Pulmonary Arterial Hypertension and Schistosomiasis

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2nd International Conference Neonatal and Childhood Pulmonary Vascular Disease, UCSF 2009

Schistosomiasis Epidemiology

- Third most prevalent parasitic dx
- 20% of the endemic population at risk
- Worldwide prevalence >200 million (600 m at risk)
- 200,000 deaths/yr in sub-Saharan Africa alone
- 1% of infected untreated individuals die
- Majority of disease burden is chronic morbidity
  - WHO: >4.5 million disability-adjusted life years

Acknowledgements

- PVRI: Ghazwan Butrous (http://pvri.info/)
- Colorado: Brian Graham
- Recife, PE, Brazil: Angela Machado
- NIH: Tom Wynn, Alan Cheever, Margaret Mentink-Kane
- Hopkins: Hunter Champion
Chronic Disease

- *S. mansoni* and *S. japonicum* → GI system
  - Granuloma formation around schistosome eggs retained in tissues
  - Chronic diarrhea, abdominal pain, colon polyps.
  - Severe (15%): hepatic fibrosis, portal hypertension, splenomegaly
    - Not liver cirrhosis: inflammation in presinusoidal areas

Pulmonary Hypertension and schistosomiasis

- Occurs in those with hepatosplenic schistosomiasis (PH: 7-30% of HS; HS: 30% of all patients)
- No differences in HS between PH vs. non-PH; also in tx vs. non-tx
- Up to 80% of severe PH in centers of reference in Brazil.
HUMAN PAH BY SCHISTOSOMIASIS

IPAH: Endothelial cell processes, proliferation
Early Lesions:

Concentric Lesions:

Immunopathology of schistosomiasis

Ghazwan et al, Circulation, 2008

Pulmonary arterial remodeling induced by a Th2 immune response

IL4 and IL13 dependent
**Experimental Goals (Brian Graham)**

- Develop and demonstrate applicability of a mouse model of the human disease
- Define the biochemical/morphological characteristics of the lung granulomatous response
- Investigate signaling pathways: interface between the granuloma and the adjacent vascular remodeling narrowing-IL13/downstream signaling

**Mouse Model: schistosomiasis**

- **Mouse models:**
  - Black 6 wild-type
  - IL13Rα1 knockout
  - IL13Rα2 knockout
- **Schisto exposure methods:**
  - Ova tail vein injection
  - Cutaneous exposure to cercariae
  - Sensitization to IP ova antigens, followed by ova tail vein injection (not used here)

TA Wynn lab at NIH (Margaret Mentink-Kane)

IL13Rα1ko: Millennium Pharmaceuticals
IL13Rα2ko: Wyeth Pharmaceuticals

**Mouse model of lung schistosomiasis**

- **Model #1**
  - Purified ova
  - Inject
  - Cercariae
  - Infected mouse
  - Worms

- **Model #2**
  - Infected mouse
  - Purified ova
  - Inject
  - Cercariae
  - Infected mouse
  - Worms
Mouse Pathology

IL4 and IL13 Receptors

- Receptor 1:
  - IL4Rα
  - Binds IL4 only

- Receptor 2:
  - IL4Rα + IL13Rα1
  - Binds IL4 and IL13
  - Found on fibroblasts, smooth muscle cells, epithelial cells
  - Regulates tissue remodeling, fibrosis, eosinophil chemotaxis
  - STAT-6 downstream signaling

- Receptor 3:
  - IL13Rα2
  - Binds IL13 only; higher affinity than IL4Rα + IL13Rα1
  - No apparent downstream signaling: decoy receptor

Overall Design

- Set 1
  - 2 wt (controls)
  - 5 wt + eggs
  - 3 IL13Rα1 ko + eggs
  - 4 IL13Rα2 ko + eggs
  - 5 wt + cercariae
  - 7 wt (external controls)
  - Ova injected by tail vein: 8 day delay
  - Cercariae percutaneous infection: 99 day delay
  - PA and RV catheterization

- Set 2
  - 4 wt (controls)
  - 3 wt + eggs
  - 3 IL13Rα1 ko + eggs
  - 4 IL13Rα2 ko + eggs
  - Ova injected by tail vein: 8 day delay
  - RV catheterization only; no placebo injection in control mice
RV Catheterization

ANOVA: p = 0.495

1 cell volume ~ 200 \text{um}^3
Ova volume ~ 30,000 \text{um}^3 (50 \times 25 \times 25 \text{um})

Smooth muscle cell

ANOVA: p < 0.001
Pairwise multiple comparisons (vs. WT, no eggs)
Cell proliferation

Fibroblasts and Collagen

9 days-eggs

99 days cercaria

Apoptosis

WT + Ova: TUNEL, DAPI + Control (DNase I)

TGF beta signaling: pSMAD 2/3

WT + Ova

pSMAD 2/3
**Conclusions:**

**Human**
- Schistosomiasis induced PAH: the most frequent form of PH
- Overall incidence: 10-20% of patient with HS form

**Experimental:**
- No clinical PAH in small numbers of mice
- **IL13Ra1 ko:**
  - DECREASED: granuloma volume; cell proliferation; Smooth muscle hyperplasia / hypertrophy
- Fibroblasts appear later in the disease course
- Little apoptosis
- TGF-beta and pSMAD 2/3 signaling
  - Not significant in smooth muscle cells

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**Th1 vs. Th2 Inflammation**

- Early post-infection: Th1 response (parasite)
- Ova laying -> Th2 response
  - IL-4, IL-5, IL-13 levels are increased
- Administering IL-12 prevents the Th2 response
  - Prevents hepatic fibrosis
- IL-13 is significant for liver fibrosis
  - Blocking or knocking out the pathway decreases fibrosis