Phenotypic and Functional Heterogeneity of Low-Density and High-Density Human Lung Macrophages

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Introduction: Macrophages are important immune cells resident of all tissues, where they play pivotal roles in tissue homeostasis. Macrophages are critical sentinels in immunity, combating infections, modulating angiogenesis and lymphangiogenesis, resolving inflammation, surveilling against tumors and are implicated in a wide spectrum of disorders, including pulmonary and cardiovascular diseases, diabetes and cancer. Although macrophages resident in different tissues and are highly heterogeneous share several common features and functions. Macrophages can be distinguished by their expression of surface antigens, by cell-intrinsic density, ultrastructural, and functional properties. Macrophages display a specific phenotype in response to microenvironmental signals. Pulmonary macrophages constitute a heterogeneous cell population localized in distinct compartments. Alveolar macrophages are found in the alveolar walls, whereas interstitial macrophages represent the majority of immune cells in the lung parenchyma. Differences in morphology, phenotype and function have been reported. In this study, we have started to characterize two distinct subpopulations of macrophages from human lung parenchyma that differ by density, size, immunological and morphometric features as well as their functional response to lipopolysaccharide (LPS).

Materials and Methods:
Macrophages were obtained from normal lung tissue of patients undergoing thoracic surgery for lung carcinoma. Macrophage fractionation by density was accomplished by centrifugation over discontinuous Percoll gradients.
Two fractions of macrophages were obtained: low-density macrophages (LDMs), floating between density bands 1.039 and 1.052, and high-density macrophages (HDMs), floating between density bands 1.065 and 1.078. Cell morphology was evaluated by cytospin (figure 1) of LDMs and HDMs; the expression of surface markers was analyzed by flow cytometry. Values were expressed as the percentage of positive and negative cells. The cut-off point between positive and negative cells was set using control antibodies of the same isotype. Fluorescence intensity was also analyzed by recording the mean fluorescence intensity expressed in linear units.

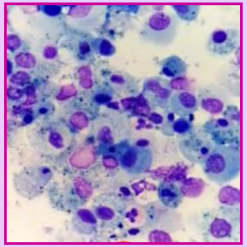

Figure 1: Cell morphology was evaluated by cytospin

Results 1: Mechanical dispersion of macroscopically normal human lung tissue yielded a cell preparation containing 5.63 ± 1.01 x 10^6 macrophages per gram of tissue with a purity of 65.4 ± 2.3% (n=10). Centrifugation of dispersed lung cells over discontinuous density gradients yielded two sets of cells at the interface between density bands of 1.039 and 1.052 (Low-Density Macrophages: LDMs) and the interface between 1.065 and 1.078 (High-Density Macrophages: HDMs). Figure 2 reports the data from five different lung preparations. There was a clear bimodal distribution of macrophages: one subpopulation had a density between 1.033 and 1.049 (white columns) whereas another had a density between 1.062 and 1.087 (black columns).

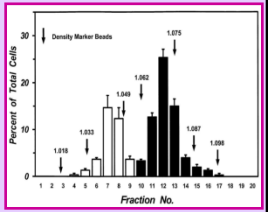

Figure 2: Separation of macrophage by density gradient

Results 2: The expression of several surface molecules (figure 3) in the two macrophage subpopulations separated by density gradients was examined. Markers expressed on more than 85% (CD11c, CD44, CD71, HLA-DR, CD206) or on less than 5% (CD1a, CD4, CD14, CD15, CD25, CD35, CD38, CD42b, CD56, CD61, CD69, CD80, CD117, CD130, CD154) of the cells in both subpopulations. The major markers of tissue macrophages (CD11c, CD44, CD71, HLA-DR and CD206) were highly expressed on both LDMs and HDMs. The low expression (< 5%) of CD1a (dendritic cells), CD4 (T cells), CD14 (monocytes), CD56 (NK cells), CD69 (eosinophils) and CD117 (mast cells) indicated that contaminating cells in HDM and LDM preparations are negligible (less than 5%). The two subpopulations showed a differential expression of relevant surface markers. A higher percentage of macrophages of HDMs expressed CD40, CD45, and CD86 as compared to LDMs, whereas larger proportions of $CD63^+$ and $CD64^+$ cells were found in LDMs. Figure 4 shows representative flow cytometry scans illustrating the differential expression of CD63 (Panel A), mostly found on LDMs, and of CD86 (Panel B), predominantly expressed on HDMs. These results indicate that the two subpopulations of human lung macrophages can be distinguished by the expression of surface molecules.

Results 3: The results on cytokine release were obtained by stimulating the cells with LPS for 24 h to allow an appropriate comparison between LDMs and HDMs. Results indicate that the kinetics of release of proinflammatory cytokines (IL-6 and TNF-α) are faster than those of immunomodulatory cytokines (IL-10 and IL-12). The time-course of the IL-6 and IL-10 production by HDMs and LDMs. The results illustrated in Figure 5 demonstrate that the release of IL-6 is faster than that of IL-10 in both macrophage subpopulations. The difference in the production of IL-6 between LPS-stimulated HDMs and LDMs was already significant after 6 h of incubation, whereas that of IL-10 required 24 h to reach significance. These data indicate that LPS-induced production of cytokines is quantitatively different between HDMs and LDMs and that it is not due to differences in the release kinetics.

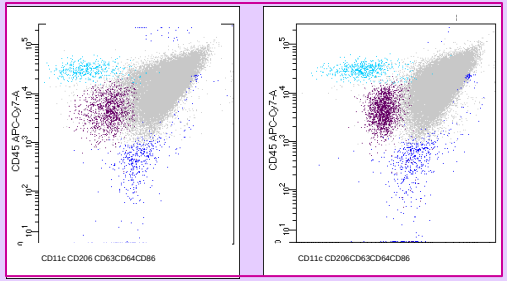

Figure 3: Flow cytometry macrofages grey dots

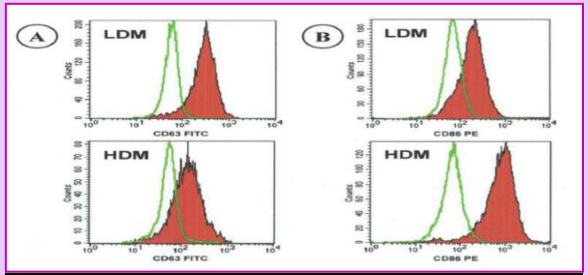

Figure 4: FACS analysis of CD63 and CD86 expression on LDMs and HDMs.

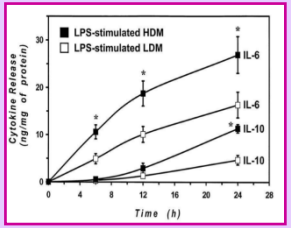

Figure 5: The time-course of cytokines

Discussion: In this study, we found that two subpopulations of human lung-resident macrophages, separated by property, differ by morphometry, phenotype as well as functional response to LPS. Our data do not allow to understand whether LDMs are derived from HDMs and whether LDMs represent a stage of macrophage differentiation in the lung tissue. It is tempting to speculate that exposure to different microenvironments may lead to the acquisition of distinct phenotypes and may confer the distinct profile to LDMs and HDMs.
Another aim of this study was to explore the responsiveness of the two subpopulations of macrophages to stimulation with LPS, a major component of the outer membrane of Gram-negative bacteria.

Conclusion: These subpopulations of macrophages probably play distinct roles in lung inflammation and immune responses. Our study shows that LMDs and HDMs produce different amounts of cytokines upon activation with the same stimulus. In fact, LPS-stimulated HDMs produce significantly higher levels of classical proinflammatory cytokines (i.e., IL-6 and TNF-α) compared to LDMs. This difference is even more marked in the case of the immunoregulatory cytokines IL-10 and IL-12. These observations support the concept that differences in the intensity rather that the quality of the response to LPS is a feature of the two subpopulations of human lung macrophages.