Inflammation Research

Increased frequencies of circulating CXCL10-, CXCL8- and CCL4-producing monocytes and Siglec-3-expressing myeloid dendritic cells in Systemic Sclerosis patients.

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Supplementary Materials and Methods

Quantification of monocytes subsets and myeloid dendritic cells

The quantification of classical, non-classical monocytes and myeloid dendritic cells (mDC) was performed in 0.2 mL of whole blood collected into K_3- Ethylenediamine tetraacetic acid (EDTA) tubes, stained with the following monoclonal antibodies combination: anti-CD45 Krome Orange (clone: J.33; Beckman Coulter – Immunotech; Marseille, France), anti-CD11c Phycoerythrin- Cyanine -5 tandem (clone: B-ly6, BD Pharmingen), anti-HLA-DR Pacific Blue (clone: Immu-357; Beckman Coulter – Immunotech), anti-CD14 Allophycocyanin- Hellite -7 tandem (clone: MφP9; BD Biosciences, San Jose, CA, USA), anti-CD16 Fluorescein Isothiocyanate (clone: CLB-Fc-gran/1; Sanquin, Amsterdam, the Netherlands), anti-CD33 Phycoerythrin- Cyanine -7 tandem (clone: D3HL60.251; Beckman Coulter – Immunotech). Sample were then incubated for 15 min at room temperature in darkness. After this incubation period, a lyse and wash protocol was followed: 2 mL of FACS Lysing Solution (BD Biosciences) diluted 1:10 (vol/vol) in distilled water was added, and after 10 min of incubation, the cells were washed with 2 mL of PBS and resuspended in 0.5 mL of PBS before acquisition.

Data acquisition was performed in an FACSCanto™II (BD Biosciences) flow cytometer equipped with FACSDiva software (version 6.1.2; BD Biosciences). For data analysis, Infinicyt (version 1.6) software (Cytognos SL, Salamanca, Spain) was used. Absolute counts were calculated using a dual platform methodology (flow cytometry and hematological cell analyzer).