Title:	Multi-Scale 3 dimensional imaging of Mycobacterium tuberculosis granuloma.
Authors:	Karishma
Keywords:	Multi-Scale 3 dimensional imaging of Mycobacterium Mycobacterium tuberculosis granuloma Mycobacterium tuberculosis tuberculosis granuloma.
Issue Date:	May-2024
Publisher:	IISER Mohali
Abstract:	Tuberculosis (TB), caused by Mycobacterium tuberculosis(Mtb), primarily targets the lungs. Mtb can persist within host tissues for decades in structures called granulomas, often with- out causing active disease. These granulomas are dynamic, 3D clusters of immune cells formed in response to infection. While not the dominant cell type, granulocytes signifi- cantly contribute to granuloma formation, with their recruitment to the lungs being a hall- mark of active TB. This recruitment can promote both immune system damage and bacterial replication. Our study aimed to establish an imaging protocol for 3D visualization of granulomas to identify cellular and subcellular structures. We focused on the granulocyte marker Ly6G in a C3HeB/FeJ (Kramnik) mouse model after treatment with the frontline TB drug isoniazid (INH). Advanced CUBIC tissue clearing was used on Mtb-infected mouse lungs, creating transparent sections for confocal microscopy imaging. This 3D imaging allowed us to quantitatively analyze immune cell populations within multiple mice with drug-treated and untreated infections. By identifying regions within granulomas and separately selecting and counting immune cells, we could quantify the specific immune cell population within these lesions. Our optimized protocol successfully imaged and performed volumetric analysis of granu- lomas using immunofluorescent staining. The results suggest that INH treatment decreases both the number of Ly6G + cells in the granuloma cuff region and the overall expression of Ly6G, while also reducing the bacterial burden within these cells. This technique of clear- ing infected lung tissue provides a powerful tool for gaining a deeper understanding of the immune system's role in TB development.
URI:	http://hdl.handle.net/123456789/5764
Appears in Collections:	MS-19

Files in This Item:

File	Description	Size	Format	
Under Embargo period.odt		9.72 kB	OpenDocument Text	View/Open

Show full item record