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

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Science and Translational Medicine, 2014
Alveolar Macrophage (AM)

Primary cell in lung defense

Phagocytosis of:
- invading microorganisms
- surfactant proteins
Pulmonary Alveolar Proteinosis (PAP)

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

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

B6 strain
- carries CD45.1 isotype
- enables tracing of cells

NSG mice
- NOD scid gamma
- immunodeficiency enables transplantation
Csf2rb\(^{-/-}\) mice display all main features of human herPAP

3-year girl

A

Chest computed tomography (CCT)

BAL fluid turbidity and proteinosis

B

C

D

mouse

C57Bl/6 WT

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

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

3-year girl

\[ \rightarrow \text{No upregulation of CD11b expression} \]

Consumption of GM-CSF in murine cells

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

Proteinosis is resolved and respiratory function is restored
Differentiation of transplanted cells

**Morphology**

- Donor-derived cells
- WT AM
- BMDM
- BMDDC

**Phagocytosis – uptake of beads**

- Donor-derived cells
- WT AM
- BMDM
- BMDDC

**FACS staining for macrophage marker**

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?

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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**

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

- **Proteinosis**
  - 2 months
  - 6.5 months

- **GM-CSF accumulation**
  - 2 months
  - 6.5 months

- **Lung-specific engraftment**

- **Lung function improvement**

- **Inspiratory volume**
Differentiation of transplanted cells

**Morphology**

(A) Donor-derived cells, Primary human AM, CD34-derived Mac, CD34-derived DC

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**

(D) Donor-derived cells, Primary human AM, CD34-derived Mac, CD34-derived DC

E) 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 differntiation
- 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