Advanced Flow Cytometry Solutions for Drug Development: *A Focus on B Cells and T Cells*

If you have trained in the field of immunology, it may not be uncommon to emerge with a self-characterisation as either a "B cell immunologist" or a "T cell immunologist." Historically, this distinction arose from years of specialisation and the degrees of separation between B cell and T cell biology research. Approaches to therapeutic design have often mirrored that, with either a B cell or T cell-specific approach.

However, as a broader understanding of immune networks and coordinated immune responses has emerged, this line is increasingly blurred. Advanced technologies like flow cytometry enable deep interrogation of both B and T cell populations. As a result, for those working in drug development and clinical research, it is more important than ever to have a comprehensive understanding of both B and T cell biology and a knowledge of how to leverage advanced technology to distinguish and resolve these populations.

In this article, we consider how flow cytometry supports an advanced understanding of B and T cell phenotype and function.

B Cells, At The Forefront of Immunology

B cells have a diverse and multifunctional role in immune response. While traditionally associated with antibody production, multifunctional B cells are also important cytokine producers and serve as professional antigen-presenting cells.

In humans, B cell genesis begins in the bone marrow, where hematopoietic stem cells (HSC) differentiate first into immature naive B cells and then into transitional B cells. Exiting the bone marrow, transitional B cells move into the periphery and develop into mature naive B cells. Following recognition of a cognate antigen, the mature B cells are activated, proliferate and further differentiate into memory B cells, short-lived plasmablasts, or long-lived plasma cells. These cells can rapidly activate, proliferate and produce antibodies and cytokines. In healthy individuals, the activation is induced by foreign antigens from infection or protective vaccination. The subsequent production of antibodies protects against pathogens and are key for vaccine-mediated immune protection.

B Cells In Autoimmunity

While B cells are essential for maintaining immunity, they can also be dysregulated and contribute to pathology and disease. In autoimmune diseases, autoreactive B cells are a critical driver in the complex immune environment found in rheumatoid arthritis (RA), multiple sclerosis (MS), systemic lupus erythematosus (SLE), inflammatory bowel disease (IBD), type 1 Diabetes and others. A dysregulated B cell response can be characterised by a break in tolerance or molecular mimicry response that results in an attack of "self." Indeed, in a variety of autoimmune diseases, the pathological impact of tissue-targeting autoantibodies is well documented.

Our understanding of autoimmune diseases is rapidly expanding, yet effective therapies remain limited. Effective treatments for B cell-driven diseases have included B cell targeted immunotherapies. One such approach has been monoclonal antibody therapies, which are safe and well-tolerated. The production of monoclonal antibody therapies in large-scale manufacturing results in a low risk of product variability. However, the relatively short therapeutic half-life requires multiple administrations, and limited overall B cell depletion has driven the need to explore therapeutic alternatives (Li, 2024).

Another approach to target B cells is the use of cellular therapies. Cell-based immunotherapies like Chimeric Antigen Receptor (CAR)-T cell therapy have been transforming the treatment of hematologic malignancies for more than a decade. Several groundbreaking studies[1] have led to a growing interest in applying these cell-based therapies to other T and B cell-mediated diseases. One emerging application of CARs is in the treatment of autoimmune disease. With recent successes in B cell malignancies, there is a growing interest in understanding how B cell targeted CAR-T cell therapy can be successfully applied to autoimmune disease. Early studies have shown promise in a variety of B cell-driven diseases such as SLE, Sjogren's and myasthenia gravis, among others. The number of clinical studies evaluating cellular therapies in autoimmune disease reflects the increased interest within the industry. According to one publication, by mid-2024, there were more than 65 ongoing clinical trials evaluating anti-CD19 and anti-BCMA CAR-T therapies in autoimmune disease. Additional trials are ongoing to assess anti-CD7 CAR-Ts, CAR-Treg and novel Chimeric Autoantigen Receptor (CAAR) T cells. Collectively, these trials target more than 25 different autoimmune diseases (Abigail Cheever, 2024).

While CAR-T therapies also have unique challenges, there is a critical need to understand the impact of CAR-T therapy on the B cell subsets in this emerging space.

T Cells

Given the emerging interest in applying CAR-T-based therapies for treating autoimmune disease and the recognition that these diseases are driven by both T and B cells, there is a need for a deep understanding of T cell subsets. Let's take a deeper look at T cells and highlight the importance of reliable cell phenotyping by high-dimensional spectral flow cytometry.

T Cells and Autoimmunity

T cells are critical mediators of adaptive immunity in a regulated immune system. Known for their central role in protection from pathogens, activated T cells have a wide array of activities, from cytokine production to cytolytic function. When properly engaged, T cells are responsible for resolving viral infections, providing effective memory against future infection, supporting B cell activation and antibody production, or, in the case of regulatory-T cells, maintaining immune tolerance.

Conversely, dysregulated T cells have been shown to contribute to the development and progression of autoimmune diseases. Key subsets such as regulatory T cells (Tregs), T helper 1 (Th1), and T helper 17 (Th17) cells are pivotal in maintaining immune homeostasis or, conversely, driving autoimmune pathology. As just one example of T cell involvement in autoimmunity, activating Th2, Th17 and follicular helper T cells is known to support B cell activation and subsequent autoantibody production in Systemic lupus erythematosus. On a broader scale, mediators of disease can be widely attributed to the complex interactions of autoreactive T cells, autoreactive B cells and autoantibodies, which are inappropriately targeting "self" antigens. Self-targeting results in a difficult-to-break cycle of chronic inflammation and cell activation, leading to long-term tissue and organ damage.

Although each autoimmune indication is relatively rare, collectively this category impacts as many as 1 out of every 10 people in the US and more than 350 million people globally. Autoimmune diseases have a significant impact on individual quality of life and can have a social and economic burden for those seeking effective treatment. Given T cells' complex role in autoimmune disease, understanding T cell dynamics is crucial for developing targeted therapies, including biologics, small molecules and advanced cell therapies.

Development of therapies targeting T cells requires characterisation of potentially rare and unique cell subsets. Technologies like spectral flow cytometry enable this type of complete characterisation. High-dimensional analysis yields deeper and more precise insights into T-cell phenotypes and functions that can finally shed light on complex mechanisms of action and fine-tuned immune responses. This capability enhances the understanding of T cell biology and can support the development of effective therapies for T cell-driven autoimmune diseases.

A Turnkey Solution: Accelerating Science with a Validated Flow Cytometry Solution

Given the need for high-dimensional T cell analysis, we recently completed validation of a 21-colour spectral flow cytometry method that is entirely focused on discrimination of T cell subpopulations in human PBMC samples. With a comprehensive phenotyping goal, this validated spectral flow cytometry method is designed to enable evaluation of T cell subpopulations, such as:

- T helper cells
- Cytotoxic T cells
- Naive and memory subsets, including central and effector memory T cells
- Activated T cells
- Follicular Helper T cells
- Regulatory T cells
- Th1, Th2 and Th17 cells
- Proliferating and PD-1+ exhausted cells

Off-the-shelf and custom methods should be rigorously validated based on the context of use. The comprehensive T cell panel is no exception. Repeatability, inter-assay, inter-analyst, inter-instrument precision and post-fixation sample stability were all evaluated in a fit-for-purpose validation. More than 150 parameters can be determined from the validated and comprehensive T cell panel. This validated method offers an efficient and scalable approach to evaluating samples with reduced costs and timelines when used as an off-the-shelf offering. When used as a starting backbone, this method offers cost and time value to sponsors by allowing efficient customisation, enabling programmes to move quickly forward.

Comprehensive T and B Cell Phenotyping by Flow Cytometry

Given the dual role that T and B cells play in health and disease, advanced, scalable research tools like high-dimensional flow cytometry are essential. A well-developed flow cytometry phenotyping panel enables precise characterisation of T and B cell subsets and functional state in diverse disease contexts. Phenotyping panels by flow can help support the needs of researchers in the pharmaceutical and biotech industries working on innovative therapies in autoimmunity, inflammation and oncology.

Final Thoughts

Precision is a cornerstone of any reliable flow cytometry assay. Recognising the importance and prevalence of T and B cell involvement in disease, there is a pressing need for advanced and scalable research tools to properly interrogate cell phenotypes, modulation and function in both healthy and diseased individuals. Thoughtfully designed methods can transform our understanding of T and B cells in health and disease.

Understanding both B and T cells through specialised technologies enables the identification of novel biomarkers, better patient stratification and more precise evaluation of therapeutic efficacy. The evolution of technology like spectral flow cytometry opens new paths for understanding the complexities of the immune response. By leveraging technologies like high-dimensional spectral flow cytometry, scientists in all stages of research, whether in discovery, pre-clinical or clinical stage programmes, can gain deeper insights into the phenotype and function of immune cells.

REFERENCES

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