Ankersmit Laboratory
for Diagnosis & Regeneration in Thoracic Diseases & Applied Immunology

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. Dick4,5,6Jens Schreiber8 Dunja Bruder1,2

Published: 09/2016 in Lung, Springer Science and Business Media New York 2016

COPD Pathogenesis

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

→ affecting predominantly the **lung parenchyma** and **peripheral airways**

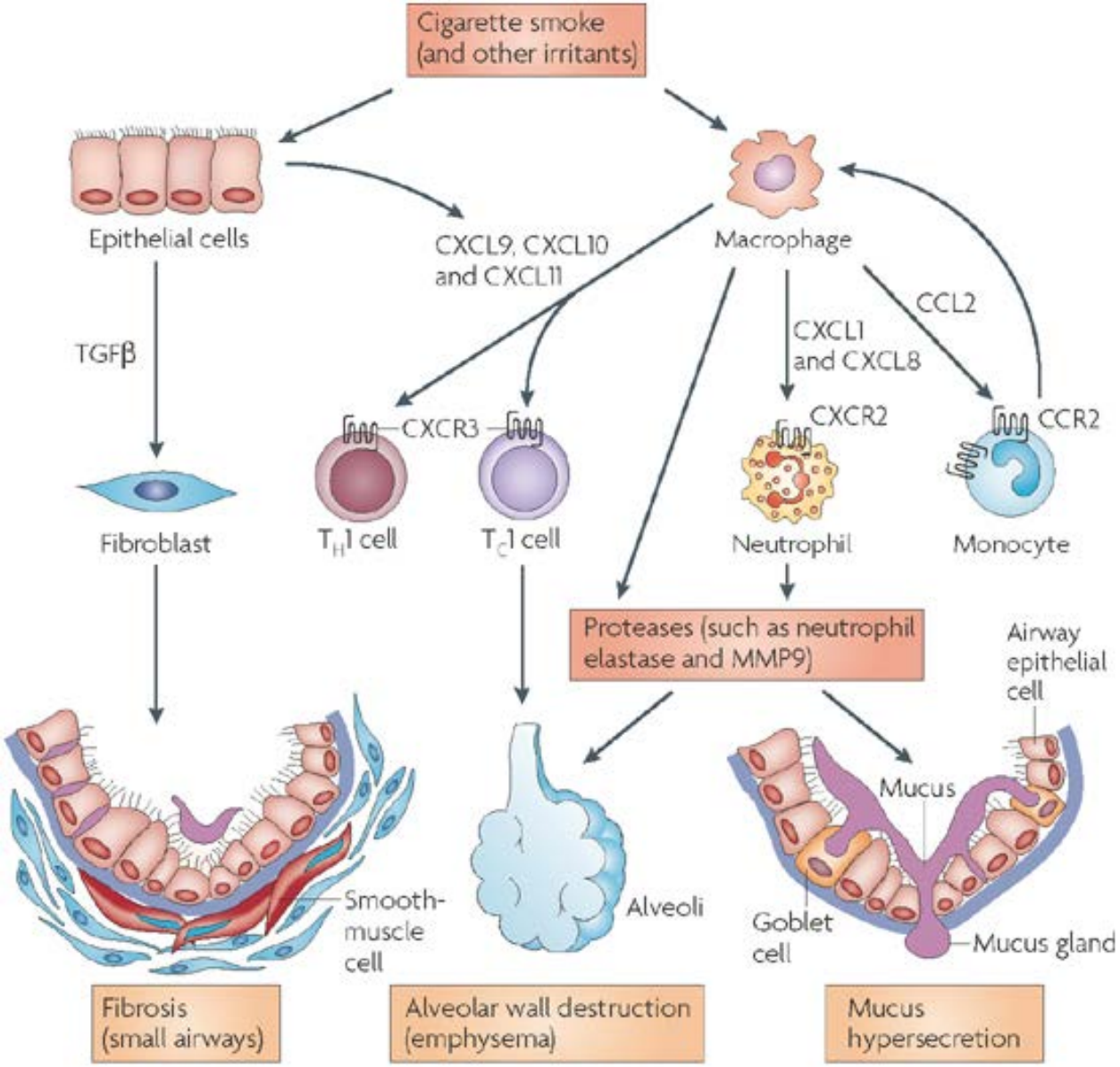
→ results in largely irreversible and progressive airflow limitation.

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

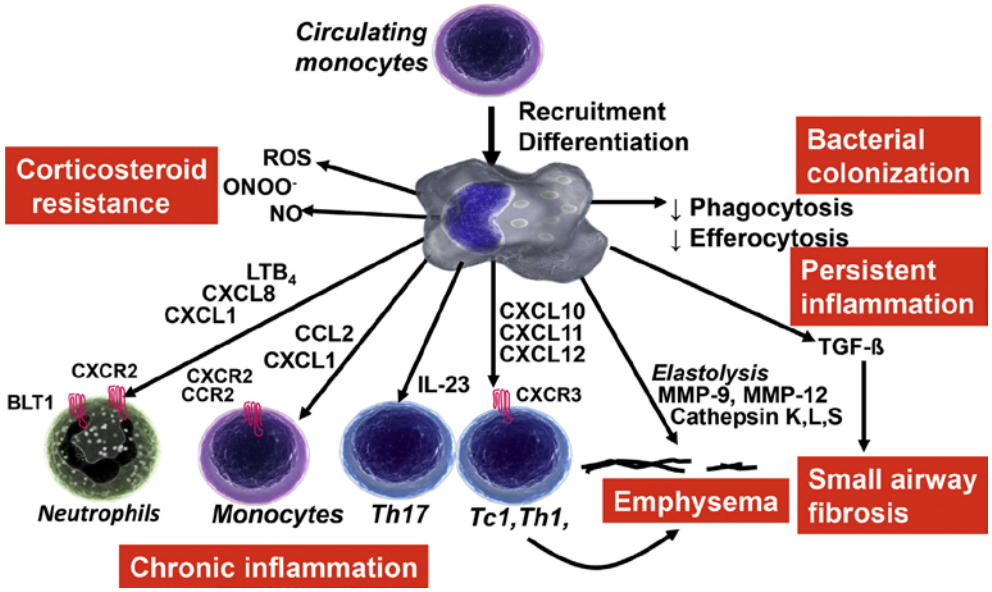
2 major pathological processes

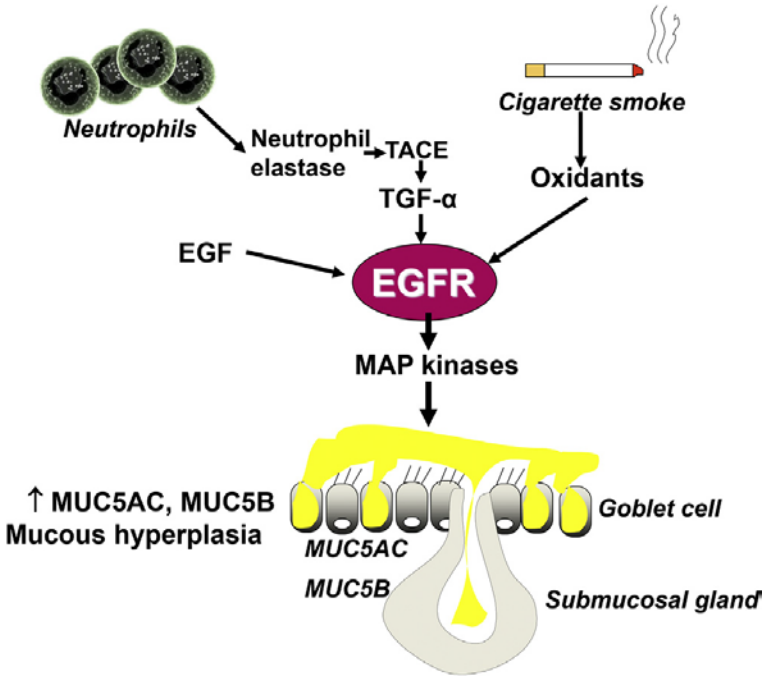
1) remodeling and narrowing of the small airways
2) destruction of the lung parenchyma with loss of the alveolar attachments
   → emphysema formatuion

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
CXCL9, CXCL10 and CXCL11
Macrophage
TGFβ
CXCL1 and CXCL8
CCL2
CXCR3
CXCR2
CCR2
Fibroblast
T_H1 cell
T_C1 cell
Neutrophil
Monocyte
Proteases (such as neutrophil elastase and MMP9)
Airway epithelial cell
Mucus
Smooth-muscle cell
Alveoli
Goblet cell
Mucus gland
Fibrosis (small airways)
Alveolar wall destruction (emphysema)
Mucus hypersecretion
Nature Reviews | Immunology

Alveolar Macrophage vs Neutrophile Granulocyte





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

Parameters	COPD patients	Normal controls
Basic parameters		
Number of subjects (n)	50	29
Age (mean years)	66.7	61.6
Gender (men/women)	36/14	19/10
BMI (kg m^2)	27 (14–42) ($n = 39$)	–
Clinical parameters		
GOLD (I/II/III/IV/nd)	(3/18/8/9/12)	–
IL-6 (pg/ml)	6 (0–41) ($n = 38$)	–
TNF-α (pg/ml)	2 (0–21) ($n = 37$)	–
CRP (ng/ml)	8 (1–75) ($n = 34$)	–
Vitamin D-1,25-OH (ng/ml)	50 (22–84) ($n = 39$)	–
Pulmonary function tests		
VC%	77 (44–134) ($n = 38$)	–
FEV_1 (%)	54 (23–113) ($n = 39$)	–
DL_{CO} (%)	53 (23–92) ($n = 35$)	–

Sera meassurements and COPD-induction in mice

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

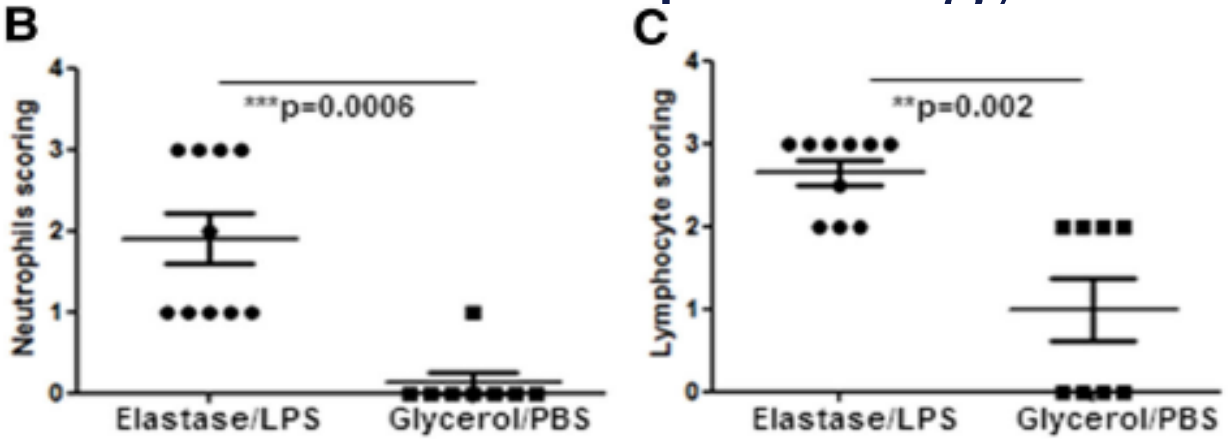
- COPD-like features were induced in both CD200KO and C57BL/6 wild-type mice

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

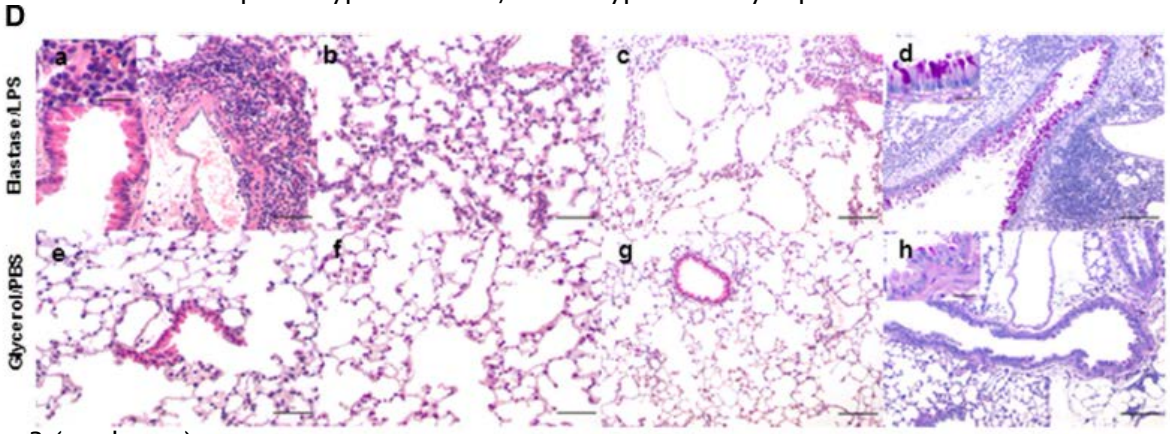
- negative control mice were exposed to 50% glycerol and phosphate-buffered saline (PBS)

- Mice were sacrificed one week after the final exposure, followed by lung extraction and histological staining

Neutrophil/Lymphocyte Influx Representative Histopathology



Induction of a COPD-like phenotype in C57BL/6 wild-type mice by repeated intranasal administration of elas



a inflammation score 3 (moderate)
b alv. macrophage infiltration: score 2,5 (mild to moderate),
c emphysema: score 3 (moderate)
d mucin staining in large bronchi: score 2 (strong cellular signal) and 2,5 (multifocal to diffuse)

e inflammation: score 0 (none)
f alveolar macrophage infiltration: score 0 (none)
g emphysema: score 1 (minimal)
h 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

correlation of COPD-relevant clinical parameters with serum sCD200 levels revealed a significant positive correlation between sCD200 and IL-6

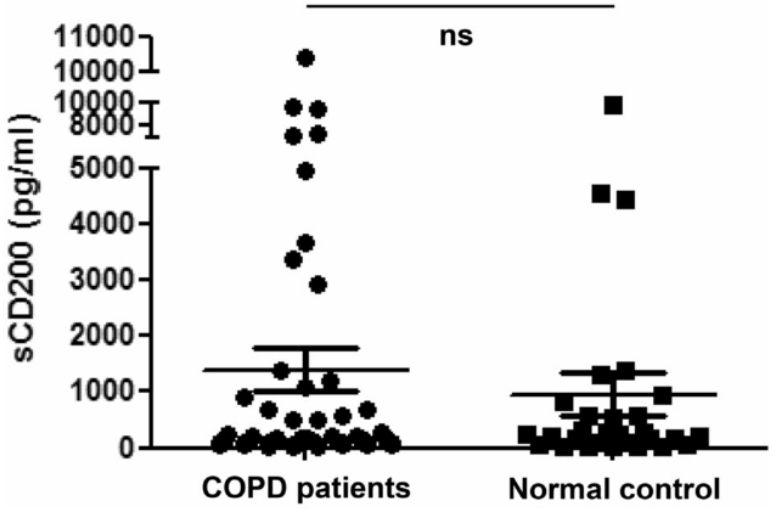
a trend towards a negative correlation with vitamin D-1, 25-OH in COPD patients

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

Parameters	sCD200
Age (*P*/*r*)	0.04*/0.27
GOLD stage (*P*/*r*)	0.49/0.11
CRP (*P*/*r*)	0.20/0.22
Vitamin D-1,25-OH (*P*/*r*)	0.07‡/−0.28
IL-6 (*P*/*r*)	0.01*/0.38
TNF-α (*P*/*r*)	0.83/−0.03

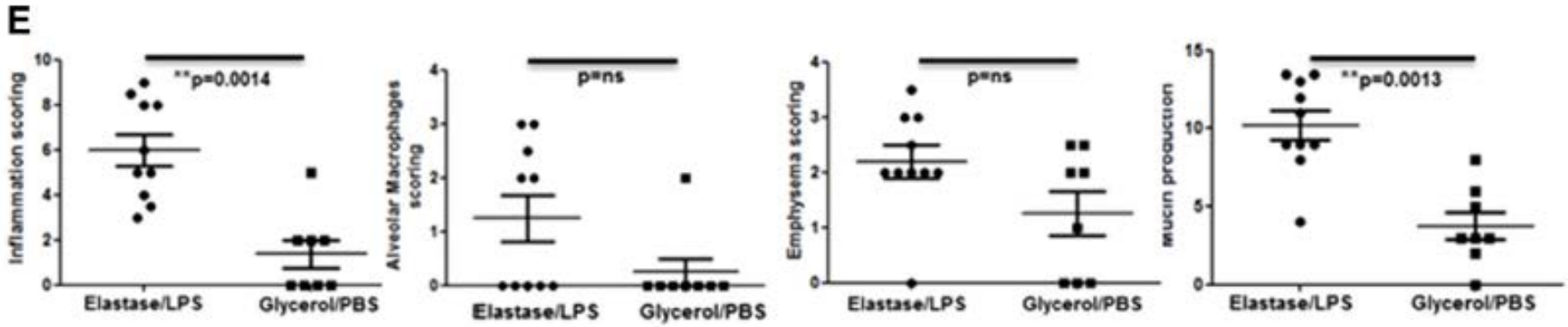
GOLD global initiative for chronic obstructive lung disease, *CRP* C-reactive protein, *IL* interleukin, *TNF-α* tumor necrosis factor (alpha)

* Significant *P* values, ‡ Tendencies



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.

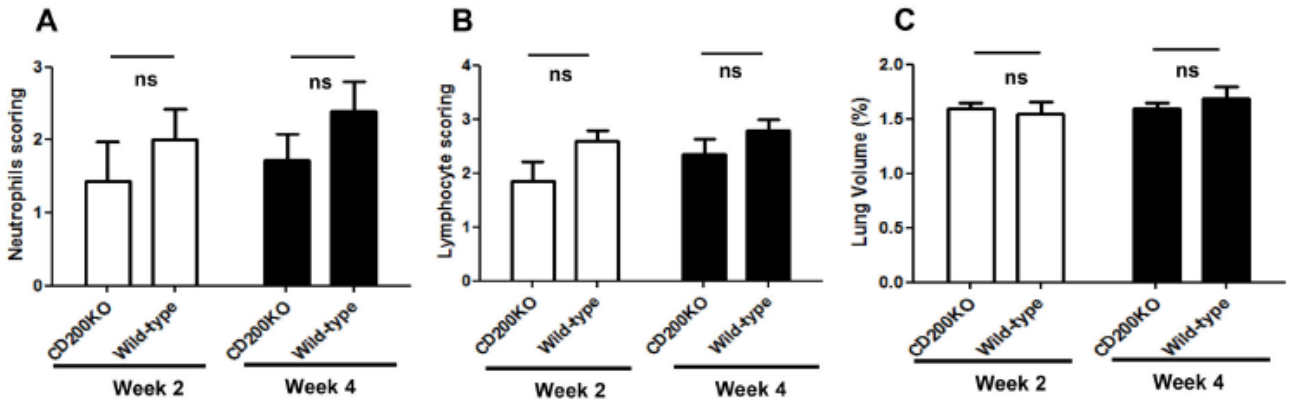
CD200 Deficiency Does Not Affect the Onset of COPD-Like Features in Mice Following Elastase/ LPS Exposure

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

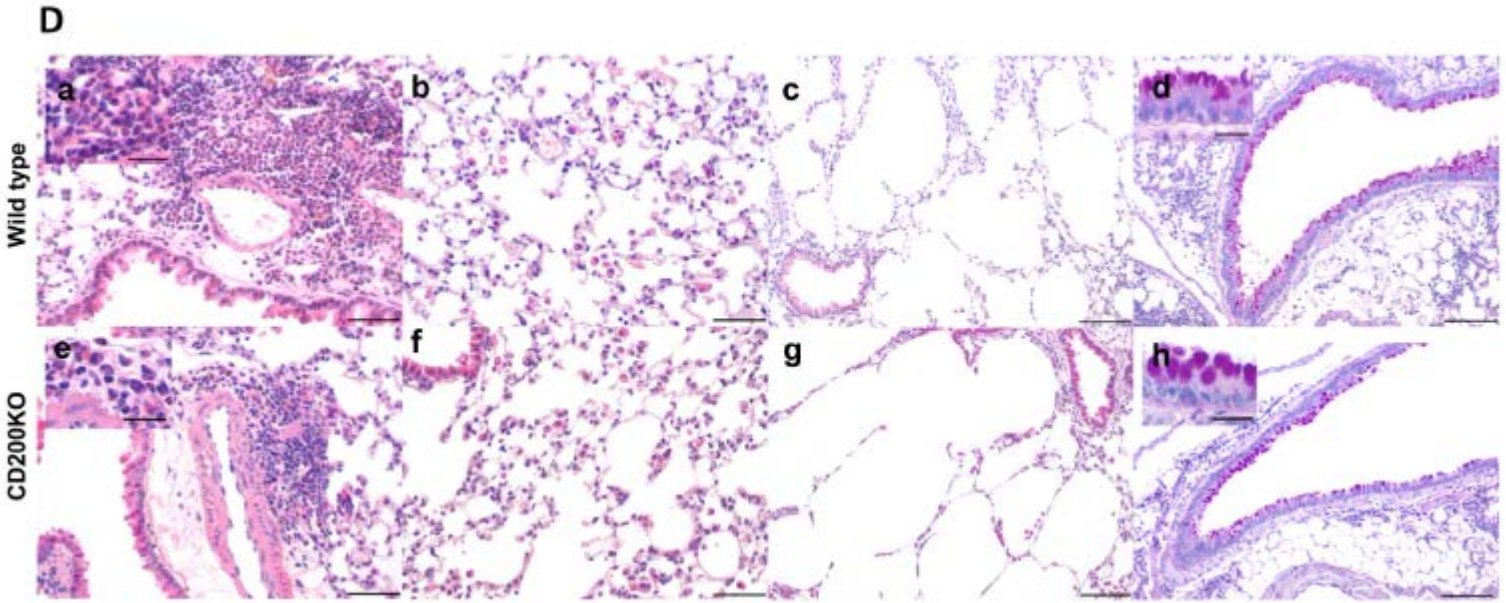
→ CD200/CD200R inhibitory axis

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

slightly reduced inflammation score in CD200KO mice compared to control mice



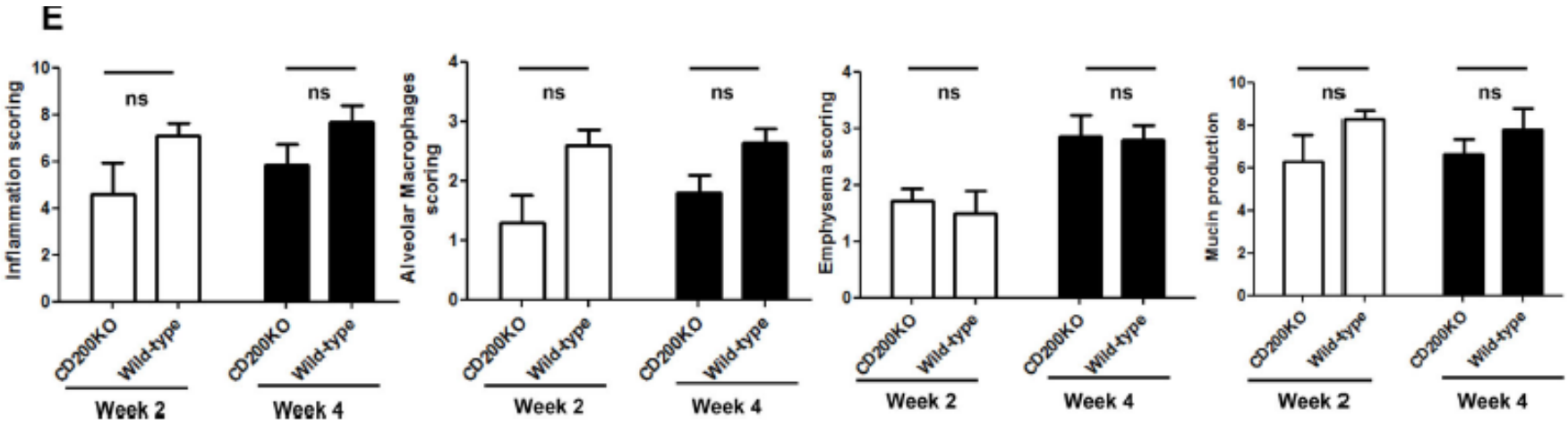
Histopathology
CD200 Deficient vs control mice after elastase/LPS exposure



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



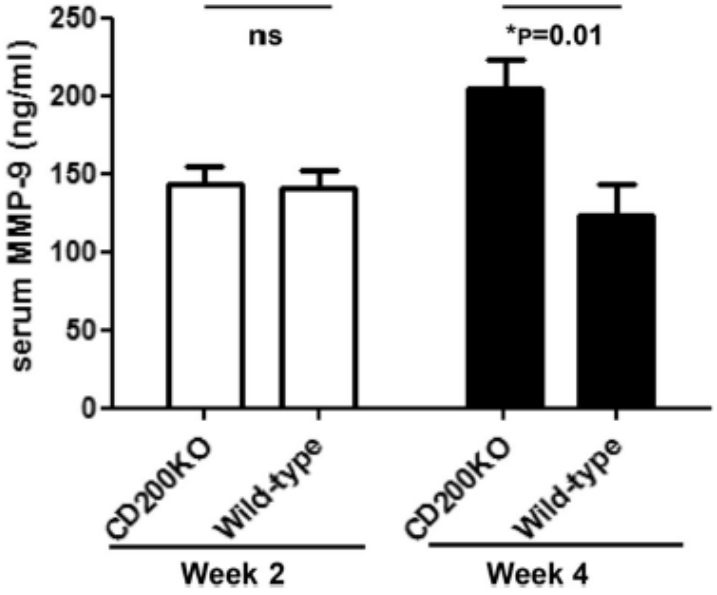
→ CD200/CD200R axis does not seem to play a dominant role in the early development of a COPD-like phenotype

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 ?