe and challenging to treat than other types of juvenile idiopathic arthritis as its origin is unknown and no specific test to diagnose disease exists.

The aim of this study was to develop a full spectral cytometry immunophenotyping panel, using known cell markers for patients with SJIA, to determine the immunophenotypic profiles of cells found in patients with SJIA and MAS. Such an immunophenotyping panel will tend to later discover the pathogenesis of these diseases and creation of a diagnostic or prognostic test. To create the panel, we used peripheral blood mononuclear cells (PBMCs) that were stained with various immune cell markers and specific fluorochromes and analyzed using full spectral cytometry. The panel was dependent upon the amount of the corresponding cell marker present within the sample. By slowly adding new markers and specific fluorochromes to the panel, we were able to control overlap in fluorescence emissions limiting interference that may arise. The future goal of this study is to optimize the immunophenotyping panel, which would then allow for analysis of SJIA and MAS patient samples.

Abstract

Systemic Juvenile Idiopathic Arthritis (SJIA) is a rare and serious subtype of arthritis that affects 10% to 15% of children with juvenile idiopathic arthritis. This disease is a more serious subtype of juvenile idiopathic arthritis with a fairly higher mortality rate. Systemic means it causes inflammation throughout the whole body. Along with inflammation other symptoms include fever, rash, and hepatosplenomegaly. For SJIA, 60% of children with this pathology go on to develop a deadly phenotype known as macrophage activation syndrome, which is caused by excessive activation and proliferation of T cells and well-differentiated, non-neoplastic macrophages. This is a severe complication of SJIA and should be treated as a life-threatening emergency, with 10% to 20% of cases being fatal. The pathogenesis of SJIA is unknown, which results in no specific test to diagnose the disease. Various immune cell types, such as B cells, T cells, and monocytes/macrophages, play a significant role in SJIA, but it is not known which subtypes of these cells play a specific role in the disease.

To address whether specific subpopulations of immune cells are key in the pathogenesis of SJIA, this project seeks to use a high number of cell surface markers to detect the presence of all known immune subtypes. In this way, we can analyze samples from patients with SJIA and MAS, which would help identify new immune cell populations that occur in patients with SJIA and MAS and identify new immune cell populations potentially driving the disease.

Introduction

Methods

For this study, we used 28-color full spectral analysis panels able to characterize many immune cell populations, or different positive and negative populations.

Figure 1: Progression of colors chosen for 28-color panel. While finalizing the 28-color panel, we compared our antibodies with others to ensure that our final panel is optimal.

Figure 2: CCR7 BUV737 Titration. Anti-human BUV737 (100:100:10:1:0.1:0) was used to determine the appropriate titer, as it provides the best signal-to-noise ratio between positive and negative populations.

Figure 3: Full parameter gating strategy used to identify different classifications of monocytes. This was collected from the 8-color stained panel tube.

Figure 4: Identification of different classifications of monocytes. This was collected from the 8-color stained panel tube.

Figure 5: Examples of identification of spread as described in Panel Design. Data was collected from a single stained tube (upper right) and the same tube after unmixing (lower left). These showed the prevalent spread in various degrees.

Gating Strategies

From this, we have also developed a strong gating strategy that allows for the identification of different classifications of monocytes including: classical, intermediate, and nonclassical monocytes.

High Parameter Panel Design

When designing a panel, it is very important to minimize the spread that occurs between each fluorochrome. Spread occurs when a fluorochrome’s emission is interfering with another fluorochrome or spilling over into another fluorochrome emission spectrum and negatively impacting gating that fluorochrome’s emission pattern to widen or spread upon detection. Spread occurs after unmixing has been applied and can be detected by comparing the signal of one fluorochrome’s single-stained (SS) tube to that in the multi-stained (MS) tube and determining how the data remains whether the fluorochrome is being measured by itself or in the presence of other fluorochromes.

Fluorochrome Spread after Unmixing

- A small panel was created that minimized the spread and interaction between different fluorochromes.
- With limited data, we were able to develop a gating strategy that identifies T cells, B cells, NK cells, classical monocytes, intermediate monocytes, and nonclassical monocytes.

Conclusion and Future Implications

As of now, the panel is still in progress, working towards adding more cell markers that can help identify the different immunophenotypic profiles of subsets of these major immune cell phenotypes. The end goal is to create a final full spectra flow cytometry panel able to characterize many different cell subsets and level of activation/toxicity production. With this, we can analyze samples from patients with SJIA and MAS, which would be compared to controls. With this information, we hope to potentially identify new immune cell populations that occur in patients with SJIA and MAS, which will in time help develop new therapeutic and treatments for patients.

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