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Cellular Microbiology: The metabolic interface between host cell and pathogen.

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Abstract

Cellular microbiology has benefited greatly from the use of immortalized cell lines as host cells for tissue culture models of infection. However, these cells lack many important characteristics of the different cell lineages that are found *in vivo*. This deficiency is particularly true of macrophages that we now know derive from several distinct ontogenic lineages. This perspective discusses these challenges, and possible approaches to overcome them.

Introduction.

The field of “Cellular Microbiology” emerged in the mid-to late 80s when a small number of laboratories started to exploit bacterial genetics to probe the nature of the interactions between pathogenic bacteria and their host cells (Isberg *et al.*, 1985, Gaillard *et al.*, 1986, Sansonetti *et al.*, 1986, Mengaud *et al.*, 1987, Bernardini *et al.*, 1989, Bielecki *et al.*, 1990, Mounier *et al.*, 1990). These early studies were very much focused on the search for virulence factors and were biased towards single gene/single function outcomes. From these studies emerged specific bacterial adhesins involved in host cell attachment and entry, and bacterial hemolysins that enabled bacteria to lyse their host cell phagosome and escape into the cytoplasm. In the late 80s, early 90s, these groups published several reviews and opinion pieces that discussed pipelines combining genetic screens with cell biological readouts to further study the nature of the host cell/pathogen interplay (Falkow *et al.*, 1987, Maurelli *et al.*, 1988, Cossart *et al.*, 1989). By this time the number of investigators operating in this area was expanding dramatically.

The term “Cellular Microbiology” was first used in a perspective published by Cossart and colleagues in 1996 (Cossart *et al.*, 1996), where they sketched out how the two disciplines, molecular microbiology and cell biology were complementing one another to provide deeper insights into both eukaryote cell processes and microbial effectors required to sustain microbial infections within their host cell. The journal Cellular Microbiology emerged in 1999 to fill the gap between the two fields, as discussed in the editorial by the founding editors, Richard Stephens, Philippe Sansonetti and David Sibley (Stephens *et al.*, 1999). Since this time our genetic screens have grown in sophistication, and our cell biological tools have increased in resolution and powers of discrimination. This is now a highly

sophisticated field rich in emergent technologies. However, having worked actively in the area of host/pathogen interplay my entire scientific career I feel that we have reached a point where we need to step back from these tissue culture models to address a more complex and nuanced aspect of host/pathogen interplay; namely the metabolic interface between host and microbe in *in vivo* infection.

Host cell metabolism.

There have been several studies published showing how the host cell environment impacts microbial metabolism and how the metabolic state of the infecting microbe bears little resemblance to its metabolism in rich microbiological media (Pethe *et al.*, 2010, VanderVen *et al.*, 2015). Transcriptional profiling, metabolomics, and transposon-insertion site mapping screens and chemical screens against intracellular microbes have generated a rich body of literature demonstrating how microbial metabolism is impacted by the host cell environment. Some bacteria, such as *Salmonella spp.*, are generalists, show extensive metabolic flexibility and can utilize a range of nutrients for anabolic and catabolic functions (Steeb *et al.*, 2013, Bumann *et al.*, 2017). In contrast, other bacilli, such as *Mycobacterium tuberculosis* appear constrained by the intracellular environment and rely almost exclusively on host cell fatty acids and cholesterol (McKinney *et al.*, 2000, Pandey *et al.*, 2008, VanderVen *et al.*, 2015, Nazarova *et al.*, 2017). Much of our current understanding of the metabolism of intracellular pathogens was generated in tissue culture infection models (Russell *et al.*, 2019). Some data were generated in primary cells but most of the information emerged from studies conducted on immortalized cell lines. These experimental systems have been invaluable and have generated our current level of understanding of this complex interplay.

But these systems do have limitations that need to be acknowledged and addressed. These limitations are of particular significance for pathogens that reside in macrophages, which can be modulated to adopt different states of polarization, commonly referred to as M1- (IFN- γ and TNF- α) and M2- (IL-4 and IL-13) type macrophages. For example, *Listeria monocytogenes* grows best in M1-polarized macrophages (Gillmaier *et al.*, 2012) and primary macrophages are activated by the TLR agonists on the bacteria during infection. The cells exhibit enhanced glycolytic activity that increases glucose uptake and the production of lactate, both exploited by *Listeria* to support bacterial growth. Significantly, this increased growth phenotype is not observed in the murine macrophage-like cell line J774A.1 (Gillmaier *et al.*, 2012). The reason is that J774A.1 cells are a neoplastic cell line that was derived from a murine reticulum sarcoma. Because it is a cancer cell line it has already undergone the Warberg shift to enhanced aerobic glycolysis and a reduction in the production of ATP through the TCA cycle, so it comes in a pre-polarized state. And as the majority of microbial infection studies are conducted on immortalized cell lines of similar oncogenic origins, the same concern applies equally to all these datasets.

Where this becomes an issue of even greater concern is *in vivo*. For decades we believed that all macrophages arose as from blood-derived monocytes that populated our tissues and were replenished during insult or disease (van Furth *et al.*, 1968, van Furth *et al.*, 1972), and that macrophages started in a neutral M0 state that was then driven by cytokines to adopt the M1

or M2 polarized states of classical and alternative macrophage activation. However, recent cell fate mapping studies have demonstrated that this is not true. Tissue resident macrophages, such as dermal macrophages and alveolar macrophages, are actually separate, myeloid lineages that emerge at different times during embryogenesis (Guilliams *et al.*, 2013, Ginhoux *et al.*, 2014, Ginhoux *et al.*, 2016, Guilliams *et al.*, 2017). These cells are both long-lived and self-renewing. But more significantly, the M1/M2 polarization paradigm observed *in vitro* does not apply to these tissue resident myeloid cells.

LPS challenge of the mouse airways drove recruitment of monocyte-derived macrophages that added to the resident alveolar macrophage population (Mould *et al.*, 2017). Transcriptional and metabolic profiling of the two discrete macrophage populations demonstrated two significant areas of divergence. Firstly, the resident alveolar macrophage population was driven into replication by the insult, whereas the recruited cells were not. Secondly, the recruited macrophage population showed up-regulation of glycolytic genes and glycolytic intermediates, whereas the resident alveolar macrophages showed increased TCA cycle intermediates. Yet one assumes that both macrophage populations are exposed to, and experience the same cytokine environment.

We had generated fluorescent fitness and replication reporter strains of *Mycobacterium tuberculosis* to probe bacterial status in the host cell populations in experimental murine infection (Sukumar *et al.*, 2014) and were interested in determining how macrophage ontogeny and metabolism might impact bacterial growth *in vivo* (Huang *et al.*, 2018b). We found that the bacilli in alveolar macrophages exhibited lower stress and higher replication indicators than those in the recruited, blood monocyte-derived macrophages. Moreover, depletion of alveolar macrophage population reduced the bacterial burden 10-fold, whilst depletion of the recruited monocyte-derived macrophages increased the bacterial load 10-fold. Transcriptional profiling indicated that the alveolar macrophages were up-regulated for OXPHOS and fatty acid oxidation, while the recruited macrophages were committed to glycolysis. In experimental infections of macrophages *in vitro* we found that inhibition of glycolysis with 2-deoxyglucose enhanced bacterial growth, while inhibition of fatty acid oxidation with Etomoxir suppressed bacterial growth, further reinforcing this link between host and pathogen metabolism (Huang *et al.*, 2018b). We have known for several years that optimal intracellular growth of *M. tuberculosis* is dependent on its ability to acquire and process cholesterol, and that chemical inhibitors of *M. tuberculosis*' capacity to degrade cholesterol block the bacterium's ability to grow inside its host cell (VanderVen *et al.*, 2015). Similar data regarding the differential permissiveness of resident macrophage lineages have been reported in cutaneous leishmaniasis, where *Leishmania major* exhibited enhanced growth and persistence in resident dermal macrophages in comparison to the recruited blood monocyte-derived macrophages (Lee *et al.*, 2018). We suspect that the different macrophage lineages are epigenetically modified to respond divergently to the same tissue and cytokine environment.

I believe that, when it comes to the metabolic interface between host cells and their pathogens, tissue culture infection models have now become a limitation, and that this limitation is most acute for intra-macrophage pathogens. We can now longer regard macrophages as a homogeneous population of cells that will respond synchronously, or

comparably to the same cytokine milieu *in vivo*. We need to develop our *in vivo* infection models to probe the metabolic interface between host and pathogen in the appropriate host cells in the appropriate environment (Russell *et al.*, 2019). The types of integrated approaches and tools that we believe are key to successful interrogation of this scientific question are diagrammed in Figure 1, and are actually already available for several microbial pathogens.

In brief, the tools required include fluorescent microbial fitness reporter strains capable of providing real-time readouts of bacterial fitness, or stress, or replication. An appropriate animal model that recapitulates the host cell heterogeneity central to real infection. The capacity to harvest and dissociate infected host tissue to generate single cell suspensions that can be analyzed and sorted by flow-cytometry. Cell sorting can be driven by either the bacterial readouts, or the identification of the salient host cell subsets using surface markers. And finally, an array of analytical platforms that include transcriptional profiling with RNA-seq, Dual RNA-seq, and single cell RNA-seq, coupled with metabolic flux analysis, and metabolomics, and the capacity to perturb the system with chemical inhibitors or host cytokines. Moreover, access to human tissue or data from human disease will help integrate and validate the data from experimental animal infections.

Understanding the metabolic interface between the host cell and pathogen is not just an intellectual exercise but one with real world application and relevance to both vaccine efficacy as well as drug development. While we may focus predominantly on immune-mediated killing mechanisms as a means of controlling infection I believe that “nutritional immunity” or nutrient limitation, as first proposed by Kochan to describe immune-mediated iron sequestration (Kochan, 1973), is likely to be of greater significance for chronic and persistent infections. And we need to understand these parameters to induce an appropriate immune response to control infection, or disease progression. It is important to perform phenotypic drug discovery screens in the context of the host environment to reveal new drug targets that are “masked” by the metabolic escape routes available to microbes grown in rich broth (VanderVen *et al.*, 2015, Huang *et al.*, 2018a), because broth-based screens can be grossly misleading (Pethe *et al.*, 2010).

Conclusions.

Initially in Cellular Microbiology we understandably sought to simplify the host component of the equation and emphasized the use of cell lines or homogeneous populations of primary cells differentiated *in vitro*. I feel strongly that not only is this no longer necessary, it is has become a limitation. Our tools have grown in sophistication and resolution and we need to embrace the full complexities of the host tissues and the diversity of the cell lineages that promote or control the infection *in vivo*.

I believe that this is part of the natural maturation of the field of Cellular Microbiology that was initiated by the insightfulness and creativity of those microbiologists that published the early host/pathogen interplay studies that motivated the rest of us to join the field!

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Figure 1.

A diagrammatic representation of the tools and technologies that would be invaluable in resolving the nature of the metabolic interface between host and pathogen *in vivo*. These include fluorescent bacterial fitness and replication reporter strains, and appropriate animal model that reproduces most of the characteristics of human disease. The capacity to isolate and dissociate infected tissues to generate single cell suspensions for analysis. The ability to flow-sort live, infected cells on the basis of the bacterial fluorescent readouts, and host cell surface markers. These cells would then be subjected to extensive characterization of transcriptional profiling, metabolomics analysis and perturbation by small molecule inhibitors or immune-modulators. Finally these data need to be integrated with our current understanding of human disease. This figure is modified from (Russell *et al.*, 2019).