

RESEARCH ARTICLE

LUNG DISEASE

Pulmonary transplantation of macrophage progenitors as effective and long-lasting therapy for hereditary pulmonary alveolar proteinosis

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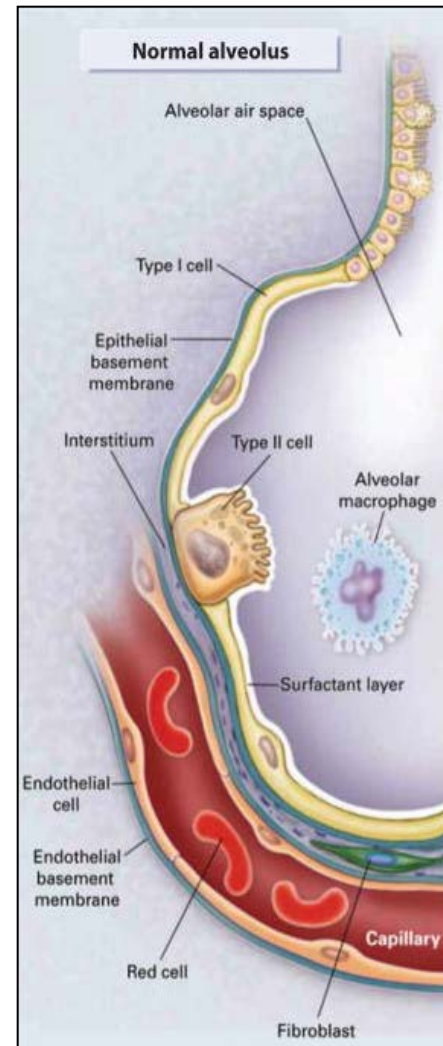


Alveolar Macrophage (AM)

Primary cell in lung defense

Phagocytosis of:

- invading microorganisms
- surfactant proteins



Genetics

Hereditary (herPAP): mutations in the **CSF2RA** or **CSF2RB** genes

- Defect in **GM-CSF** signaling
- Blockade in terminal alveolar macrophage differentiation
- Ineffective phagocytosis
- Protein aggregation
- Accumulation of surfactant

Consequences

- Massive protein accumulation in the lungs
- Respiratory failure
- Susceptibility to infections

- Rare
- Life-threatening
- Onset: pre-school age



Analysis of the therapeutic potential of intrapulmonary transplanted macrophage progenitors

Mouse model of organotropic transplantation of myeloid progenitor cells in PAP

Csf2rb^{-/-} mice

- knock-out for CSF2RB gene
- PAP model



B6 strain

- carries CD45.1 isotype
- enables tracing of cells

huPAP mice

- targeted replacement of murine by human IL-3/GM-CSF



NSG mice

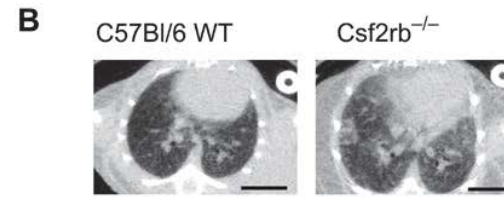
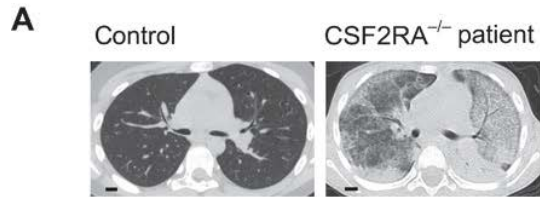
- NOD scid gamma
- immunodeficiency enables transplantation

Csf2rb^{-/-} mice display all main features of human herPAP

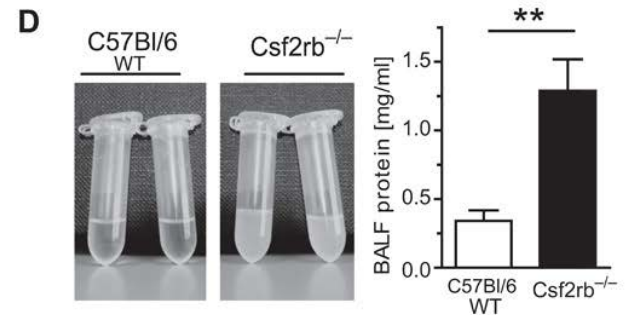
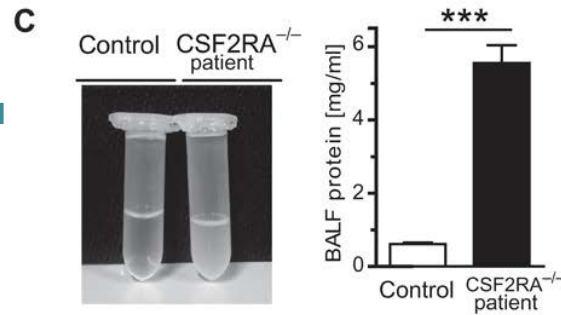
3-year girl

mouse

Chest computed tomography (CCT)



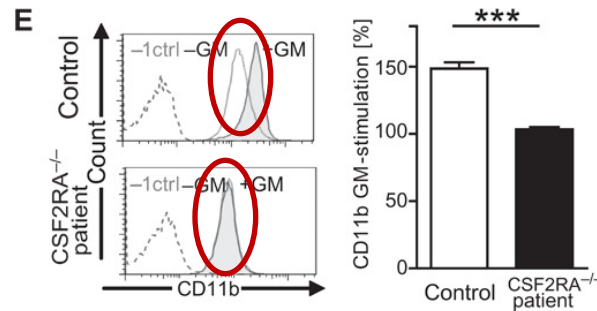
BAL fluid turbidity and proteinosis



Csf2rb^{-/-} mice display all main features of human herPAP

Stimulation of hu-granulocytes or m-bone marrow cells with GM-CSF

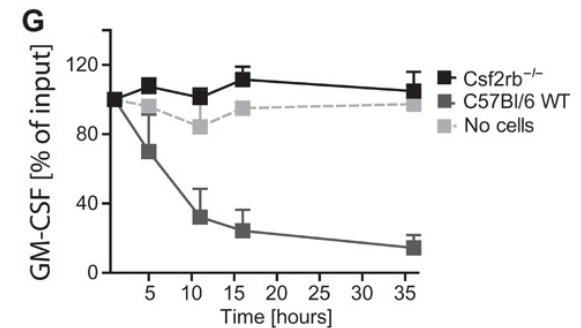
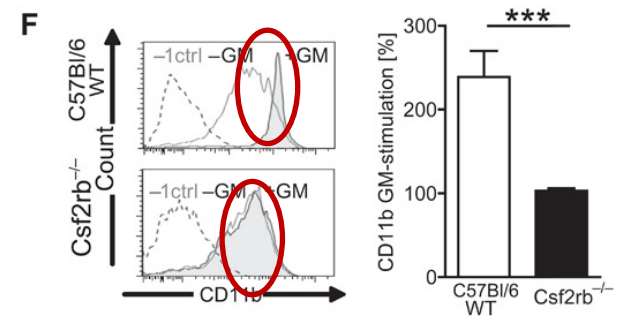
3-year girl



→ No upregulation of CD11b expression

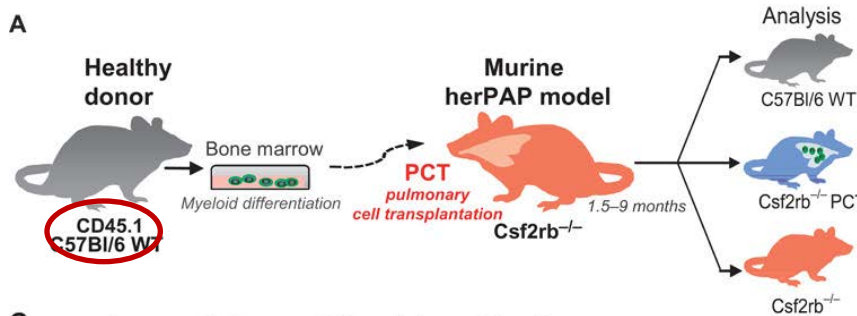
Consumption of GM-CSF in murine cells

mouse

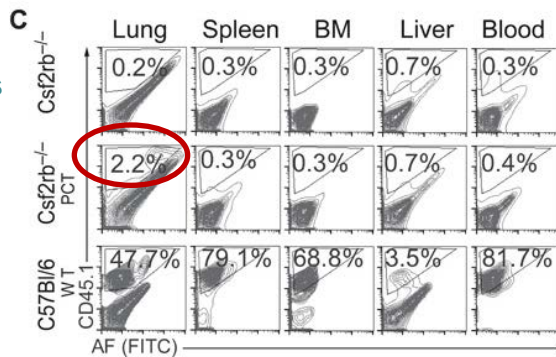
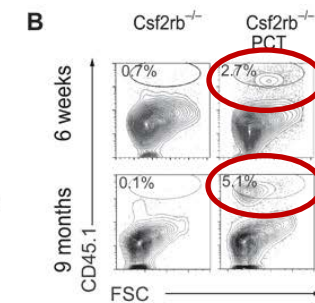


Csf2rb^{-/-} Mouse model mimics human disease in regard to protein accumulation and defect in GM-CSF signaling

Experimental design



Engraftment of CD45.1 donor-derived cells



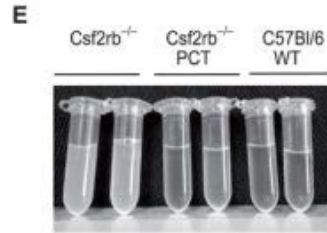
Donor-derived cells exclusively found in the lungs

Donor-derived cells can be detected up to 9 months after transplantation

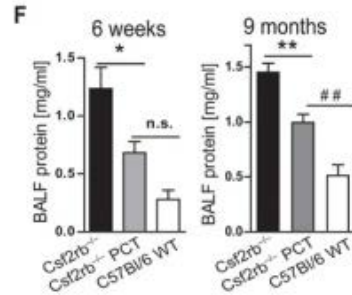


Improvement of PAP-phenotype

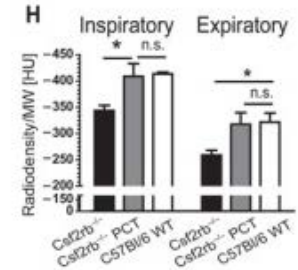
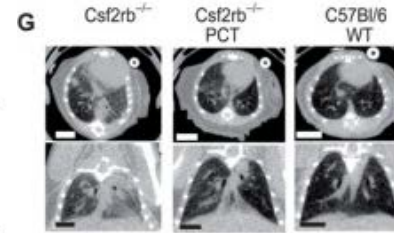
BAL fluid turbidity



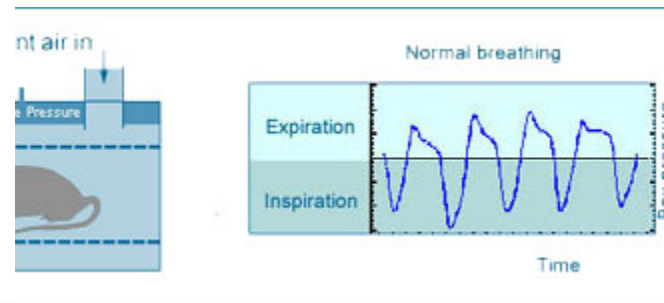
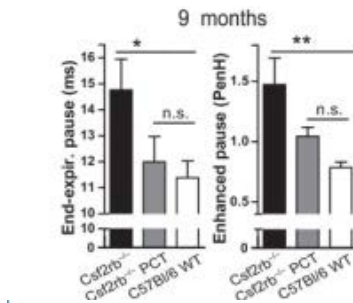
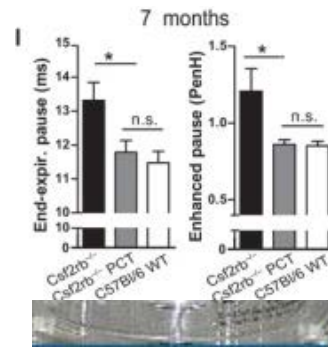
Proteinosis



CCT



Lung function – whole body plethysmography



Proteinosis is resolved and respiratory function is restored

Differentiation of transplanted cells

CD45.1 isolated from *Csf2rb*^{-/-} recipient lungs

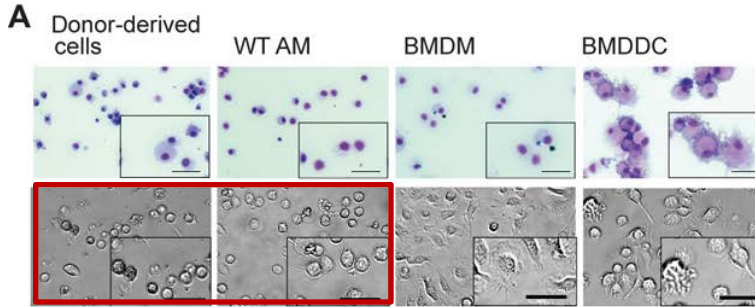
BM of CD45.1⁺ donor mice

B6 mice

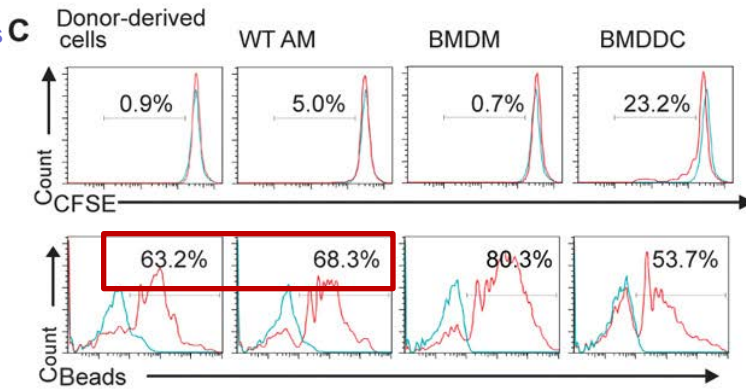
+M-CSF
macrophage

+GM-CSF
dendritic cells

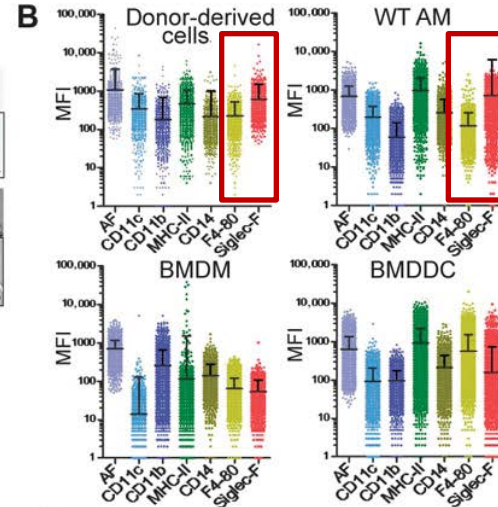
Morphology



Phagocytosis – uptake of beads

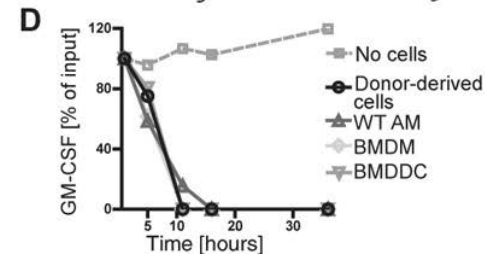


FACS staining for macrophage marker



FACS staining for macrophage marker

GM-CSF consumption in culture



GM-CSF consumption in culture

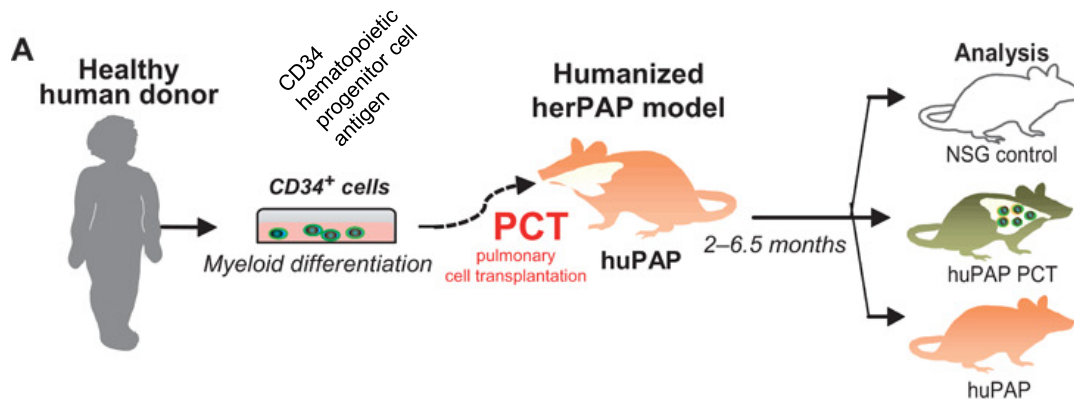
CD45.1+ isolated from *Csf2rb*^{-/-} recipient lungs undergo differentiation into functional macrophages

Transplantation of human macrophage progenitors

Mouse model

- ✓ Long-term engraftment of donor cells
- ✓ Improvement of PAP phenotype
- ✓ Differentiation in functional macrophages

Also working for human cells?



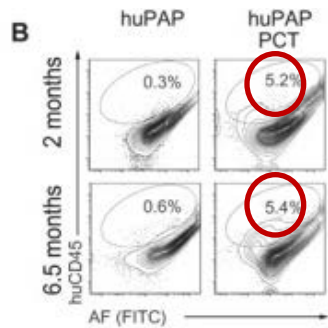
NSG mice

- NOD scid gamma
- immunodeficiency enables transplantation

huPAP mice

- targeted replacement of murine by human IL-3/GM-CSF

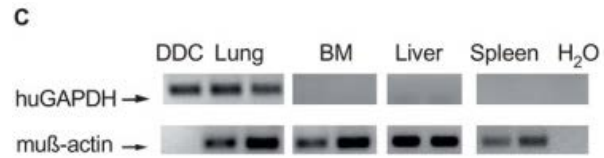
Transplantation of human macrophage progenitors



huCD45⁺ cells in recipient mice

Long-term engraftment of donor cells ✓

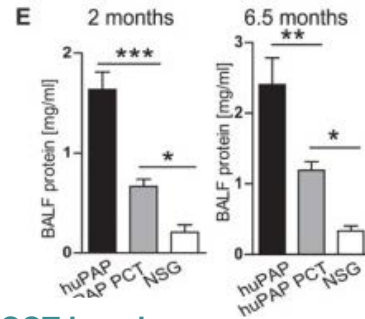
RT-PCR with primers for human or murine cells



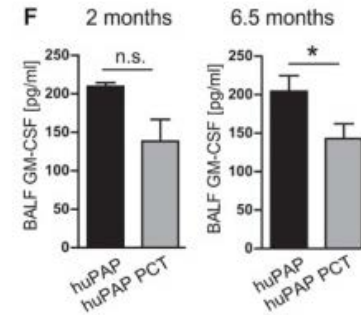
Lung-specific engraftment ✓

Lung function improvement ✓

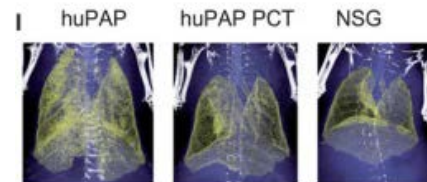
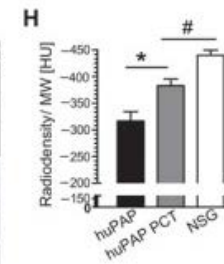
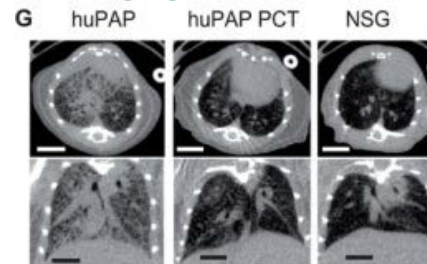
Proteinosis



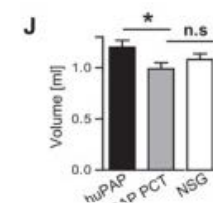
GM-CSF accumulation



CCT imaging



3D rendering of CCT data depicting lung density and structural changes

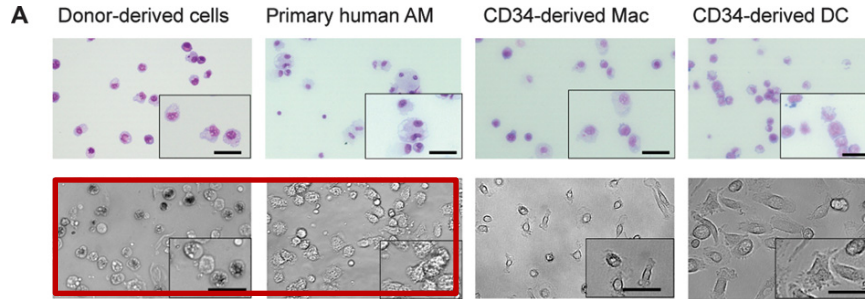


Inspiratory volume



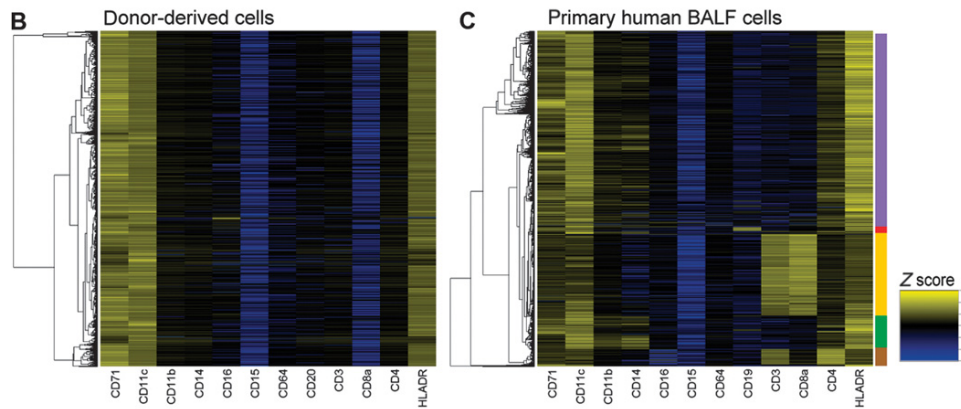
Differentiation of transplanted cells

Morphology



Donor-derived cells resemble primary human AM morphology

Heat map

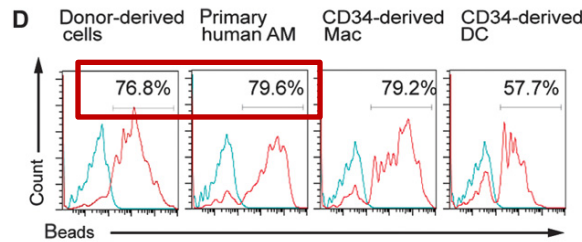


(B) Transplanted cells express markers characteristic for AM

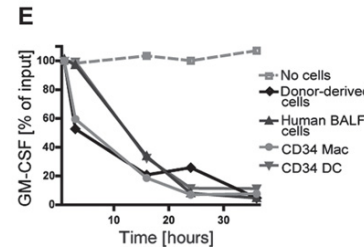
- high expression of CD71, CD11c, and MHC-II
- low expression of CD11b, CD14

(C) Hierarchical clustering of CD45⁺: clear clusters of T cells, B cells and macrophages

Phagocytosis – uptake of beads



GM-CSF consumption in culture



Two mouse models for organotropic transplantation of macrophage progenitors cell in herPAP were established

- single transplantation
- exclusive pulmonary engraftment
- *in situ* differentiation
- long-term persistence of donor-derived cells

no monitoring beyond 9 months age

- transplantation of progenitor cells may reduce risk of secondary cancer development compared to HSC transplant
- HSC-based gene therapy for herPAP in $Csf2br^{-/-}$ mice model

**Thank you for
your attention!**