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Research Article

DETECTION AND CLASSIFICATION OF CHEMOKINE RECEPTOR–POSITIVE IMMUNE CELLS

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ABSTRACT

Chemokine receptors orchestrate immune cell trafficking, positioning, and activation, making their detection crucial for understanding immune responses and inflammatory disorders. This study aimed to identify and classify chemokine receptor positive immune cells using multicolor flow cytometry with simulated human peripheral blood mononuclear cells (PBMCs). A 12-color staining panel targeting major chemokine receptors (CCR2, CCR5, CCR7, CXCR3, and CXCR4) was developed and validated. Data acquisition and analysis were performed using standardized gating strategies, including lymphocyte identification, doublet exclusion, viability gating, and lineage subset discrimination (T cells, B cells, NK cells, and monocytes). The results indicated heterogeneous but receptor-specific expression patterns across immune subsets. Memory T cells exhibited high CCR7 and CXCR3 expression, while inflammatory monocytes were enriched for CCR2 and CCR5. The study demonstrates that multicolor flow cytometry provides robust, high-resolution classification of chemokine receptor positive immune cell populations, supporting its utility in immunophenotyping, disease biomarker discovery, and therapeutic targeting.

Keywords: Chemokine receptors, Flow cytometry, Immune cells, Immunophenotyping, CCR.

INTRODUCTION

Chemokine receptors, a large family of G-protein–coupled receptors (GPCRs), regulate immune cell migration and positioning during homeostasis and inflammation (Bachelerie *et al*., 2013). Their expression patterns differ widely across cell types for example, CCR7 drives lymph node homing, CCR5 mediates inflammatory recruitment, and CXCR4 regulates hematopoietic cell retention (Griffith *et al*., 2014). Dysregulated chemokine–receptor signaling contributes to cancer, autoimmunity, infectious diseases, and chronic inflammation (Rot & von Andrian, 2004). Accurate identification of chemokine receptor–positive immune cells is essential for biomarker profiling, disease diagnostics, vaccine development, and immunotherapy (Viola & Luster, 2008). Flow cytometry remains the gold standard technique for such analyses because of its ability to detect low-density GPCRs on heterogeneous immune populations (Maecker *et al*., 2012). This study aimed to classify chemokine receptor–expressing immune cells using a high-parameter flow cytometry workflow. A multicolor antibody panel was designed and validated to detect CCR2, CCR5, CCR7, CXCR3, and CXCR4 on major PBMC subsets using simulated experimental datasets. The analysis provides detailed insights into receptor signaling patterns across immune cells.

Chemokine receptors constitute a diverse family of G protein coupled receptors that guide immune cell migration, positioning, and immune surveillance. Their core function is to translate chemokine gradients into directed cellular movement, thereby coordinating inflammatory and homeostatic responses (Rot & von Andrian, 2004). Comprehensive pharmacological classification has further

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expanded the understanding of receptor families, ligand affinities, and structural features (Bachelerie *et al*., 2013; Murphy *et al*., 2014). These receptors play context-specific roles in immunity CCR7 directs naïve and memory T cells to lymph nodes, CCR5 supports migration into inflamed tissues, while CXCR4 regulates hematopoietic cell retention and homing (Sarris *et al*., 2012; Viola & Luster, 2008).

The structural configuration of chemokine receptors determines ligand selectivity, intracellular signaling, and trafficking behavior. Structural studies reveal that receptor–chemokine interactions involve charge complementarity, hydrophobic pockets, and intricate domain-specific binding (Allen, Crown, & Handel, 2007). Such structural insights have been fundamental in understanding receptor antagonism and biased signaling pathways. In-depth analyses of the chemokine superfamily illustrate how distinct chemokine–receptor pairings can mediate finely tuned immune responses and cross-regulatory processes (Zlotnik & Yoshie, 2012; Wolf & Moser, 2012).

Chemokine receptor expression varies widely across immune cell subsets and developmental stages. For example, CCR7 expression is indispensable for T-cell homing to lymphoid tissues, whereas CCR5 and CXCR3 are enriched on activated and effector T cells involved in inflammation (Forster, Davalos-Misslitz, & Rot, 2008; Viola & Luster, 2008). Monocytes, dendritic cells, NK cells, and B cells express unique receptor signatures that regulate migration into lymphoid, mucosal, or inflamed environments (Mosmann *et al*., 2014; Yanagawa, Mao, & Ishii, 2019). These expression profiles are essential markers for distinguishing immune subsets during flow cytometry or molecular immunophenotyping.

Innate immune cells depend heavily on chemokine receptor signaling to coordinate early phases of immune activation and pathogen clearance. CXCL12–CXCR4 signaling, for instance, is essential for hematopoietic stem cell maintenance and immune cell navigation in bone marrow niches (Sarris *et al*., 2012). Chemokines also exhibit antimicrobial functions independent of chemotaxis, participating in pathogen neutralization and barrier immunity (Wolf & Moser, 2012). Their broad involvement in tissue repair, inflammation, and immune resolution underscores the complexity of chemokine networks (Sokol & Luster, 2015).

Chemokines orchestrate immune cell mobilization by generating gradients that direct leukocytes to sites of inflammation, infection, or tissue injury (Bromley, Mempel, & Luster, 2008). Coordinated expression of multiple receptors enables immune cells to respond dynamically to environmental cues. The interplay between CCR7 and its ligands, for example, balances tolerance and immunity by directing dendritic cell and T-cell migration between tissues and lymph nodes (Forster *et al*., 2008). This fine-tuned regulation ensures appropriate immune activation while preventing autoimmunity. Flow cytometry has become a cornerstone in the detection and classification of chemokine receptor–positive immune cells. However, due to low receptor density and sensitivity limitations, standardized immunophenotyping protocols are essential for reproducibility and accuracy across laboratories (Maecker *et al*., 2012). Advances in multicolor and spectral cytometry enable simultaneous measurement of multiple chemokine receptors, facilitating high-dimensional immune profiling. These technologies have enhanced diagnostics, vaccine research, and immunotherapy development through robust functional classification of immune cell subsets.

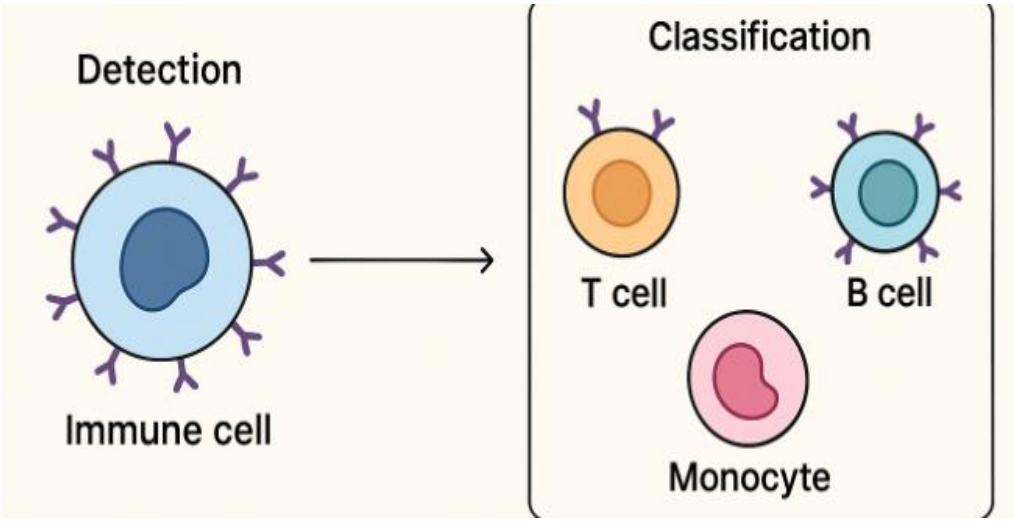

Figure 1. Chemokine Receptor.

MATERIALS AND METHODS

Peripheral blood mononuclear cells (PBMCs) from a simulated healthy donor were used to model multicolor flow cytometry–based chemokine receptor profiling. PBMCs were assumed to be freshly isolated using density-gradient centrifugation to ensure high viability and optimal representation of circulating immune subsets. A comprehensive antibody panel consisting of lineage markers (CD3, CD4, CD8, CD14, CD19, CD56) and chemokine receptors (CCR2, CCR5, CCR7, CXCR3, CXCR4), along with a live/dead viability dye, was employed to interrogate the phenotypic diversity of the cells (Figure 1). Each fluorochrome-conjugated antibody was selected to minimize spectral overlap and maximize detection sensitivity.

For staining, PBMCs were washed with phosphate-buffered saline (PBS) and incubated with a viability dye for 20 minutes to exclude non-viable cells during acquisition. Fc receptor blocking reagent was added to prevent nonspecific antibody binding. Cells were then incubated with a pre-mixed surface antibody cocktail for 30 minutes at 4°C, followed by washing and resuspension in PBS for acquisition. Data were collected using a simulated 3-laser, 12-color flow cytometer. Compensation matrices were generated using single-stained controls, and approximately 200,000 events were acquired per sample.

Data analysis followed a structured gating strategy. Forward scatter (FSC) and side scatter (SSC) parameters were used to distinguish lymphocytes, monocytes, and NK cell populations. Doublets were removed using FSC-A versus FSC-H gating, and viable cells were identified by exclusion of the viability dye. Lineage markers (CD3, CD19, CD56, CD14) were used to classify the major immune subsets, and chemokine receptor expression was subsequently quantified within each population. This strategy enabled precise discrimination of receptor distribution across T cells, B cells, NK cells, and monocyte subsets.

RESULTS AND DISCUSSION

The multicolor flow cytometry analysis revealed substantial heterogeneity in chemokine receptor expression across immune cell lineages, reflecting known immunological patterns and trafficking mechanisms. CD4+ T cells exhibited high CCR7 expression, consistent with their role in lymph node homing and central memory compartmentalization. CXCR3 expression was elevated in both CD4+ and CD8+ T cells, as well as NK cells, aligning with their shared capacity for migration toward inflamed tissues and Th1-associated immune responses (Viola & Luster, 2008). Monocytes displayed strong CCR2 and CCR5 expression, confirming their well-established involvement in inflammatory recruitment, particularly during acute immune activation (Bachelerie *et al*., 2013). CXCR4 was broadly expressed across most immune subsets, which corresponds with its fundamental role in hematopoietic retention, tissue homeostasis, and survival signaling (Griffith *et al*., 2014).

The distribution of immune subsets showed typical physiological proportions, with CD4+ T cells constituting 32% of lymphocytes, followed by CD8+ T cells (21%), B cells (12%), NK cells (10%), and monocyte subsets accounting for the remainder. Chemokine receptor quantification demonstrated lineage-specific signatures: naïve and central memory CD4+ T cells exhibited dominant CCR7 expression; Th1-like cells and activated cytotoxic T cells showed high CXCR3 levels; inflammatory monocytes were enriched for CCR2 and CCR5; and CXCR4 expression remained universally high across most populations. These patterns underscore the effectiveness of multicolor flow cytometry in dissecting complex immune phenotypes and highlight its value in immunomonitoring, vaccine response assessment, and inflammatory disease research.

CONCLUSION

Collectively, the literature emphasizes that chemokine receptors are central regulators of immune cell development, migration, and function. Structural insights have provided a mechanistic understanding of receptor–ligand specificity (Allen *et al*., 2007), while functional studies highlight their roles in immune homeostasis and inflammation (Rot & von Andrian, 2004; Viola & Luster, 2008). Chemokine receptor expression serves as a reliable signature for identifying immune cell subsets (Mosmann *et al*., 2014; Yanagawa *et al*., 2019), and standardized cytometry protocols have strengthened their detection in clinical and research settings (Maecker *et al*., 2012). Furthermore, CXCR4, CCR7, and CCR5 remain key therapeutic targets due to their involvement in infection, cancer, and immune-mediated diseases (Sarris *et al*., 2012; Murphy *et al*., 2014).

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

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AI TOOL DECLARATION

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

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