Soluble CD200 Correlates With Interleukin-6 Levels in Sera of COPD Patients: Potential Implication of the CD200/CD200R Axis in the Disease Course

Priya Sakthivel1,2 Angele Breithaupt3 Marcus Gereke1,2 David A. Copland4,5,6 Christian Schulz7 Achim D. Gruber3 Andrew D. Dick4,5,6 Jens Schreiber8 Dunja Bruder1,2

COPD Pathogenesis

Chronic obstructive pulmonary disease (COPD) is associated with chronic inflammation

- affecting predominantly the lung parenchyma and peripheral airways

- results in largely irreversible and progressive airflow limitation.

An Initial inflammatory trigger causes tissue damage mediated by mucosal immune cells

2 major pathological processes

1) remodeling and narrowing of the small airways
2) destruction of the lung parenchyma with loss of the alveolar attachments
   → emphysema formation

Neutrophile Granulocytes, Macrophages, T-cells and structural cells (epithelial and endothelial cells and fibroblasts) secrete a variety of proinflammatory mediators
Cigarette smoke (and other irritants)

Epithelial cells

TGFβ

Fibroblast

CXCL9, CXCL10 and CXCL11

Macrophage

CXCL1 and CXCL8

CCL2

Neutrophil

T₁₁₁ cell

T₉₋₁ cell

CXCR3

CXCR2

CCR2

Proteases (such as neutrophil elastase and MMP9)

Airway epithelial cell

Mucus

Goblet cell

Mucus gland

Fibrosis (small airways)

Alveolar wall destruction (emphysema)

Mucus hypersecretion

Nature Reviews | Immunology
Alveolar Macrophage vs Neutrophile Granulocyte

Corticosteroid resistance

Recruitment Differentiation

Phagocytosis

Efferocytosis

Circulating monocytes

Rx

CCL2

CXCL1

IL-23

CXCR2

CXCR3

CXCL10

CXCL11

CXCL12

ROS

ONOO-

NO

LTB₄

CXCL8

Neutrophils

Monocytes

Th17

Tc1, Th1,

Emphysema

Small airway fibrosis

Persistent inflammation

Bacterial colonization

↑ MUC5AC, MUC5B
Mucous hyperplasia

Submucosal gland

EGF

TACE

TGF-α

Oxidants

Cigarette smoke

MAP kinases

MUC5AC

MUC5B

↑ MUC5AC, MUC5B
Mucous hyperplasia

Goblet cell

COPIC Dragan
WHY the CD200/CD200R axis?

CD200 is expressed on airway epithelial cells, endothelial cells, and T cells

CD200R molecule is predominantly expressed on airway macrophages and neutrophils → inhibition of airway macrophage activation

soluble (s) forms of CD200 and its receptor CD200R in human sera reported → produced either by membrane shedding or by mRNA splicing

CD200(-/-) mice were shown to develop excessive lung inflammation with enhanced neutrophil and T-lymphocyte infiltration into the lung

Therefore they analyzed the serum concentrations of sCD200 in COPD patients and normal controls and correlated the data with COPD-relevant clinical parameters
Characteristics of COPD patients and normal controls

<table>
<thead>
<tr>
<th>Parameters</th>
<th>COPD patients</th>
<th>Normal controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basic parameters</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of subjects (n)</td>
<td>50</td>
<td>29</td>
</tr>
<tr>
<td>Age (mean years)</td>
<td>66.7</td>
<td>61.6</td>
</tr>
<tr>
<td>Gender (men/women)</td>
<td>36/14</td>
<td>19/10</td>
</tr>
<tr>
<td>BMI (kg m²)</td>
<td>27 (14–42) (n = 39)</td>
<td>–</td>
</tr>
<tr>
<td>Clinical parameters</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GOLD (I/II/III/IV/nd)</td>
<td>(3/18/8/9/12)</td>
<td>–</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>6 (0–41) (n = 38)</td>
<td>–</td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td>2 (0–21) (n = 37)</td>
<td>–</td>
</tr>
<tr>
<td>CRP (ng/ml)</td>
<td>8 (1–75) (n = 34)</td>
<td>–</td>
</tr>
<tr>
<td>Vitamin D-1,25-OH (ng/ml)</td>
<td>50 (22–84) (n = 39)</td>
<td>–</td>
</tr>
<tr>
<td>Pulmonary function tests</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VC%</td>
<td>77 (44–134) (n = 38)</td>
<td>–</td>
</tr>
<tr>
<td>FEV₁ (%)</td>
<td>54 (23–113) (n = 39)</td>
<td>–</td>
</tr>
<tr>
<td>DL_CO (%)</td>
<td>53 (23–92) (n = 35)</td>
<td>–</td>
</tr>
</tbody>
</table>
Sera measurements and COPD-induction in mice

- ELISA was performed in order to determine Serum levels of sCD200, MMP-9 (only in mice), TNF- alpha

- COPD-like features were induced in both CD200KO and C57BL/6 wild-type mice

  → elastase/ lipopolysaccharide (LPS) exposure for two and four consecutive weeks

- negative control mice were exposed to 50% glycerol and phosphate-buffered saline (PBS)

- Mice were sacrificed one week after the final exposure, followed by lung extraction and histological staining
Induction of a COPD-like phenotype in C57BL/6 wild-type mice by repeated intranasal administration of elastase and LPS exposure.

- Inflammation score 3 (moderate)
- Alveolar macrophage infiltration: score 2.5 (mild to moderate)
- Emphysema: score 3 (moderate)
- Mucin staining in large bronchi: score 2 (strong cellular signal) and 2.5 (multifocal to diffuse)
- Inflammation: score 0 (none)
- Alveolar macrophage infiltration: score 0 (none)
- Emphysema: score 1 (minimal)
- Mucin staining in large bronchi: score 1 (weak) and 2 (multifocal)
RESULTS

• Serum sCD200 Concentration is Positively Correlated to the Abundance of the Proinflammatory Cytokine IL-6 in Human COPD Patients.

• Exposure to LPS/elastase resulted in hallmark features of COPD

• CD200 Deficiency Does Not Affect the Onset of COPD-Like Features in Mice Following Elastase/ LPS Exposure

• Increased Serum MMP-9 Levels Were Detected in CD200-Deficient Mice Following Elastase/LPS Exposure
Serum sCD200 Concentration is Positively Correlated to the Abundance of the Proinflammatory Cytokine IL-6 in Human COPD Patients

sCD200 was well detectable in the sera of normal and diseased donors. The correlation of COPD-relevant clinical parameters with serum sCD200 levels revealed a significant positive correlation between sCD200 and IL-6. There was a trend towards a negative correlation with vitamin D-1, 25-OH in COPD patients.

Table 2  P values and r values from Spearman’s rank correlation test between sCD200 and clinical parameters in COPD patients

<table>
<thead>
<tr>
<th>Parameters</th>
<th>sCD200</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (P/r)</td>
<td>0.04*/0.27</td>
</tr>
<tr>
<td>GOLD stage (P/r)</td>
<td>0.49/0.11</td>
</tr>
<tr>
<td>CRP (P/r)</td>
<td>0.20/0.22</td>
</tr>
<tr>
<td>Vitamin D-1,25-OH (P/r)</td>
<td>0.07*/-0.28</td>
</tr>
<tr>
<td>IL-6 (P/r)</td>
<td>0.01*/0.38</td>
</tr>
<tr>
<td>TNF-α (P/r)</td>
<td>0.83/-0.03</td>
</tr>
</tbody>
</table>

GOLD global initiative for chronic obstructive lung disease, CRP C-reactive protein, IL interleukin, TNF-α tumor necrosis factor (alpha)

* Significant P values, † Tendencies

![Graph showing correlation between serum sCD200 and clinical parameters]
Exposure to LPS/elastase resulted in hallmark features of COPD

- Immune cell infiltration, emphysematous changes, and mucus overproduction were observed

- elastase/ LPS exposure resulted in the induction of COPD-like features in wild-type mice and could be used to study the role of the CD200/CD200R axis in COPD-like lung inflammation.
CD200 Deficiency Does Not Affect the Onset of COPD-Like Features in Mice Following Elastase/LPS Exposure

expected an early and augmented inflammatory immune cell infiltration in KO mice

→ CD200/CD200R inhibitory axis

a slight reduction in neutrophil, lymphocyte, and alveolar macrophage recruitment observed

slightly reduced inflammation score in CD200KO mice compared to control mice
Histopathology
CD200 Deficient vs control mice after elastase/LPS exposure

a inflammation including neutrophils and lymphocytes: score 3 (moderate)
b alveolar macrophage infiltration: score 2,5 (mild to moderate)
c emphysema: score 3 (moderate)
d mucin staining in large bronchi: score 2 (strong) and 2,5 (multifocal to diffuse)
e inflammation: score 2 (mild)
b alveolar macrophage infiltration: score 2,5 (mild to moderate)
c emphysema: score 3 (moderate), H&E staining, scale bar
d mucin staining: score 2 (strong) and 2 (multifocal)
• No differences were observed regarding emphysematous changes, lung volume, and mucus production between CD200-deficient and wild-type animals

→ CD200/CD200R axis does not seem to play a dominant role in the early development of a COPD-like phenotype
Increased Serum MMP-9 Levels Were Detected in CD200-Deficient Mice Following Elastase/LPS Exposure

MMPs are crucially involved in the development of emphysematous lesion in COPD

→ MMP-9 level measurement

significantly increased concentrations of MMP-9 in CD200KO mice exposed to elastase/LPS for 4 weeks compared to wild-type control animals

Thus, serum MMP-9 concentration in CD200KO mice is elevated compared to wild-type mice and animals with established COPD-like features
Discussion

• The trend for inverse correlation with vitamin D3 provided hint for a potential proinflammatory role of sCD200 in COPD pathogenesis

• that circulating sCD200 might block the cell surface interactions between CD200 and CD200R to mediate their immune inhibitory functions

→ Functional studies are required to prove this notion

→ this study lacks the required power of analysis, which could be substantiated with a larger COPD cohort

Expected CD200KO mice exhibit increased myeloid cell influx following elastase/LPS exposure and a more severe COPD progression.

→ no significant differences in COPD-relevant histopathological parameters were observed

→ other lung regulatory mechanisms that might compensate?