Monocyte-derived immunoregulatory macrophages contribute to the alveolar macrophage pool in humans

Abstract

Alveolar macrophages (AM) are functionally important innate cells involved in lung homeostasis and immunity. While the idea that AM merely represent a homogenous population has been recently challenged, it remains unclear whether ontogenically and functionally distinct subpopulations of AMs can be found in healthy human lungs, and to what extent conditions like smoking or chronic obstructive pulmonary disease (COPD) trigger changes in the AM compartment. Here, we analyzed bronchoalveolar lavage fluid (BALF) cells from healthy non-smokers, healthy smokers and COPD patients by flow cytometry, bulk and single-cell(sc) RNA-sequencing (RNA-seq). We found that BALF CD206+ macrophage subpopulations could be distinguished based on their level of auto-fluorescence. Bulk RNA-seq analysis of blood monocytes, CD206+ autofluorescentlow (AFlo) and AFhi macrophages supported that AFlo macrophages derived from monocytes that were imprinted by the alveolar niche to differentiate into AM expressing both monocyte- and AM-related genes. ScRNA-seq analyses performed on total BALF cells from the same patients highlighted the dual monocytic and self-proliferative origin of AM, as well as the functional diversity of the AM pool in healthy and COPD lungs. Of note, monocyte-derived AFlo AM were equally represented in each category of subjects and exhibited an immunoregulatory profile, including the ability to secrete the immunosuppressive cytokine interleukin(IL-10). Finally, we provide evidence that the monocyte-associated marker CCR2 can be used by flow cytometry to distinguish AM according to their origin, thereby opening new perspectives to better understand AM complexity and functions in different contexts, such as infections, inflammatory disorders or cancer.

Introduction

We were further interested in investigating the identity of AF-low and AF-high AM using high-throughput technologies. For this purpose, we recruited 3 healthy non-smokers, 3 healthy smokers and 3 COPD patients, as presented in Table 1. Individuals were matched for age. While healthy subjects did not exhibit any abnormalities in forced expiratory volume (FEV) or in the FEV/forced vital capacity (FVC) ratio, COPD patients showed low FEV and FEV/FVC values and were diagnosed stages II or III according to the GOLD COPD stage (Table 1 in manuscript). BALF were collected and BALF cells were divided into 3 fractions (Figure 2A in manuscript). The first fraction was used to perform a total and differential cell count, which showed no obvious signs of inflammation in each of the patients analyzed (Table 1 in manuscript). The second and third fractions were used for bulk and single cell mRNA-sequencing, respectively (Figure 2A in manuscript). For bulk mRNA-sequencing (RNA-seq), CD14^+ monocytes were isolated from the blood and AF-low and AF-high CD45^+ CD206^+ cells were FACS-sorted from the BALF of the same patients (Figure 2A). Notably, AF-low AM were present in each patient analyzed (Figure 2B). Here present the source codes for all single-cell analyses from 10X Cellranger output to figure production.

Data import, QC and cell typeing

Data availability

Source data containing counts matrix, features and barcodes for each samples can be downloaded from following links: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE183982

Source data for individual samples:

- NGS19-I415_Dim_LBA-Hum3 https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSM5575227

- NGS19-I679_Dim_LBA-Hum4 https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSM5575228
- NGS19-J028_Dim_LBA-Hum5 https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSM5575229
- NGS19-J141_Dim_LBA-Hum7 https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSM5575230
- NGS19-J142_Dim_LBA-Hum8 https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSM5575231
- NGS19-J263_Dim_LBA-Hum9 https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSM5575232
- NGS19-J264_Dim_LBA-Hum10 https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSM5575233
- NGS19-K359_Dim_LBA-Hum11 https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSM5575234

Processed results can be accessed via online visualization platform: https://gigaimmunophysiology.shinyapps.io/Human_monocytes

load data with Seurat package

Unzip data and load.

```
library(Seurat)
library(dplyr)

# source data paths:
sample.list <- c(
  NGS19_I415_Dim_LBA_Hum3=
    "NGS19-I415_Dim_LBA-Hum3/outs/filtered_feature_bc_matrix/",
  NGS19_I679_Dim_LBA_Hum4=
    "NGS19-I679_Dim_LBA-Hum4/outs/filtered_feature_bc_matrix/",
  NGS19_J028_Dim_LBA_Hum5=
    "NGS19-J028_Dim_LBA-Hum5/outs/filtered_feature_bc_matrix/",
  NGS19_J141_Dim_LBA_Hum7=
    "NGS19-J141_Dim_LBA-Hum7/outs/filtered_feature_bc_matrix/",
  NGS19_J142_Dim_LBA_Hum8=
    "NGS19-J142_Dim_LBA-Hum8/outs/filtered_feature_bc_matrix/",
  NGS19_J263_Dim_LBA_Hum9=
    "NGS19-J263_Dim_LBA-Hum9/outs/filtered_feature_bc_matrix/",
  NGS19_J264_Dim_LBA_Hum10=
    "NGS19-J264_Dim_LBA-Hum10/outs/filtered_feature_bc_matrix/",
  NGS19_K359_Dim_LBA_Hum11=
    "NGS19-K359_Dim_LBA-Hum11/outs/filtered_feature_bc_matrix/",
  NGS19_K360_Dim_LBA_Hum12=
    "NGS19-K360_Dim_LBA-Hum12/outs/filtered_feature_bc_matrix/"
  )
```

System Configuration

```
sessionInfo()
```

```
## R version 4.0.3 (2020-10-10)
## Platform: x86_64-pc-linux-gnu (64-bit)
## Running under: Ubuntu 20.04.3 LTS
##
## Matrix products: default
```

```
## BLAS:   /usr/lib/x86_64-linux-gnu/openblas-pthread/libblas.so.3
## LAPACK: /usr/lib/x86_64-linux-gnu/openblas-pthread/liblapack.so.3
##
## locale:
##  [1] LC_CTYPE=en_US.UTF-8       LC_NUMERIC=C
##  [3] LC_TIME=en_GB.UTF-8        LC_COLLATE=en_US.UTF-8
##  [5] LC_MONETARY=en_GB.UTF-8    LC_MESSAGES=en_US.UTF-8
##  [7] LC_PAPER=en_GB.UTF-8       LC_NAME=C
##  [9] LC_ADDRESS=C               LC_TELEPHONE=C
## [11] LC_MEASUREMENT=en_GB.UTF-8 LC_IDENTIFICATION=C
##
## attached base packages:
## [1] stats     graphics  grDevices utils     datasets  methods   base
##
## other attached packages:
## [1] RColorBrewer_1.1-2 pheatmap_1.0.12    ggplot2_3.3.5      dplyr_1.0.7
## [5] SeuratObject_4.0.2 Seurat_4.0.3
##
## loaded via a namespace (and not attached):
##   [1] nlme_3.1-152          spatstat.sparse_2.0-0 matrixStats_0.60.0
##   [4] RcppAnnoy_0.0.19      httr_1.4.2            sctransform_0.3.2
##   [7] tools_4.0.3           utf8_1.2.2            R6_2.5.0
##  [10] irlba_2.3.3           rpart_4.1-15          KernSmooth_2.23-20
##  [13] uwot_0.1.10.9000      mgcv_1.8-33           DBI_1.1.1
##  [16] lazyeval_0.2.2        colorspace_2.0-2      withr_2.4.2
##  [19] gridExtra_2.3         tidyselect_1.1.1      compiler_4.0.3
##  [22] plotly_4.9.4.1        scales_1.1.1          spatstat.data_2.1-0
##  [25] lmtest_0.9-38         ggridges_0.5.3        pbapply_1.4-3
##  [28] goftest_1.2-2         stringr_1.4.0         digest_0.6.27
##  [31] spatstat.utils_2.2-0  rmarkdown_2.9         rticles_0.20
##  [34] pkgconfig_2.0.3       htmltools_0.5.1.1     parallelly_1.27.0
##  [37] fastmap_1.1.0         htmlwidgets_1.5.3     rlang_0.4.11
##  [40] shiny_1.6.0           generics_0.1.0        zoo_1.8-9
##  [43] jsonlite_1.7.2        ica_1.0-2             magrittr_2.0.1
##  [46] patchwork_1.1.1       Matrix_1.3-4          Rcpp_1.0.7
##  [49] munsell_0.5.0         fansi_0.5.0           abind_1.4-5
##  [52] reticulate_1.20       lifecycle_1.0.0       stringi_1.7.3
##  [55] yaml_2.2.1            MASS_7.3-53           Rtsne_0.15
##  [58] plyr_1.8.6            grid_4.0.3            parallel_4.0.3
##  [61] listenv_0.8.0         promises_1.2.0.1      ggrepel_0.9.1
##  [64] crayon_1.4.1          deldir_0.2-10         miniUI_0.1.1.1
##  [67] lattice_0.20-41       cowplot_1.1.1         splines_4.0.3
##  [70] tensor_1.5            knitr_1.33            pillar_1.6.2
##  [73] igraph_1.2.6          spatstat.geom_2.2-2   future.apply_1.7.0
##  [76] reshape2_1.4.4        codetools_0.2-18      leiden_0.3.9
##  [79] glue_1.4.2            evaluate_0.14         data.table_1.14.0
##  [82] png_0.1-7             vctrs_0.3.8           httpuv_1.6.1
##  [85] polyclip_1.10-0       spatstat.core_2.3-0   gtable_0.3.0
##  [88] RANN_2.6.1            purrr_0.3.4           tidyr_1.1.3
##  [91] scattermore_0.7       future_1.21.0         assertthat_0.2.1
##  [94] xfun_0.24             mime_0.11             xtable_1.8-4
##  [97] later_1.2.0           survival_3.2-7        viridisLite_0.4.0
## [100] tibble_3.1.3          cluster_2.1.0         globals_0.14.0
## [103] fitdistrplus_1.1-5    ellipsis_0.3.2        ROCR_1.0-11
```