Classical Dendritic Cells Are Drivers of Hypoxia-Induced Pulmonary Hypertension

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Introduction

- Pulmonary hypertension (PH) is characterized by elevated right ventricle pressures, increased vascular remodeling and it is often fatal.
- Inflammation is a hallmark of PH and there is increasing evidence that bone-marrow derived cells are required for the development of PH.
- Dendritic cells (DCs) are professional-antigen presenting cells that scan and sense their tissue microenvironment, coordinating innate and adaptive immune responses.
- Classical dendritic cells (**cDCs**) are divided in two different subsets: cDC1 (CD11b-/CD103+) and cDC2 (CD11b+).
- Activated DCs modify their immediate tissue microenvironment by secreting chemokines and cytokines that attract other inflammatory cells, including monocytes/ macrophages.
- Three interstitial macrophage populations in the naïve lung have been described and characterized as **IM1, IM2, IM3**.
- There is a substantial body of evidence indicating that dendritic cells are orchestrators of the PH-related immune response, including their augmented presence around remodeled vessels in different etiologies of PH; however, *there are few studies that address pathogenic mechanisms in which these cells could participate in PH.*
- ***Our hypothesis is that bone-marrow classical lung dendritic cell populations, in the hypoxia setting, drive an inflammatory cascade, with monocyte recruitment and changes in the perivascular microenvironment, resulting in pulmonary vascular remodeling and Pulmonary Hypertension (PH).***

Methods

Experimental Design:

zbtb46 (zinc finger and BTB domain containing 46) is expressed exclusively by classical dendritic cells. We generated ZBTB46 DTR chimera mice (ZBTB DTR CH), which are depleted from cDCs upon diphtheria toxin (DT) injection. In addition, we used a ZBTB46^{GFP} reporter mouse for all our flow cytometry experiments. Animals treated with either vehicle or DT were hypoxia-challenged (10% FiO2 Denver altitude) for 7 days. Alternatively, we hypoxia-challenged the ZBTB46 DTR chimeras for 2 weeks to establish PH, and subsequently depleted cDCs (with DT). We then assessed right ventricle systolic pressure (RVSP) by right heart catheterization and monocyte recruitment by flow cytometry.

Results

FIG1: cDC2s are increased in the lungs after 3 days hypoxia

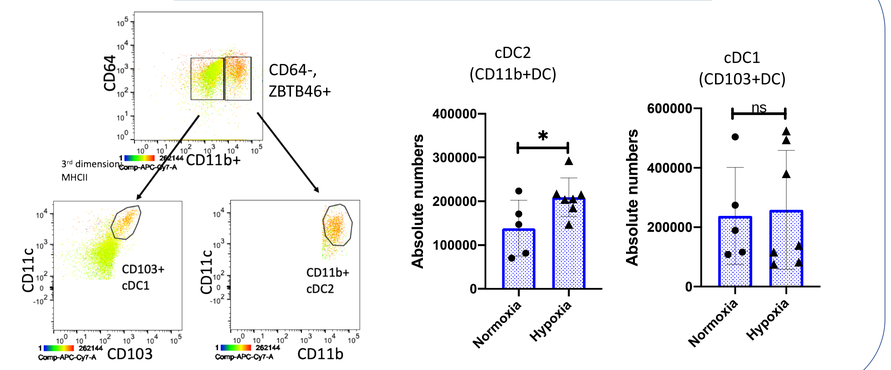


FIG2: cDC2s , but not cDC1s, are activated in the lungs after 3 days hypoxia

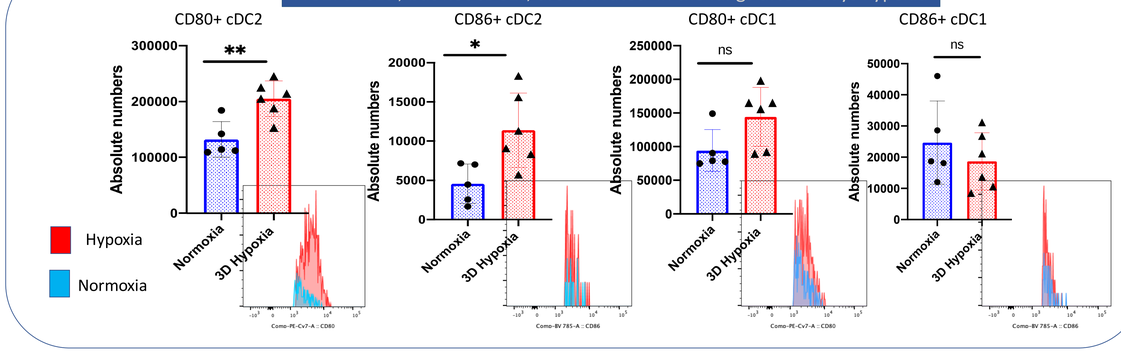


FIG3:Bone-marrow cDCs are determinant in driving PH

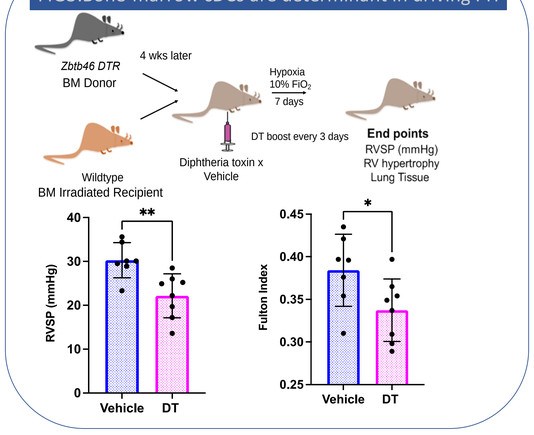


FIG4: Depletion of bone-marrow cDCs reverse established PH

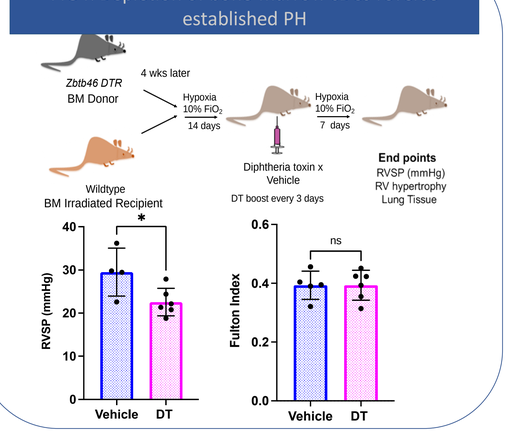
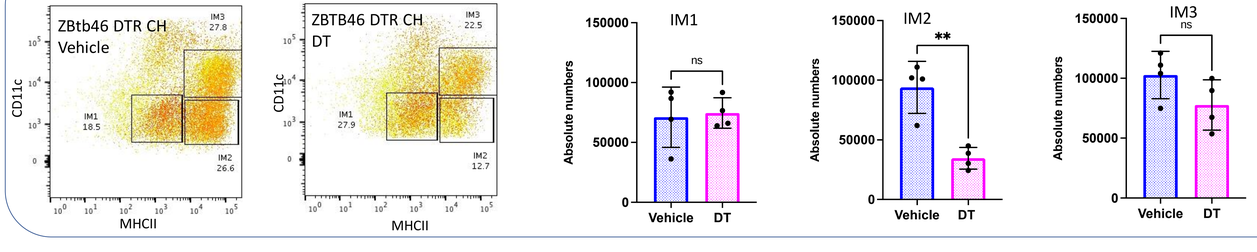


FIG.5: IM2 recruitment is dependent of bone-marrow derived cDCs



Discussion

- There is strong evidence that DCs are involved in perivascular inflammation, which is a hallmark of PH.
- Bone-marrow derived cDCs are increased after acute hypoxia challenge (FIG.1), indicating that they are involved in the initiation of the disease and inflammation.
- The cDC2 subset is primarily activated, with an increased number of cells and also expression of CD80 (FIG.2), indicating that this subset is involved in PH pathogenesis.
- **Mice deficient in bone marrow-derived cDCs are protected from hypoxia-induced inflammation and PH (FIG.3)**
- **Mice deficient in bone-marrow derived cDCs after established hypoxia-induced PH reversed their phenotype (FIG. 4).**
- Recruitment of classical dendritic cells into the lung during inflammation may drive a pro--inflammatory perivascular environment by directly secreting cytokines and chemokines, which may lead to the recruitment of pathogenic monocytes to the site. Remarkably, we observed ablation of the IM2 subpopulation after cDC depletion in *ZBTB* DTR chimeras , which strongly supports that cDCs are drivers of pathologic IM recruitment into the lungs

Conclusions

1- Bone-marrow derived cDCs are drivers in hypoxia-induced PH and depletion of bone marrow cDCs reverse established PH.

2- cDC2s are activated in acute hypoxia exposure and are most likely involved in hypoxia-PH pathogenesis.

3- Bone-marrow derived cDCs recruits IM2 to the lungs which may change the perivascular microenvironment leading to vascular remodeling and hypoxia-induced PH.

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