A High Content Screen to Identify Novel Factors That Restore Phagocytosis in COPD Alveolar Macrophages

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Abstract

Background: COPD is a chronic progressive airway disease characterized by airway inflammation, bronchial obstruction and airflow limitation. Alveolar macrophages (AMs) from COPD patients have been reported to be defective in bacterial phagocytosis, which contributes to increased bacterial load in the lungs and the increased severity and frequency of COPD exacerbations. We sought to develop an image-based, high-content screen to identify novel potential therapeutic targets that may enhance AM phagocytosis in COPD patients.

Methods: Healthy volunteers (n=46), smokers with normal spirometry (>30-pack years of smoking), and patients with COPD (n=10) were recruited. Freshly isolated alveolar macrophages (AMs) were prepared and cultured in a high-throughput platform for the study. Phagocytosis was assessed using a high-content screen. To screen for factors that may regulate phagocytosis, AMs were cultured in 384-well plates and each well was treated with supernatants containing protein-free FBS or protein-free FBS containing increasing concentrations of the factor of interest. Bacterial uptake was assessed by imaging AMs and quantifying the pHrodo density in pHrodo positive cells (accumulated intracellular bacteria).

Results: AMs from COPD patients were defective in bacterial phagocytosis. To screen for factors that may regulate phagocytosis, AMs were cultured in 384-well plates and each well was treated with supernatants containing protein-free FBS or protein-free FBS containing increasing concentrations of the factor of interest. Bacterial uptake was assessed by imaging AMs and quantifying the pHrodo density in pHrodo positive cells (accumulated intracellular bacteria). Mean ± SD shown.

Table 1: Demographic characteristics of participants

<table>
<thead>
<tr>
<th>Donor Group</th>
<th>Donor Numbers</th>
<th>Male/Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy males</td>
<td>10</td>
<td>52</td>
</tr>
<tr>
<td>Healthy females</td>
<td>10</td>
<td>53</td>
</tr>
<tr>
<td>COPD smokers</td>
<td>10</td>
<td>53</td>
</tr>
<tr>
<td>COPD non-smokers</td>
<td>10</td>
<td>52 ± 14.3</td>
</tr>
</tbody>
</table>

Figure 1: Bacterial uptake in AMs occurred in a dose- and time-dependent manner. Alveolar macrophages from a COPD smoker were challenged with intracellular pHrodo-positive NTHi or commercial NTHi Escherichia coli for 2 hours. Increase in uptake was measured by an increase in signal in all three key readouts: % responding cells, pHrodo index and phagocytic activity (A). In addition, time-dependent bacterial uptake in COPD AMs was observed up to 6 to 8 hours post challenge (B). Signal is background (BFU at 48 h) / BFU at 2 h. Significant differences are indicated by an asterisk (p < 0.05) using ANOVA followed by Tukey’s multiple comparison test. Each dot represents a single assay plate. 100 cells were counted per assay plate. Each assay was repeated at least three times. Data are presented as mean ± SD. E.coli NTHi

Figure 2: Differential expression of surface markers and uptake of phagocytic NTHi was observed in different donor groups. Significantly higher levels of CD163 and CD14 and significantly lower levels of CD11c and CD64 were observed in COPD smokers compared to the healthy never-smokers (n=10, p<0.05, student t-test). In COPD smokers, significant decreases in CD14 and CD16 were detected compared to those of COPD ex-smokers (n=10, p<0.05, student t-test). These results suggest an effect of active smoking on these markers in both smokers with normal spirometry and COPD smokers. (A) To understand the differences in bacterial uptake among these donor groups, AMs were challenged with live or killed pHrodo-positive NTHi for 6 hours. Uptake of the bacteria was analyzed in all four donor groups and a significant decrease in phagocytic activity was observed in the healthy never-smokers compared to the COPD smokers. (B) A scatter plot showing the correlation between the phagocytic activity and the expression of CD11c. Each dot represents a single donor. Data are presented as mean ± SD.

Figure 3: High content imaging screen identified potential protein modulators of phagocytosis. Screening data on over 570 candidate proteins were normalized to the plate mean and represented as Z scores centered around the plate mean for each of the three key readouts. A threshold for protein activity of 1.5 sigma (dotted line) was selected based on the effect size of the assay controls. Examples of proteins with activity (red boxes) demonstrate the dynamic range of the screen. Poorly performing controls (green boxes) trend to the plate mean. Significant differences are indicated by an asterisk (p < 0.05) using ANOVA followed by Tukey’s multiple comparison test. Each dot represents a single assay plate. 100 cells were counted per assay plate. Each assay was repeated at least three times. Data are presented as mean ± SD. Examples of protein modulators of phagocytosis

Conclusions and Future Directions

We observed differential expression levels of selected surface markers on alveolar macrophages from COPD and healthy individuals, suggesting suppressive effects of active smoking on CD11c (differentiation marker), CD14 (monocyte marker) and CD64 (marker for M2 macrophages). Additionally, we demonstrated defective phagocytosis of bacteria in COPD smokers, but not other smokers, in AAMs from COPD smokers compared to AAMs from healthy never-smokers using a phagocytosis-based assay. We successfully developed and validated a high content screen of bacterial uptake in COPD donors and reported the application of this system. These results suggest that the screen may hold promise to identify novel therapeutic targets that may enhance AM phagocytosis in COPD patients.

References

3. C.S. Berenson et al, J Infective Disease (2016)

Acknowledgements

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