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Chemokine receptor expression on monocytes from healthy individuals

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ABSTRACT

Chronic immune mediated inflammation is characterized by continuous chemokine mediated recruitment and activation of pro-inflammatory cells, monocytes in particular. We believe that an evaluation of the recruitment profile of monocytes during healthy condition is essential for the understanding of cellular response in disease. For this, we have established normal reference values and 95% confidence intervals for receptor expression of 20 chemokine receptors on monocyte subsets; classical ($CD14^+CD16^-$), non-classical ($CD14^+CD16^+$) and $HLA\text{-}DR^{hi}$ monocytes from 20 healthy controls using flow cytometry. We demonstrate significant differences in the chemokine receptor expression profiles and high correlation between fraction of cells and level of expression. This is the first global approach to provide a platform for comparable evaluation of cell recruitment during normal and under inflammatory conditions. This will be useful when exploring chemokine–chemokine receptor interactions, inhibition of chemokine signaling and selective removal of migrating cells, which are new treatment strategies in immune mediated diseases.

1. Introduction

In the search for novel therapeutic interventions growing evidence point towards the importance of chemokine–chemokine receptor interactions due to their ability of directing the transmigration of peripheral circulating cells to the site of inflammation in the pathogenesis of life-long immune mediated inflammatory diseases (IMIDs).

Chemokines are chemotactic cytokines, 8–10 kDa small molecules that act as mediators for an effective adaptive immune response. First by inducing migration of antigen-presenting cells (APC) and lymphocytes into lymph nodes for surveillance and activation upon antigen presentation and second by inducing chemo-attraction, "homing", of activated effector cells to the site of inflammation This migratory apparatus is often organ specific and the chemokines induce chemotaxis by recruiting cells that express the appropriate G-protein-coupled chemokine receptor; thereby accomplishing both tissue- and cell-specific migration [1].

Chronic immune mediated inflammation is characterized by continuous recruitment and activation of immune cells such as monocytes in response to a persistent stimulus. Monocytes constitute 5–10% of the leukocyte population in human peripheral blood and stand for the highest production of pro-inflammatory mediators responsible for fueling the inflammatory process.

It was early suspected that the monocyte population was composed of different subsets and that these should be separated; by size, density or by the expression of CD14 (part of the lipopolysaccharide receptor) and CD16 (low affinity Fcγ-receptor) [2,3]. Accordingly, the monocyte population is regarded to consist of two main subsets; The classical monocytes ($CD14^+CD16^-$), which constitute of 80–90% of circulating blood monocytes; and the non-classical monocytes ($CD14^+CD16^+$) which are normally low in number however increased in patients with inflammation and infectious diseases compared to healthy individuals [4].

In addition, a subset of monocytes that has drawn interest to our research group is the $CD14^+$ $HLA\text{-}DR^{hi}$-expressing monocyte [5]. HLA-DR (MHC class II), is required for antigen presentation to $CD4^+$ T-lymphocytes, providing the link between the innate and the adaptive immune response [6]. We have recently demonstrated that circulating $HLA\text{-}DR^{hi}$ monocytes are elevated in patients with inflammatory bowel disease (IBD) and they display a pro-inflammatory phenotype with high production of TNF-α [5], bringing the $HLA\text{-}DR^{hi}$ monocytes in focus for several IMIDs.

We believe that a comparable evaluation of the migration profile of the three monocyte subsets during healthy conditions is essential for the understanding of monocyte subsets in disease. For this, we have established normal reference values for chemokine receptor expression of 20 chemokine receptors on three monocyte subsets; classical ($CD14^+CD16^-$), non-classical ($CD14^+CD16^+$) and $CD14^+$ $HLA\text{-}DR^{hi}$

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monocytes from 20 healthy controls using multicolor flow cytometry. This will be particularly useful for exploring the pathological role of various aspects of chemokine–chemokine receptor interactions in IMIDs and inhibition of chemokine signaling or selective removal of migrating cells, which are shown to be new treatment strategies [7–9].

2. Material and methods

2.1. Study population

We included 20 healthy individuals, 9 women and 11 men, all healthy blood donors with a mean age of 37 years. The study was approved by the Stockholm Regional Ethics Review Board in Stockholm, Sweden (http://www.epn.se).

2.2. Flow cytometry and statistical data analysis

Two heparin tubes of 9 ml peripheral blood were collected from each healthy control. The red blood cells were lysed and the leukocytes preserved using Fix Buffer (Phosphate Buffer Saline (PBS) citrate with 4% paraformaldehyde) for four minutes at 37 °C followed by treatment with lysing buffer (PBS with 10 mM Tris and 160 mM NH_4Cl, pH 7.5) for 15 min at 37 °C. The cells were washed in PBS with 2% Bovine Growth Serum, and incubated with 10% human serum for 15 min at room temperature and stained with monoclonal antibodies CCR6 PE, CCR4 PerCPCy5.5, CCR6 PerCPCy5.5, CD16 PE Cy7, CD123 PE Cy7, CD56 APC, CD3 V450, CD86 V450, CD19 V500, CD14 V500, CXCR4 APC (BD Bioscience), CD14 FITC (Beckman Coulter), CCR5 PE, CCR3 PE, CXCR7 PE, CXCR2 PE, CCR2 PerCP Cy5.5, CCR7 PerCP Cy5.5, CXCR5 PerCP Cy5.5, CXCR3 PerCP Cy5.5, CX3CR1 APC, CCR1 Alexa Fluor 647, CXCR1 APC, HLADR APC Cy7 (Biolegend), CD303 FITC (Miltenyi), CXCR6 PE, XCR1 PE, CCR10 PE, CCR9 APC, ChemR23 APC, CCR8 APC (R&D Systems) at 4 °C for 30 min. The cells were subjected to flow cytometry using a BD FACS Canto flow cytometer and data was analyzed using the FACS Diva software (BD Biosciences) and FlowJo software (Tree Star). Confidence intervals, p-values and correlation coefficient were calculated and graphs were made in Graph Pad Prism (Graph Pad Software).

The p-values for differences in chemokine receptor expression between different monocyte subtypes were calculated using Wilcoxon Signed Rank Test. This test was chosen on recommendation from The Statistical consultant group, Gothenburg.

The correlation coefficient between percentage of cells expressing various receptors and median fluorescent intensity (MFI) was calculated using Spearman's rank correlation coefficient.

2.3. Gating strategies

Monocytes were identified by localization in dot plots of leukocytes analyzed in forward versus side scatter (Fig. 1A). Monocytes were gated for CD14 expression (Fig. 1B). The monocytes were further subdivided in classical and non-classical monocytes by gating for $CD14^+CD16^-$ and $CD14^+CD16^+$ monocytes (Fig. 1C). The HLA-DR^{hi} monocyte population was identified by gating $CD14^+$ monocytes with the highest HLA-DR expression (Fig. 1D). Having identified the three monocyte subsets of interest, the monocytes were gated for chemokine receptor expression using IgG isotype antibodies as controls (Supplemental Fig. 1).

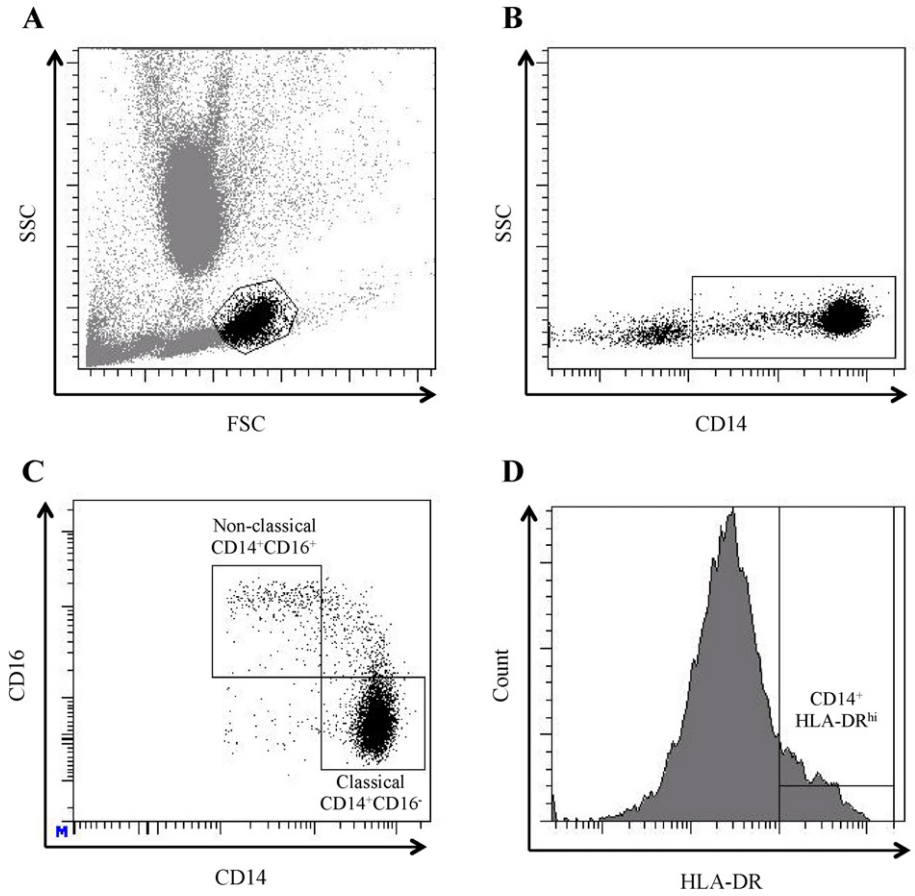

Fig. 1. Gating strategies for monocyte subsets. Flow cytometry plots show representative data from one healthy control. (A) The monocyte cell population is identified by side scatter (SSC) vs forward scatter (FSC). (B) The plot shows the defined CD14 expressing monocyte population. (C) The subsets classical monocytes ($CD14^+CD16^-$) and non-classical monocytes ($CD14^+CD16^+$) are defined by their expression of CD14 and CD16. (D) The pro-inflammatory monocyte subset is defined by high expression of HLA-DR.