Role of Inflammation in Pulmonary Hypertension

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Prominent Fibroproliferative Changes are Observed in the Lung Vasculature of Infants With Pulmonary Arterial Hypertension (PAH)
**Traditional Hypothesis**

Injury

Resident Pulmonary Artery Cell Activation

Modulation of Resident Cell Phenotype

↑ Proliferation, ↑ Migration, ↑ Matrix deposition

Structural Vascular Remodeling

**Inflammatory Cells, Including Monocytes / Macrophages (CD68+), Accumulate In and Around Vascular Lesions of Patients with PAH**
Accumulation of Inflammatory (CD68+) Cells is Associated with Fibroproliferative Vascular Changes in Infants with PAH

Progenitor-like (CD133+, cKit+) Cells Accumulate in the Vascular Lesions of Patients with PAH
Complimentary Hypothesis

Traditional Hypothesis

Injury

Resident PA Cell Activation

Circulating Inflammatory & Progenitor Cell Recruitment

Modulation of Cell Phenotype

Direct & Indirect Contribution to Cell Proliferation, Differentiation, Fibrosis

Pulmonary Vascular Remodeling

Experimentally-Induced Pulmonary Vascular Remodeling Varies Considerably Between Animal Species

Perivascular Inflammation

Vascular Remodeling (Medial / Adventitial Thickening)

Mouse  Rat  Calf

CD11b

α-SM-actin
Sustained Hypoxia Induces Robust Accumulation of Monocytes in the PA Wall

Hypoxia-induced Accumulation of Monocytes in the PA Adventitia is Time-Dependent
Observation: Perivascular accumulation of monocytes is closely associated with fibrosis in PH

Question: What is the “cellular link” between monocytic accumulation and fibrosis in PH?

“Fibrocytes”

A subset of circulating leukocytes that co-express leukocytic (CD45, etc) and mesenchymal (collagens) markers and:
- Rapidly recruited to the site of tissue injury;
- Transition into fibroblasts / myofibroblasts;
- Produce extracellular matrix proteins;
- Secrete inflammatory and pro-angiogenic factors

(Bucala et al. 1994; 2005; Abe et al. 2001; Hartlapp et al. 2001; Yang et al. 2002; Schmidt et al. 2003; Metz 2003, etc.)
Some of the Recruited Fibrocytes Transition into a "Myofibrocyte" Phenotype (CD68+ / α-SM-actin+)

Two Experimental Approaches Demonstrate that Fibrocytes in the Hypoxic Vessel Wall Originate from Circulation / Bone Marrow.

Approach 1:
- In vivo Dil-labeling of monocytes in circulation via Dil-liposomes;
- Identifying Dil-labeled monocytes in the adventitia of pulmonary (but not systemic) arteries of hypoxic rats

Approach 2:
- Using a natural bovine XY/XX chimera (freemartin calf), identify Y-chromosome+ cells recruited to hypoxic PA wall.

**What is the Contribution of Circulating Monocytes / Fibrocytes to Pulmonary Vascular Remodeling and Fibrosis?**

**Approach:** *In Vivo* Depletion of Circulating Monocytes / Fibrocytes via i.v. Injections of Liposome-Encapsulated Clodronate

- When i.v.-injected, clodronate-liposomes are taken up only by circulating phagocytic cells (monocytes/macrophages/fibrocytes) - and intracellular excess clodronate induces apoptosis of these cells
- Not toxic to non-phagocytic cells, no reduction in the number or activation of T-cells

**Hypoxia-Induced Perivascular Remodeling & Fibrosis are Attenuated by Depletion of Circulating Monocytes/Fibrocytes via Clodronate-Liposomes (Clo-L)**

What are the Local Mediators that:

1. **Induce Recruitment and Retention of Monocytes / Fibrocytes in the PA Wall?**

![Image of PA wall with CD14+ and fibrocytes]

2. **Participate in the Differentiation of the Recruited Fibrocytes into Mesenchymal (collagen-producing) Cells?**

![Image of CD45, procollagen I, and composite PAS staining]

**Hypoxia Induces PA-Specific mRNA Upregulation (blue bars) of Chemokines & Receptors Known to be Involved in Monocyte Recruitment and Activation**

Moreover, regression of PH vascular remodeling is associated with downregulation of mRNA for these chemokines / receptors (green bars).
Hypoxia Induces PA-Specific mRNA Upregulation (blue bars) of Adhesion Molecules, Growth and Differentiation Factors Known to be Involved in Monocyte Retention and Fibrocyte Differentiation

- Osteopontin
- VCAM-1
- TGFβ1
- Endothelin-1
- PDGF-A
- VEGF

Hypoxia Induces Upregulation (at the protein level) of Chemokines, Adhesion Molecules, and Differentiation Factors Known to be Involved in Monocyte / Fibrocyte Recruitment, Retention and Differentiation

- SDF-1
- OPN
- VCAM-1
- TGFβ1
- VEGF
- Fibronectin (ED-A isoform)
- Hsp-47 (pro-Collagen I)
Why is Inflammation Largely Perivascular in Nature?

Hypothesis: Fibroblasts Orchestrate the Initiation and Perpetuation of Inflammation in the Vessel Wall

Fibroblasts From the Hypoxic Hypertensive Calves Exhibit in vitro A “Constitutively” Activated Proinflammatory Phenotype

PA Adventitial Fibroblasts From Hypoxic Hypertensive Calves Exhibit Constitutive Activation of pAkt and p38MAPK Under Basal Conditions
Fibroblasts From Hypoxic Hypertensive Calves Induce Greater Adhesion of Monocytes Than Control Fibroblasts

Rapamycin (mTOR inhibitor) Attenuates Monocyte Recruitment and Remodeling in Hypoxic Rats
Inhibition of the SDF1/CXCR4 Axis Prevents and Reverses PH in Neonatal Hypoxic Mice

Myocardial Inflammation and Fibrosis Contribute to Right Ventricular Dysfunction in Chronically Hypoxic Calves
Conclusions/Speculations

- Vascular remodeling in PAH is due, in part, to recruitment of circulating inflammatory/progenitor cells.
- The pathways involved in the recruitment and retention of these cells may serve as selective pharmacologic targets for PAH treatment.

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Question: What are the mechanisms through which the recruited inflammatory / progenitor cells contribute to the remodeling process?
**Cell Culture Model:** Non-Resident (Recruited = “R”) Cells Have Been Isolated from Distal PA of Hypoxic Calves

In Culture, “R”-cells Transition (Differentiate) into Myofibroblasts (α-SM-actin+)
“R”-Cells Exhibit Augmented Proliferative (including Autocrine Growth) and Migratory Capabilities

Proliferation

Migration

Autocrine Growth of “R” Cells is Due, in part, to PDGF and SDF-1 Production
“R”-Cells Produce Potent Pro-Mitogenic Activity for Resident PA Cells (SMC & Fibs)

Pro-Mitogenic Effects of “R”-Conditioned Medium are Due, in part, to PDGF(s), SDF-1 and S100A4 (effect of neutralizing Abs)
In culture, “R”-Cells Express mRNA for Progenitor-Associated Markers and Inflammatory Mediators

![Graphs showing relative gene expression for CD34, CD73, cKit, IL-6, MCP-1, and OPN comparing “S” and “R” cells.]

What are the Routes of Delivery of Circulating Cells to the Pulmonary Vasculature?

Appearance of CD11b+ Cells in Adventitia Correlates with eNOS+ Vasa Vessels

72 hr Hypoxia
Extensive Neovascularization is Observed in PA Adventitia of Hypoxia Calves

Marked expansion of the Bronchial Circulation

Davie et al. Am. J. Physiol., 2004