Data in Brief

Gene expression profiling of the Peyer's patch mononuclear phagocyte system

Johnny Bonnardel, Clément Da Silva, Marion Masse, Frederic Montañana-Sanchis, Jean-Pierre Gorvel, Hugues Lelouard *

Centre d'Immunologie de Marseille-Luminy, Aix Marseille Université UM2, Inserm, U1104, CNRS UMR7280, 13288 Marseille, France

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ABSTRACT

Peyer's patches (PPs) are primary inductive sites of mucosal immunity. The PP mononuclear phagocyte system, which encompasses both dendritic cells (DCs) and macrophages, is essential for the initiation of the mucosal immune response. We recently developed a method to isolate each mononuclear phagocyte subset of PP (Bonnardel et al., 2015). We performed a transcriptional analysis of three of these subsets: the $CD11b^+$ conventional DC, the lysozyme-expressing monocyte-derived DC termed LysoDC and the $CD11c^{hi}$ lysozyme-expressing macrophages. Here, we provide details of the gating strategy we used to isolate each phagocyte subset and show the quality controls and analysis associated with our gene array data deposited into Gene Expression Omnibus (GEO) under GSE65514.

Specifications [*standardized info for the reader*] where applicable, please follow the Ontology for Biomedical Investigations: http://obi-ontology.org/page/Main_Page

Organism/tissue/cell type	Mus musculus/Peyer's patch/dendritic cells and macrophages
Sex	Female
Sequencer or array type	Affymetrix GeneChip® Mouse Gene 1.0 ST array
Data format	Raw data (CEL files); Robust Multi-array Average algorithm normalized data (Matrix table)
Experimental factors	No treatment
Experimental features	Isolation and gene expression profiling of Peyer's patch phagocyte subsets of C57BL/6 mice, 6–8 weeks old.
Consent	N/A
Sample source location	Marseille, France

1. Direct link to deposited data

Deposited data can be found at: http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE65514.

* Corresponding author at: Centre d'Immunologie de Marseille-Luminy, 13288 Marseille, France. Tel.: +33 4 91 26 91 16; fax: +33 4 91 26 94 30.
E-mail address: lelouard@ciml.univ-mrs.fr (H. Lelouard).

2. Experimental design, materials and methods

2.1. Experimental design

PP DCs encompass 5 different subsets: $CD8\alpha^+$ DC, $CD11b^+$ DC, double negative DC (DN DC), lysozyme-expressing DC (LysoDC) and plasmacytoid DC [2–5]. The latter is the only subset that does not express high levels of CD11c [2]. Among the other subsets, both LysoDC and $CD11b^+$ DC express CD11b in addition to high levels of CD11c and MHCII [1]. There are also PP lysozyme-expressing macrophages termed LysoMac which display at their surface CD11b and CD11c but only low levels of MHCII [1]. The goal of this study was to design a gating strategy to sort each $CD11c^{hi}$ $CD11b^+$ phagocyte subset in order to analyze its gene expression profile and compare it with the other subsets.

2.2. PP macrophage and dendritic cell isolation procedure

PPs were collected from 42 C57Bl/6 mice per replicate (Charles River Laboratories, 6–8 week-old). Groups of PP from 3 mice were cut into pieces and digested in 7 mL of RPMI containing 100 μg/mL of type 2 collagenase (Worthington) and 140 μg/mL of DNase I (Sigma) for 40 min at room temperature. $CD11c^+$ cells were enriched using anti-CD11c microbeads and the program posselD2 of an autoMACS pro separator according to the manufacturer's instructions (Miltenyi Biotec). $CD11c^+$ cells were incubated on ice for 5 min with the 2.4G2 antibody

to block Fc receptors before staining for the following surface markers: CD8α (Brilliant Violet 785; clone 53–6.7; BD Biosciences), BST2 (FITC; clone 927; Biolegend), CD11b (APC-Cy7; clone M1/70; Biolegend), CD11c (PE-Cy7; clone N418; Biolegend), CD4 (PE-Cy5.5; clone RM4-5; eBioscience), SIRPα (APC; clone P84; eBioscience) and MHCII (Alexa Fluor 700; clone M5/114.15.2; eBioscience). Cell viability was evaluated using Fixable Viability Dye eFluor 506 (eBiosciences). Multiparameter fluorescence-activated cell sorting was performed using a FACSAria III (BD Biosciences). Data were analyzed with the BD FACSDiva software (BD Biosciences). First, cells were gated according to their CD11c and MHCII expression (Fig. 1). Then, LysoDC and LysoMac were separated from conventional DC (cDC) based on their BST2 expression (Fig. 1). LysoDC and LysoMac were subsequently distinguished using their CD4 and MHCII differential expression (Fig. 1). Finally, dome CD11b$^+$, CD8α$^+$ and DN cDC as well as dome-associated villus (DAV) DC were separated using a combination of CD11b, SIRPα and CD8α staining (Fig. 1). Sorted LysoDC, LysoMac and dome CD11b$^+$ cDC were collected in tubes containing 90 μL RLT PLUS buffer (Qiagen) and stored at −80 °C until further used.

2.3. RNA isolation and microarray

The total RNA of LysoDC, LysoMac and dome CD11b$^+$ cDC from 3 independent experiments was extracted with a Qiagen micro RNAeasy PLUS kit. Quantity, quality and absence of genomic DNA contamination were assessed with a Bioanalyzer 2100 (Agilent). Microarray experiments were performed by the Plateforme Biopuces de l'IGBMC of Strasbourg (France). Biotinylated double strand cDNA targets were prepared, starting from 5 to 17 ng of total RNA using the Ovation Pico WTA System V2 Kit (NuGEN) followed by the Encore Biotin Module Kit (NuGEN) according to the manufacturer's recommendations. Following fragmentation and end-labeling, cDNAs were hybridized for 16 h at 45 °C on GeneChip® Mouse Gene 1.0 ST arrays (Affymetrix) interrogating 28.853 genes represented by approximately 27 probes spread across the full length of the gene. The chips were washed and stained in the GeneChip® Fluidics Station 450 (Affymetrix) and scanned with the GeneChip® Scanner 3000 7G (Affymetrix) at a resolution of 0.7 μm. Raw data (.CEL intensity files) were extracted from the scanned images using the Affymetrix GeneChip® Command Console (AGCC) version 3.2. CEL files were further processed with Affymetrix Expression Console software version 1.1 to calculate probe set signal intensities using Robust Multi-array Average (RMA) algorithms with default settings or using RMA via the oligo package, through Bioconductor (release 2.13) in the R statistical environment (version 3.0.2).

2.4. Quality control and data analysis

Raw data were quality assessed using Relative Log Expression (RLE) and Normalized Unscaled Standard Error (NUSE) boxplots. RLE plot showed boxes centered to 0 with similar spread for all arrays (Fig. 2A) and NUSE plot was centered close to 1 for all arrays (Fig. 2B). Thus, both RLE and NUSE indicated that all arrays were of good quality. Boxplot and density plot of unprocessed and RMA processed probe intensities across all arrays shown in Fig. 2C indicated similar distribution of signal intensities between arrays. Hierarchical clustering (HC) by Pearson correlation distance and Ward's aggregation (Fig. 2D) and principal component analysis (PCA, Fig. 2E) were performed after selection of probes with a differential expression in any combination of array > 1.5 (2035 probes). Both HC and PCA showed that replicates clustered together (Fig. 2D and E). By HC, two main clusters were observed: one composed by dome CD11b$^+$ cDC and the other by monocyte-derived cells (Fig. 2D). Among the latter, LysoDC clustered apart from LysoMac. Similarly, by PCA, the first principal component, which explained 86% of the overall variability of the samples, separated dome CD11b$^+$ cDC from monocyte-derived cells and the second principal component, which
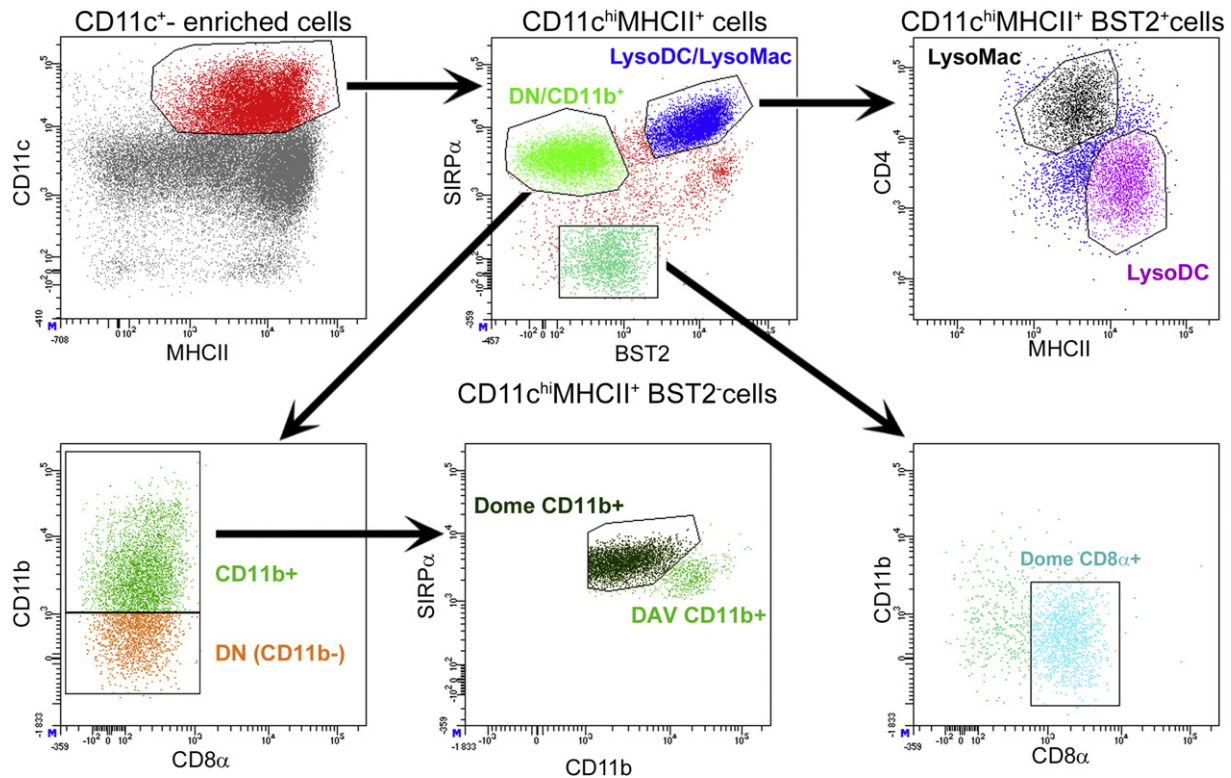
Fig. 1. Gating strategy to isolate dome CD11b$^+$ cDC, LysoDC and LysoMac. CD11c^{hi}MHCII$^+$ cells were selected among PP CD11c$^+$-enriched cells and analyzed for SIRPα and BST2 expression. Unlike cDC (i.e. CD11b$^+$, CD8α$^+$ and DN DC), LysoDC and LysoMac expressed BST2. Among CD11c^{hi}MHCII$^+$ SIRPα^{hi}BST2$^+$ cells, LysoDC expressed high levels of MHCII but no CD4 whereas LysoMac expressed CD4 and lower levels of MHCII. Among CD11c^{hi}MHCII$^+$ BST2$^-$ cells, DN and CD11b$^+$ cDC expressed SIRPα whereas CD8α$^+$ cDC did not. Finally, surface expression of CD11b and SIRPα on CD11c^{hi}MHCII$^+$ BST2-SIRPα$^+$ cells allowed to distinguish dome DN, dome CD11b$^+$ and DAV CD11b$^+$ cDC.
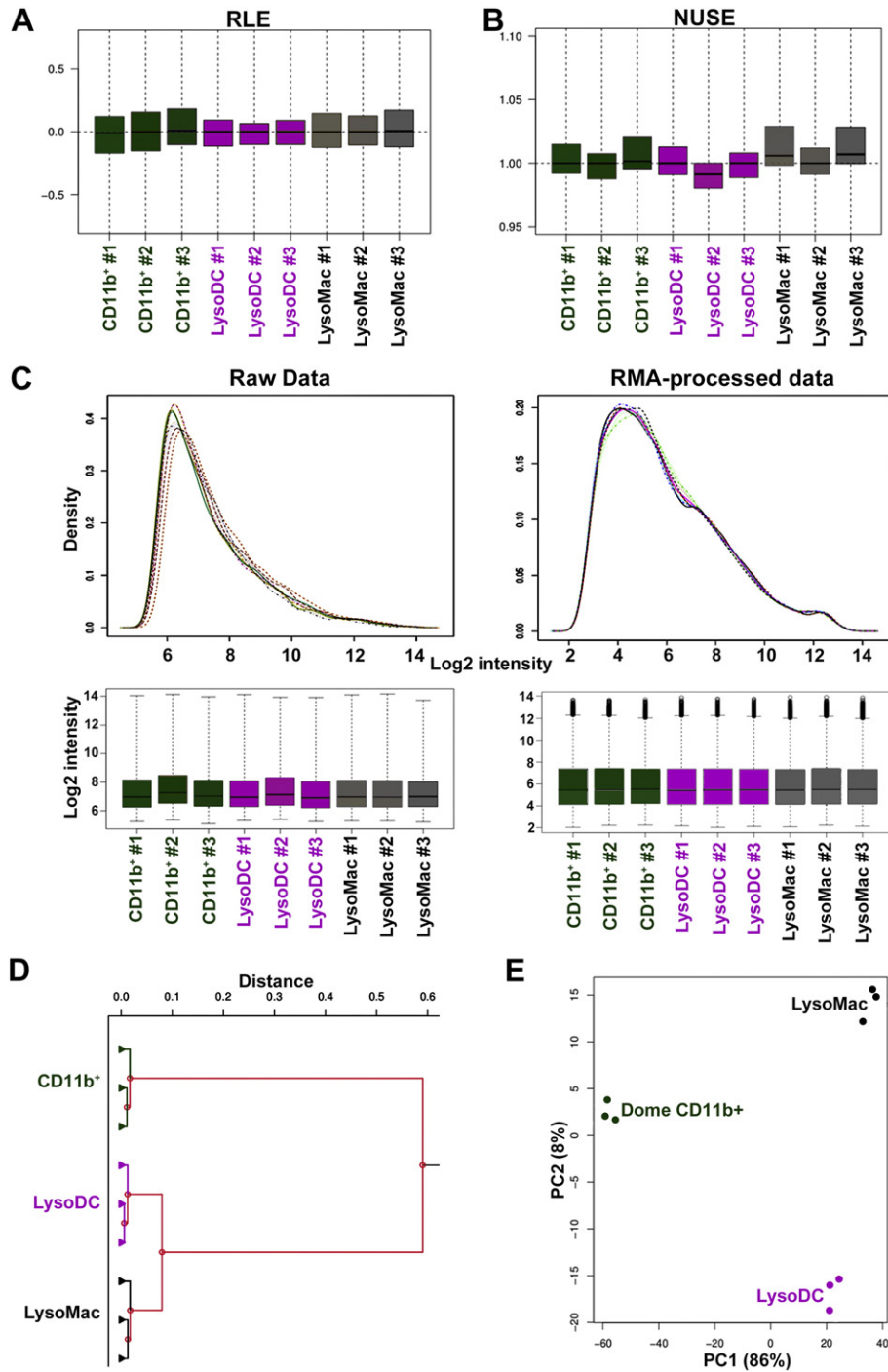
Fig. 2. Quality controls and analysis of microarrays. A) and B) The quality of arrays for CD11b+ cDC, LysoDC and LysoMac triplicates was assessed using Relative Log Expression (RLE; A) and Normalized Unscaled Standard Error (NUSE; B) boxplots. C) Histograms (upper row) and boxplots (lower row) of log2 transformed probe intensity before (raw data, left column) and after RMA processing (right column). D) Hierarchical clustering by Pearson correlation distance and Ward's aggregation showed the clustering of the replicates and the close genetic relationship between LysoDC and LysoMac. E) Principal component analysis of replicates. The first principal component (PC1) allowed the distinction between CD11b+ cDC and monocyte-derived cells whereas the second principal component (PC2) distinguished LysoDC from LysoMac with CD11b+ cDC replicates clustering in between. The percentage of variability explained by each component is indicated in brackets.

explained 8% of the overall variability, separated LysoDC from LysoMac (Fig. 2E).

3. Discussion

In this study we have sorted the different CD11c^hi CD11b+ subsets of the PP mononuclear phagocyte system in triplicates and subjected them to gene-expression profiling. These subsets include CD11b+ cDC, LysoDC and LysoMac. The transcriptional proximity between them determined by HC and PCA reveals a close genetic relationship between LysoDC and LysoMac apart from CD11b+ cDC. This is in line with their ontogeny since we have recently demonstrated that, unlike CD11b+ cDC which are derived from the common DC precursor, both LysoDC and LysoMac arise from monocytes [1]. We believe that future investigations of the genes specifically expressed by the PP mononuclear phagocyte system will likely help to understand the mechanistic and the pathways involved in the mucosal immune response initiated in PP.

Acknowledgements

References

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