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Microdissection approaches in tuberculosis research

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Abstract

Mycobacterium tuberculosis, a globally significant pathogen, results in active or latent tuberculosis. The granuloma is the characteristic lesion that offers insight into host-pathogen interactions in these distinct states. Microdissection provides a way to isolate and consequently investigate specific tissue sections. We review various techniques available and in use.

Keywords

Mycobacterium tuberculosis; non-human primate; granuloma; dissection; mesodissection

Introduction

Tuberculosis (TB), caused by aerosol infection with *Mycobacterium tuberculosis* (*Mtb*), results in around 1.5 million deaths annually(9). A majority of infected individuals control the infection in an asymptomatic condition known as latent TB infection (LTBI). Disease can result from either an acute early failure to control *Mtb* in the LTBI state, or due to immune failure following long-term LTBI.

A better insight into *Mtb* biology requires its study in highly reproducible models of human TB infection. Nonhuman primates (NHPs) are excellent models of human TB (5, 10, 16, 20-23) as they display the entire breadth of human pulmonary pathology and clinical infection (14). LTBI reactivation is usually driven by HIV co-infection, and can be modeled in primates with *Mtb*/Simian Immunodeficiency Virus (SIV) co-infection (21). There is a great potential to study the fundamental bacterial as well as host drivers of latency and reactivation in this model.

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The granuloma is the hallmark of *Mtb* infection (24). Primate granulomas are often necrotic, highly organized and evident in morphologically distinct forms. These include solid lesions, which contain lymphocytes and macrophages without necrosis; caseous lesions, with necrosis in the center surrounded by macrophages and lymphocytes; and calcified/mineralized granulomas. Cavitory lesions are associated with high bacterial numbers, but are not seen in murine models. There is clear evidence that the different clinical phases of the infection are driven by the evolution of the granuloma (24). Caseous, necrotic lesions provide a hypoxic environment. Compared to growth in normoxic environment, this shift likely has a profound impact on *Mtb* metabolism and physiology(13), which in turn, likely transcends into pathogenic differences. The pathogen physiologically undergoes distinct phases during different infective stages. Molecular cues may predict the different clinical phases of infection. For example, *Mtb* is predicted to undergo rapid replication, moderate replication in acidic conditions within macrophages and become non-replicatively persistent during long-term granulomatous residence(11). Therefore, different types of granulomas can serve as markers for bacterial physiology, metabolic state and thus potentially virulence.

NHPs can be leveraged to study the fundamental physiology of *Mtb* during different infective stages. One such approach involves studying the global transcriptome of *Mtb* within the various compartments of primate granulomas. One challenge to this approach is the paucibacillary nature of the infection and non-replicative state of most bacilli. The granuloma is compartmental, and it has been postulated that the *Mtb* present in different regions may represent different phases. Thus, while bacilli in the central, acellular core of the granuloma are likely to be in the non-replicative phase, those in the peripheral phagocytes may exhibit either a high rate of replication or the signatures associated with the acid phase. Laser capture microdissection (LCM) has been extensively used to isolate tissue subsections for downstream analyses.

Laser Capture Microscopy

LCM allows for the isolation of pure cell populations from heterogeneous tissue(2,6). The six basic components of the system are as follows: infrared laser, control unit, inverted microscope, microscope stage control, digital camera, and a monitor(12). In one commercially available system, a dehydrated tissue section is overlaid with a thermoplastic membrane that is then focally melted by a laser(7, 12). In another system, tissue enters the tube via gravity post microdissection. There are also catapulting systems where the laser energy catapults the cells out of the section of tissue(8).

LCM has been instrumental in furthering the understanding of TB pathogenesis. Examples of studies where LCM was used within TB research are listed in the table below (Table 1). These studies have primarily focused on host responses, due to the greater abundance of host RNA within granulomas. These findings have improved the understanding of cytokine responses, macrophage cellular responses, and diagnostic approaches. LCM is however, an expensive, time-consuming and labor-intensive process. Since RNA is inherently unstable, the procurement of high quality RNA obtained from LCM samples can prove difficult(7).

Mesodissection

In 2011, a new approach, mesodissection, was introduced to overcome the disadvantages of previous techniques(1). It employs a consumable mill bit to dispense a buffer recovery solution, cut tissue and aspirate the cut tissue fragment(1). The machine contains a digital microscope, which directs the dissection as well as software for the documentation of each step. A representative hematoxylin and eosin (H&E) stained slide is used to create a reference image and aligned with an unstained slide of the same section for dissection. The captured sample can then be aspirated into a collection tube and used for desired downstream applications.

A striking advantage to this new system is that it allows for easy documentation of each subsequent step. The software provided generates a report that depicts the actual region dissected with respect to an H&E reference slide. The reference slide does not need to be H&E stain based, but could theoretically use any desired stain and tissue collection buffer that best appropriates the goals of the particular experiment. Additionally, it can be used on slides generated from formalin fixed paraffin embedded tissue blocks. Archives of these blocks exist at most institutions, thus the technique opens up untapped tissue sources for further investigation without the need of additional expensive experiments. When compared to LCM, this approach is more time efficient, user friendly, and cost effective. The primary limitation includes the inability to focus on single cell areas.

Future Directions

Granulomas within the lungs of the very same host often develop different outcomes based on the local microenvironment(19). Immune capabilities in the different parts of the lung differ(3). Hence, a considerable variety exists in the architecture of TB granuloma lesions. Due to their anatomy, granulomas, which are masses of highly activated, recruited cells, like tumors, can be readily separated from the adjoining normal tissue. Microdissection is particularly applicable to the study of the granuloma. We are interested in identifying the gene-expression signature of *Mtb* within primate lesions, as a window to understanding the biology of this remarkable pathogen *in-vivo*. These studies are complicated by the presence of different bacterial populations. Using our method, it will be possible to separate these distinct, small populations of bacilli.

Ethics statement

The Tulane National Primate Research Center (TNPRC) facilities are accredited by the American Association for Accreditation of Laboratory Animal Care and licensed by the US Department of Agriculture. All animals were routinely cared for according to the guidelines prescribed by the NIH Guide to Laboratory Animal Care. Humane endpoints were pre-defined in this protocol and applied as a measure of reduction of discomfort. The TNPRC Institutional Animal Care and Use Committee approved all animal-related procedures and activities. The Tulane Institutional Biosafety Committee approved all procedures involving *Mtb*.

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Table 1

Year	Approach	Advancement/Significance	Reference
2003	Analyzed inflammatory response genes in mice. Discovered the downregulation of specific immunological gene expression at the end of bacterial exponential growth.	Developed a system using LCM and molecular beacon based real-time PCR to assess host gene expression in FFPE mice granulomas.	27
2004	Used LCM to microdissect granulomas from suspected TB patients.	The PCR method as a rapid, sensitive, and specific method for TB diagnosis, but culture is still the gold standard.	25
2007, 2008	Used LCM to microdissect granulomas from guinea pigs to assess cytokine milieu and cytokine profiles.	Found varying cytokine profiles depending on vaccination status.	17,18
2010	Used LCM to microdissect granulomas from mice to assess the role of Secondary Lymphoid Organs (SLO) in T-cell development in TB immune response.	In the absence of SLO, protective immune responses can be generated. T-cells also function locally in the lungs to defend the host.	4