3D Biomimetic Human Tuberculosis Granulomas

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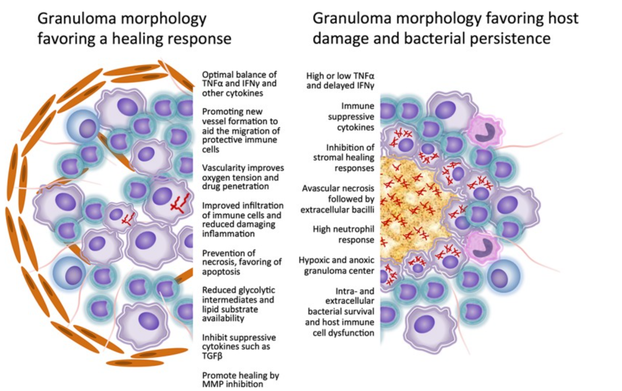
INTRODUCTION

BACKGROUND

Granulomas are the critical sites of host-pathogen interactions that determine tuberculosis (TB) disease outcomes.

Research suggests that certain factors in the granuloma microenvironment promote a healing granuloma morphology, while others favor the loss of the walled-off granuloma structure and eventually lead to bacterial dissemination.

The knowledge about such local environmental factors is sparse, mainly due to the lack of human experimental models. A better understanding of these could reveal novel therapeutic targets.



Local environmental factors determine whether a protective or destructive granuloma morphology is favored. Kiran, D. *Seminars in Immunopathology* 38, 2016.

HYPOTHESIS

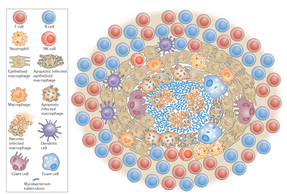
A 3D vascularized human granuloma model will allow us to uncover the local environmental factors determining the outcomes of TB infections.

OBJECTIVES

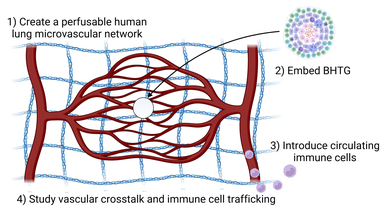
1) Develop a new experimental system that recapitulates key characteristics from human tissue granulomas

- Create a perfusable human lung microvascular network
- Create biomimetic human TB granulomas (BHTGs) for incorporation

2) Study the role of local environmental factors in TB progression and clearance



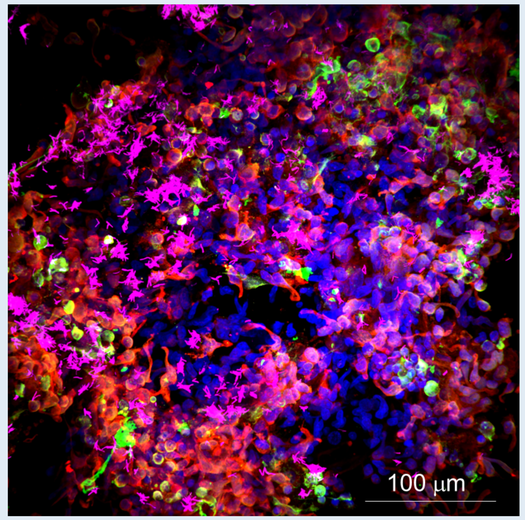
The composition of a mature tuberculous granuloma. Ramakrishnan, L. *Nat Rev Immunol* 12, 2012.



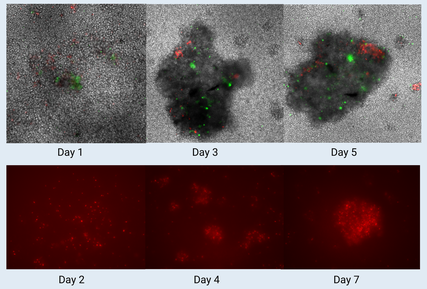
RESULTS

BHTGs recapitulate key characteristics of human tissue granulomas:

- Time-dependent organization into expanding three-dimensional structures
- A central core of macrophages containing live mycobacteria
- Peripheral localization of T lymphocytes
- Uninfected peripheral blood mononuclear cells (PBMCs) added after the initial BHTG formation migrate toward the lesion



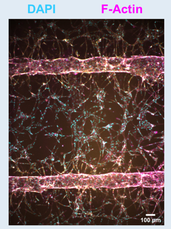
Confocal microscopy image of a BHTG. Macrophages are shown in red, T lymphocytes in green, BCG in magenta, and cell nuclei in blue.



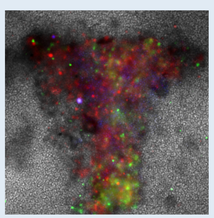
Serial imaging demonstrates time-dependent BHTG maturation.

Top: The well contains 13k monocytes infected with mCherry-expressing BCG and stained with CFSE (green signal) and 200k autologous PBMCs. Phase contrast images have been overlaid with fluorescent images.

Bottom: Fluorescent images showing the aggregation of 1.6k monocytes infected with mCherry-expressing *M. tuberculosis*.

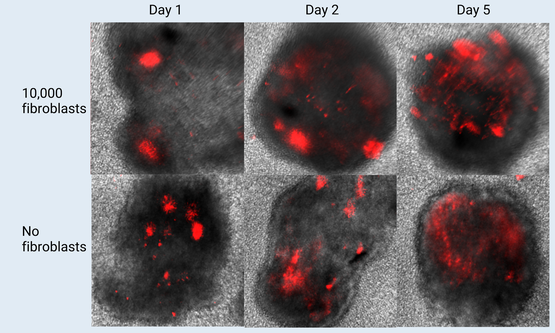


A new microfluidic platform permitting the perfusion of BHTGs has been developed for future studies of how TB granulomas affect vascular morphogenesis and endothelial activation.

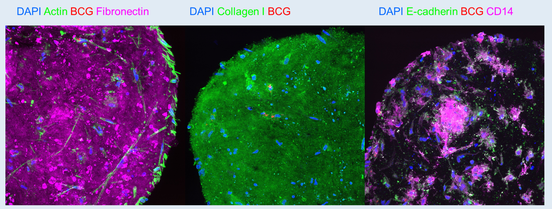


Fresh autologous PBMCs added on day 9 (blue signal) migrate to the BHTG. Image from day 14.

Human lung fibroblasts (hLFs) and collagen have been incorporated into the model, enabling studies of the interplay between the extracellular matrix and the granuloma, a largely unexplored field.

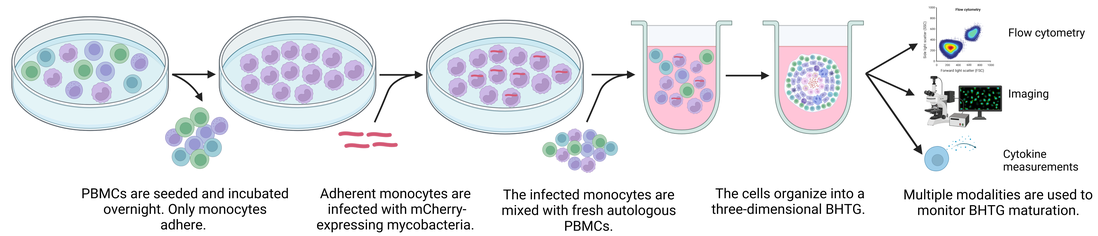


The addition of hLFs increases BHTG diameter on day 1. Later, the hLFs seem to organize more compactly.



The addition of collagen increases BHTG size from approx. 400 µm to 1-2 mm and the amount of acellular space between cells. hLFs organize in an outer layer. Aggregates of infected cells seem to occur within the BHTG.

METHODS



PBMCs are seeded and incubated overnight. Only monocytes adhere.

Adherent monocytes are infected with mCherry-expressing mycobacteria.

The infected monocytes are mixed with fresh autologous PBMCs.

The cells organize into a three-dimensional BHTG.

Multiple modalities are used to monitor BHTG maturation.

How BHTGs are made from human PBMCs.

CONCLUSION

1. BHTGs generated from human PBMCs represent a new experimental system that recapitulates the key features of human tissue granulomas.
2. Given the capacity to integrate extracellular matrix and microvasculature, this tissue-engineered model provides a promising avenue for investigating the local environmental factors that mediate the control and progression of granulomatous lesions.
3. Identifying critical determinants of TB disease outcome could reveal novel targets for host-directed TB therapies.

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