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Immunometabolism at the interface between macrophages and pathogens

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Abstract

It is generally regarded that the progression of an infection within host macrophages is the consequence of a failed immune response. However, recent appreciation of macrophage heterogeneity, with respect to both development and metabolism, indicates that the reality is more complex. Different lineages of tissue-resident macrophages respond divergently to microbial, environmental and immunological stimuli. The emerging picture that the developmental origin of macrophages determines their responses to immune stimulation and to infection stresses the importance of *in vivo* infection models. Recent investigations into the metabolism of infecting microorganisms and host macrophages indicate that their metabolic interface can be a major determinant of pathogen growth or containment. This Review focuses on the integration of data from existing studies, the identification of challenges in generating and interpreting data from ongoing studies, and a discussion of the technologies and tools that are required to best address future questions in the field.

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Different lineages of macrophages respond divergently to immune stimuli and microbial infection. This review explores our current knowledge of how the different metabolic states of macrophage lineages impacts the control or progression of intracellular bacterial infections.

Subject categories

Biological sciences; Microbiology; Bacteria; Bacterial host response; Physiology; Metabolism; Immunology; Innate immune cells; Monocytes and macrophages; Phagocytes; Antimicrobial responses

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Introduction

It has long been known that macrophage metabolism is extremely plastic and frequently reflects the pathology that is associated with specific states of disease[1–3]. For example, atherosclerosis is associated with the accumulation of foamy macrophages [G], increased lipid metabolism and fatty acid oxidation (FAO). By contrast, tumour-associated macrophages have a marked metabolic shift to glycolysis, fatty acid synthesis and altered nitrogen cycle metabolism. Until recently, these divergent metabolic responses of macrophages were thought to be controlled by environmental cues and the local cytokine milieu, partly as a result of the long-held belief that all macrophages had a common origin in the blood[4,5]. This concept was extended by the paradigm of M1 or M2 macrophages [G], which proposed that macrophages in a ground-state of M0 can be driven to either M1 or M2 states of activation following exposure to defined cytokines[6]. Although this model has generated robust in vitro data, the in vivo reality is of much greater nuance and complexity than can be accommodated by a simple model with only two polarized states of activation[7–9].

Different types of macrophage are now known to arise from distinct cell lineages emerging at different stages in embryonic development[10–12]. Blood monocytes are replenished from the bone marrow throughout life, and these cells can also invade and populate various organs as monocyte-derived macrophages upon specific recruitment. By contrast, tissue-resident macrophages are specific lineages derived from fetal stem cells. Finally, data are accumulating to indicate that these different macrophage lineages are programmed to respond divergently to both tissue injury and disease.

Much of the current literature on the metabolism of microorganisms within their host cells has been generated in tissue culture models using cell lines; however, as researchers have started to investigate the metabolism of host macrophages and pathogens in vivo it is becoming clear that the simplicity of these models has become a limitation. The development of dual RNA-sequencing [G] (dual RNA-seq) of both host and pathogen at the single cell level and the use of fluorescent bacterial fitness reporters are enabling the in vivo heterogeneity of responses to be investigated in a more holistic manner. In this Review, we summarize our existing knowledge of the metabolism of macrophage sub-populations and the metabolism of intracellular microorganisms. We focus predominantly on bacterial pathogens because they represent the more complete body of available literature, and we highlight the impact of host cell metabolism on pathogen growth or restriction. Finally, we discuss those tools and technologies that are enabling a greater understanding of the immunometabolic interplay between host macrophages and pathogens in in vivo infection models.

Macrophage immunometabolism

Immunometabolism — in particular, the metabolic reprogramming of macrophages — is now established as a rapidly growing field in immunology[2,13–15]. The key metabolic and

functional differences between the M1 and M2 macrophage polarization states in vitro are summarized briefly in FIG. 1.

M1 macrophages, which are induced by stimulation with tumour necrosis factor (TNF) or IFNγ, have marked upregulation of glycolytic metabolism for generating ATP to support phagocytic and microbicidal functions while feeding an interrupted mitochondrial tricarboxylic acid cycle [G] (TCA cycle). This blocked TCA cycle drives the accumulation of citrate[16], itaconate[17] and succinate[16]. Lipopolysaccharide (LPS) stimulation drives the downregulation of expression of the TCA enzyme isocitrate dehydrogenase (IDH), which leads to the production of excess citrate to support fatty acid synthesis and the generation of pro-inflammatory lipid intermediates. Downregulation of IDH also promotes the accumulation of citrate-based precursors to fuel the production of itaconate, which ultimately inhibits the succinate dehydrogenase complex [G] (SDH complex) in mitochondria. To compensate for the decreased expression of IDH, LPS-activated host cells increase glutamine metabolism for the production of the key metabolic intermediates, α-ketoglutarate and succinate. α-ketoglutarate sustains cellular amino acid synthesis and the excess succinate perturbs immune function by stabilizing hypoxia-inducible factor 1α (HIF1α) to promote the production of the pro-inflammatory cytokine IL-1β. In addition, these cells also have increased activity of the pentose phosphate pathway that is needed to convert glucose-6-phosphate into nucleotides and proteins while generating NADPH, which supports lipid biosynthesis as well as the production of reactive oxygen species (ROS) and reactive nitrogen intermediates. These macrophages have an enhanced antimicrobial response, including up-regulation of the superoxide burst generated by the NADPH oxidase complex[3], the generation of reactive nitrogen intermediates through inducible nitric oxide synthase (NOS2)[18] and increased production of antimicrobial effectors such as cationic peptides, lysosomal hydrolases and metabolic inhibitors[19], including itaconate (antibacterial)[17] and 25-hydroxycholesterol (antiviral)[20].

By contrast, M2 macrophages, which are induced by stimulation with IL-4 and IL-13, have an intact TCA cycle and enhanced mitochondrial oxidative phosphorylation (OXPHOS) activity. Whereas it was previously thought that this metabolic process was mainly fueled by fatty acid oxidation (FAO), it is now understood that glucose also fuels OXPHOS in M2 macrophages[21]. Multiple studies have shown that the perturbation of key metabolic enzymes in these pathways by either small molecule inhibitors or genetic manipulation can markedly affect the major immune functions of macrophages and other myeloid cells (recently reviewed in REF.[15]). The M2 macrophage state is frequently associated with the generation of anti-inflammatory products such as glucocorticoids, IL-10, IL-13 and colony-stimulating factor 1 (CSF1; also known as M-CSF), but it is an oversimplification of their complex biological properties to view these cells as a polar counterpart of the M1 macrophage activation state[22].

The emerging field of immunometabolism has particular relevance to the study of intracellular infections. Many microbial pathogens are phagocytosed by macrophages early in the infection cycle. Although most microorganisms are killed by phagocytosis and fusion with acidic lysosomes in host cells, some professional pathogens have evolved to survive and even grow in the apparently hostile environment within phagocytes. For these

microorganisms, particularly those that maintain a stable relationship with host cells, the coupled metabolism between the host and pathogen is of considerable importance. Thus, the metabolic divergence of the different macrophage lineages will affect pathogen survival and growth, and is likely to be a key factor in determining disease control or progression.

Pathogen metabolism in macrophages

The greatest body of data available with regards to host-pathogen metabolism refers to bacterial pathogens, so although we include discussion of eukaryotic pathogens where appropriate, this article emphasizes bacteria as illustrative examples. It also focuses predominantly on the central carbon metabolism of host and pathogen because the relationship between immunometabolism and macrophage ontogeny is fundamental to a full appreciation of how pathogens maintain their infection within heterogeneous macrophage populations in vivo. The major metabolic pathways that are favored by a range of eukaryotic and prokaryotic pathogens of macrophages are summarized in TABLE 1. Disease progression is invariably linked to growth of the pathogen population, but the persistence of microorganisms in a non-replicative, but metabolically active, state is also required for the maintenance of chronic infections by many intracellular pathogens. Therefore, it should be noted that the optimal conditions for intracellular growth of a pathogen detailed in TABLE 1 do not necessarily describe the only metabolic state that is relevant to infection and disease.

Many enteric bacteria exhibit carbon catabolite repression, which is a regulatory mechanism to restrict metabolism to a single, preferred carbon source, such as glucose. It is suggested that this enables bacteria living in a mixed population to outcompete their neighbors[23]. However, intracellular bacteria are not under the same evolutionary pressure as enteric bacteria owing to their relatively sterile intracellular environment. As shown by the following examples, these intracellular pathogens usually have an increased capacity to co-metabolize different carbon sources, which provides greater metabolic flexibility[24].

This section provides a brief insight into the diverse metabolic solutions that have evolved amongst pathogenic bacteria to enable them to survive and multiply within host macrophages. The solutions are complex and some bacteria rigorously compartmentalize their use of nutrients, whereas other bacteria retain extensive metabolic flexibility. An understanding of the metabolic state of the pathogen is required to appreciate the complexity of the interplay with different macrophage subsets and with an evolving immune environment. We believe that the challenges facing host cells in terms of limiting the growth of these pathogens reflect the diverse metabolism of these infectious microorganisms.

Salmonella Typhimurium.

Salmonella enterica serovar Typhimurium can invade and disseminate systemically in its hosts. In tissues, these bacteria tend to reside and proliferate in tissue-resident macrophages, although they can invade and grow in many different cell types[25]. Inside the cell, *S.* Typhimurium remains predominantly intravacuolar but remodels the intracellular compartment into an extensive, filamentous network[26]. Metabolically, intracellular *S.* Typhimurium seems to be a ‘generalist’ with an extremely flexible metabolic capacity, such

that few single mutations in metabolic pathways have a major effect on bacterial survival[27,28].

Legionella pneumophila.

L. pneumophila also remains within a membrane-bound compartment in its host cell[29], and, similarly to *S.* Typhimurium, it can infect several different cell types *in vivo.* Different isotope and carbon flux analyses indicate that *L. pneumophila* has a bipartite metabolism [G] using serine as a major carbon source, while using glycerol and glucose for anabolic processes[30,31]. In tissue culture models, *L. pneumophila* has two differential growth phases, with an exponential growth phase that depends on serine[32] and a post-exponential phase that relies more heavily on glucose and glycerol[33].

Mycobacterium tuberculosis.

Similarly to *S.* Typhimurium and *L. pneumophila, M. tuberculosis* also remains predominantly intravacuolar. The bacterium can escape from the vacuole, but this transition culminates with host cell death in tissue culture model systems[34–36]. Access to the cytosol depends on the ESX-1 bacterial secretion system and induces a type I interferon response in the host cell[37]. Early genetic studies identified the glyoxylate shunt enzyme isocitrate lyase[38] and the bacterial cholesterol transporter Mce4[39] as being important for the establishment and maintenance of intracellular *M. tuberculosis* infection. An extensive empirical screen for compounds that blocked the growth of intracellular *M. tuberculosis* identified inhibitors of the bacterial cholesterol degradation pathway[40]. Recent data indicate that cholesterol and fatty acid breakdown are balanced, which suggests that there is complex regulation of how the bacterium uses these substrates within the host cell environment[41,42]. Finally, *M. tuberculosis* has also been shown to use glucose[43] and C3 glycolytic substrates[44] to sustain in vivo and intracellular infections.

Listeria monocytogenes.

L. monocytogenes, which is the canonical ‘cytosolic’ pathogen, escapes from its vacuole rapidly following uptake by phagocytosis. Studies with *L. monocytogenes* have shown that the host cell cytosol can be nutritionally restrictive and that the bacterium requires specific co-factors, such as lipoate, for a functional pyruvate dehydrogenase complex[45]. In this cellular compartment, *L. monocytogenes* also seems to operate a bipartite metabolism, using glycerol predominantly for energy generation and glucose-6-phosphate for anabolic functions[46]. The bacteria metabolize pyruvate and lactate poorly, despite their apparent abundance in the cytosol.

Metabolic restriction of pathogens

In addition to the direct antimicrobial mechanisms of macrophages, such as the superoxide burst, generation of ROS, autophagy and production of antimicrobial peptides (not discussed here), macrophages also control microbial growth through nutrient limitation. The concept of nutritional immunity was first proposed in 1973 in reference to iron sequestration as a means of restricting bacterial growth in vivo[47]. Since then, this mechanism has been found to be relevant in many different microbial infections. In addition to iron sequestration,

restricted access to other metal ions such as Zn^{2+}, $Cu^{2+}$and Mn^{2+} can also reduce microbial fitness and growth[48–51]. Most intracellular bacteria are auxotrophic [G] for purines, pyridines and/or amino acids[52–55], which —coupled with the known metabolic restrictions of immune cells[56] — indicates an obvious link between host cell metabolism and the growth limitation of intracellular pathogens. In pivotal early experiments, IFNγ-induced degradation of host cell tryptophan was shown to restrict the growth of *Toxoplasma gondii*[57]. The enzyme involved, indoleamine 2,3-dioxygenase (IDO), has been shown to have a role in inhibiting growth of several intracellular pathogens, including *M. tuberculosis* and *Chlamydia trachomatis* [58].

One of the more intriguing pathways that controls the growth of intracellular pathogens involves the production of itaconate. Immunoresponsive gene 1 (*Irg1*) encodes an enzyme that produces itaconate from *cis*-aconitate, a citrate-derived intermediate in the TCA cycle[17] (the biology of which is discussed extensively in a companion Review[59]). In addition to its activities as a mitochondrial SDH inhibitor and an immunomodulator[60–62], itaconate is a potent inhibitor of bacterial isocitrate lyase activity. Isocitrate lyase is required to facilitate the retention of carbon from fatty acids through the glyoxylate shunt[38,63] in many Gram-negative bacteria. In *M. tuberculosis,* the enzyme also functions as a methyl-isocitrate lyase, which is required for processing propionyl-CoA generated from cholesterol breakdown, and inhibition of the enzyme leads to intoxication of the bacteria[64,65]. Several intracellular microorganisms, including *M. tuberculosis, S.* Typhimurium[17] and *L. pneumophila*[66] have reduced virulence and growth when exposed to itaconate, and IRG1-deficient mice have markedly increased susceptibility to *M. tuberculosis* infection[67]. Moreover, several pathogenic bacteria encode a pathway for the detoxification of itaconate through transfer of a CoA group from a succinyl-CoA donor, which indicates that at least some pathogens can counteract this host-derived metabolic poison[68].

Metabolic reprogramming by pathogens

Infection of macrophages by microbial pathogens results in marked changes to the physiology and metabolism of host cells. Some of these changes are mediated by the innate immune response of host cells, which detects and responds to pathogen-associated molecular patterns (PAMPs) through pattern-recognition receptors (PRRs) and generates an inflammatory response against infection (FIG. 2). However, other changes to host cell metabolism are mediated by pathogen-derived effectors, or ‘virulence factors’, that have evolved to modulate host cell function in a targeted, specific manner. Both types of change affect the outcome of the infection process.

Mediated by PAMPs.

Mammalian cells in resting state generally have low levels of catabolic and anabolic activity and efficient generation of ATP through mitochondrial respiration (OXPHOS)[69], by engaging a complete TCA cycle. By contrast, proliferating cells, including cancer cells, exhibit a shift to aerobic glycolysis, known as the Warburg effect, which involves increased uptake of glucose, the energetically inefficient generation of ATP and accumulation of by-products of glycolysis and fermentation[70].

Mammalian cells have both surface and cytosolic sensors — for example, PRRs such as Toll-like receptors (TLRs) and NOD-like receptors (NLRs) — that respond to a range of microbial products from cell wall components (LPS and peptidoglycan) to modified DNA and RNA (dsRNA and CpG), and the stimulation of these sensors drive cells into a Warburg-like response[16,71–73]. The Warburg shift is closely associated with fueling many of the antimicrobial responses that are generated following the acquisition of an M1-like macrophage phenotype. However, there are significant differences in metabolism between proliferating cancer cells and LPS-stimulated macrophages[14]. Cancer cells use glucose-6-phosphate to fuel both lipid and nucleotide synthesis, and they metabolize pyruvate to support amino acid and fatty acid synthesis for cell growth. By contrast, the inflammatory response induced by TLR ligands such as LPS yields lactate and results in the generation of nitric oxide, ROS, prostaglandins and itaconate, all of which are linked to the antimicrobial activities of phagocytes (as detailed above for itaconate). So, although cancer cells and infected macrophages are superficially similar with respect to increased glycolysis, the downstream consequences of their metabolic reprogramming are disparate.

In addition, a recent study used human monocytes to show that the metabolic switch induced by stimulation with whole-pathogen lysates from *Escherichia coli, Staphylococcus aureus* and *M. tuberculosis,* and with the TLR2 ligand Pam_3CysSK_4, was not comparable to that induced by stimulation with the TLR4 ligand LPS[74]. Measurements of the oxygen consumption rate [G] (OCR) and the spare respiratory capacity [G] (SRC) indicated that whereas all stimuli induced an increased OCR and therefore increased glycolysis, only LPS triggered a reduction in the SRC and therefore OXPHOS. These data indicate that different stimuli that induce an M1-like macrophage phenotype are not necessarily equivalent and that the metabolic response(s) to such stimuli need to be assessed individually.

Glycolytically active macrophages differentially support pathogen growth. M1-like macrophages have increased microbicidal capacity, but some pathogens benefit from the enhanced glycolytic activity. For example, replication of intracellular *L. monocytogenes* is enhanced by the increased glucose uptake in primary macrophages that is triggered upon infection. Interestingly, this increased growth rate of *L. monocytogenes* was not observed in J774A.1 cells. However, J774A.1 cells are a macrophage-like neoplastic cell line that constitutively expresses MYC and, therefore, these cells are already shifted to a Warburg-like metabolism prior to infection[75]. These data raise a note of caution for investigators using transformed cell lines to study the metabolism of intracellular pathogens. Similarly, infection of human macrophage-like THP-1 cells with *Brucella abortus* also triggers a shift to aerobic glycolysis [G] and increased lactate production[76]. *B. abortus* can use lactate as a sole carbon and energy source, and the bacterium grows robustly in THP-1 cells; blocking host glycolysis and lactate dehydrogenase using chemical inhibitors resulted in a marked decrease in pathogen number[76]. In addition, infection with *B. abortus* leads to a reduction in mitochondrial function and a metabolic shift away from OXPHOS towards aerobic glycolysis, which presumably increases lactate production in host cells.

An emerging concept of direct relevance to the metabolic reprogramming of macrophage lineages by microorganisms in vivo is that of ‘trained immunity’ (BOX 1). The induction of a trained immune response by the fungal cell wall component p-glucan involves a shift from

OXPHOS to glycolysis mediated through the AKT-mechanistic target of rapamycin (mTOR) signalling pathway and HIF1α[77]. Moreover, the epigenetic programming of these cells is modulated by the metabolic intermediates acetyl-CoA, NAD, α-ketoglutarate and succinate, all of which are extensively altered by macrophage lineage and polarization, and are directly relevant to immune-mediated and vaccine-induced pathways of control[78]. The major themes and salient points of PAMP-induced conditioning of phagocyte populations are summarized in BOX 1.

Mediated by specific microbial effectors.

In addition to the modulation of macrophage metabolism through the activation of mammalian PRRs, some pathogens have evolved more direct mechanisms to modulate the metabolism of host cells.

L. pneumophila causes pulmonary infections and replicates within human lung macrophages. The bacterium encodes a type IV secretion system [G] that can inject more than 300 bacterial effector proteins into host cells[29], which carry out a wide range of overlapping and apparently redundant activities. One feature of infected macrophages is the close apposition between *L. pneumophila*-containing vacuoles and mitochondria. A recent study reports that the subsequent fragmentation of these vacuole-associated mitochondria depends on the bacterial effector protein MitF, which is released into host cells[79]. Mitochondrial fusion and fission [G] are tightly regulated processes in mammalian cells that are mediated in part through the activity of the GTPase dynamin 1-like protein (DNM1L; also known as DRP1), and the activity of this host enzyme is increased during infection with *L. pneumophila.* The resulting increased fragmentation of mitochondria leads to reduced mitochondrial respiration and increased host cell glycolysis, and, as a result, increased growth of bacteria within the host cell.

The specific targeting of mitochondrial function is not unique to *L. pneumophila.* For example, *L. monocytogenes* uses its toxin Listeriolysin O (LLO) to escape from the phagosome and access the host cell cytosol, where LLO targets host cell mitochondria and induces their fragmentation[80]. Interestingly, in contrast to the mitochondrial fragmentation induced by *L. pneumophila*, this phenomenon is transient in *L. monocytogenes-infected* cells, leading to decreased cellular respiration without the activation of cell death pathways. It is thought that mitochondrial fragmentation is required for optimal early infection with *L. monocytogenes* as infection is impaired in cells with previously fissioned mitochondria and is enhanced in cells with mitochondria that were already fused[80].

Macrophage heterogeneity *in vivo*

The data discussed so far were generated predominantly using in vitro infection of tissue cultures consisting of relatively homogeneous cell populations, comprising either macrophage-like cell lines or primary cells such as human monocyte-derived macrophages matured from peripheral blood monocytes, or murine bone marrow-derived macrophages. Although it is understandable and necessary to have started with such homogeneous challenge models to establish what is possible, the extrapolation of in vitro data to the heterogeneous nature of the in vivo environment presents many challenges. To do so

requires a capacity to detect and assess the functional relevance of heterogeneity, and there are two main routes to this that researchers have explored so far. The first is through the generation of fitness reporter organisms [G] (BOX 2) that provide information regarding the metabolic and replicative state of the pathogen in a specific host cell or host tissue environment[81–88]. The second is single cell transcriptional profiling, specifically dual RNA-seq methodology, that enables simultaneous transcriptional profiling of host and pathogen[89–91]. These two, mutually informative methods are still in development and, so far, they have only been applied in combination to models of infection with *S.* Typhimurium and *M. tuberculosis.* Both of these pathogens can result in persistent infections that are thought to be maintained by bacteria that are live but non-replicative, with reduced metabolic activity[86,92,93]. Furthermore, the acquisition of this 'persister' phenotype is closely associated with the development of drug tolerance, resulting in the reduced effectiveness of antibiotics *in* vivo[83,94–96]. Therefore, understanding the physiological states of host macrophages and the infecting bacteria during persistence and disease progression is key to the development of new effective strategies for treatment. The different biological phenotypes that have been documented in *in vivo* infection models for *S.* Typhimurium and *M. tuberculosis* are illustrated in FIG. 3 and FIG. 4, respectively.

During S. Typhimurium infection.

S. Typhimurium causes a systemic infection in mice, infecting macrophages in multiple tissues including the Peyer's patches, mesenteric lymph nodes (MLNs), spleen and liver. A recently developed fluorescence dilution reporter strain [G] to quantify relative rates of bacterial replication has been used to determine the relative replication rates of S. Typhimurium in different tissue environments[85] (FIG. 3). This study reported that the proportion of non-replicating bacteria isolated over the first 24 hours post challenge was markedly lower in macrophages isolated from the Peyer's patches than in those isolated from the MLNs. The viability of these non-replicating bacteria was shown by subsequent in vitro outgrowth. Activation of infected macrophages in vitro with IFNγ led to the generation of a non-replicative bacterial phenotype and to the acquisition of drug tolerance in *S.* Typhimurium. Moreover, treatment of macrophages with inhibitors of the vacuolar ATPase that is responsible for phagosomal acidification caused a marked decrease in the frequency of the 'persister' bacterial phenotype, which indicates that pH-mediated stress is a primary driver of reduced bacterial replication and increased drug tolerance in intracellular bacteria.

Subsequent publications have connected macrophage polarization in vitro to the growth rates of intracellular *S.* Typhimurium. Mouse bone-marrow-derived macrophages were infected with the fluorescence dilution reporter strain of *S.* Typhimurium and sorted into populations at the extremes of fluorescence, which represent 'controller' host cell populations and 'permissive' host cell populations, for single-cell sequencing analysis[89]. All infected macrophages showed down-regulation of replication markers, which is consistent with previous reports that PAMP-mediated activation of macrophages drives differentiation into a non-replicative state. Infected macrophages containing non-growing bacteria were, by principle component analysis (PCA), most similar to uninfected bystander macrophages. By contrast, macrophages with growing populations of *S.* Typhimurium had increased expression of M2 macrophage-associated transcripts, such as *Arg1, Cd206* and *Il4ra,*

although the analysis was not extended to cellular metabolism. Recent dual-RNA-seq analysis of the splenic macrophages from *S.* Typhimurium-infected mice confirmed the permissiveness of the M2 host macrophage phenotype in vivo and demonstrated that this correlated with the increased expression of SPI2 effectors in the bacteria in these cells suggesting that the bacteria actively modulate host macrophage polarization[97].

Another study used a fluorescent protein variant known as TIMER [G], which spontaneously changes its emission fluorescence from green to orange as a function of age[83]. By quantifying the fluorescence ratio of the green to orange signal, one can estimate the division rate of individual bacteria in culture. Analysis of the *S.* Typhimurium fluorescence signal from infected macrophages and in mice confirmed the presence of bacterial populations with differing growth rates that tended to vary between host macrophages, although this was not correlated with the activation state of the host cells. Proteomic analysis showed that more actively replicating bacteria contained higher levels of ribosomal proteins, whereas slower replicating bacteria had higher levels of expression of the transcription factor cAMP receptor protein (CRP), the expression of which is linked to metabolic regulation under starvation conditions[98]. As reported in other studies, slower replicating bacteria had higher levels of phenotypic tolerance to antibacterial drugs[83].

These data suggest that the phenotype of the infecting bacteria, with respect to both growth state and drug sensitivity, is determined predominantly by the polarized state of their host macrophage. Macrophages expressing M1 macrophage-associated markers control bacterial growth and induce drug tolerance, whereas macrophages expressing M2 macrophage-associated markers are more permissive for bacterial growth.

During M. tuberculosis infection.

Researchers have long been aware of the heterogeneity within *M. tuberculosis* populations during infection (reviewed recently in REF.[93]), and it is known that *M. tuberculosis* has evolved hard-wired genetic mechanisms to generate inequality between its daughter cells to maximize the benefits of phenotypic heterogeneity[96,99,100]. It is thought that this bacterial heterogeneity better equips *M. tuberculosis* to exploit the environmental diversity found in host cells and host tissues.

Recent studies of early low-dose infection in mice show that *M. tuberculosis* is internalized by alveolar macrophages that subsequently invade the underlying tissue[101]. Immunohistology and flow cytometric analysis of infected tissues from mice and non-human primates (NHPs) have shown that *M. tuberculosis* becomes established in several types of host phagocyte, primarily alveolar macrophages, recruited interstitial macrophages and neutrophils[102]. The bacteria are also found in dendritic cells, which are known to have a role in the development of an adaptive immune response through the presentation of mycobacterial antigens[103] but are not thought to be an important host cell population for bacterial replication[104]. Extensive analysis of macrophage subsets in individual granulomas of NHPs infected with *M. tuberculosis* has documented different populations of macrophages that express M1 macrophage-associated markers, such as endothelial nitric oxide synthase (NOS3) and inducible nitric oxide synthase (NOS2), or M2 macrophage-associated markers, such as arginase (ARG1 and ARG2)[105] (FIG. 4a). In a disease outcome

model based on these data, the authors suggest that an increased ratio of M2 to M1 macrophage subsets is an accurate predictor that an individual granuloma is likely to progress to active disease[106]. Similarly, when *M. tuberculosis*-infected macrophages were isolated from mouse lung tissue and flow-sorted into activated ($CD80^{hi}$) versus resting ($CD80^{low}$) cells, the bacteria in the activated host macrophages had much higher levels of phenotypic drug tolerance[95], which suggests that these bacteria were in a state of low-level replication.

These findings were extended recently to examine the metabolic state induced by *M. tuberculosis* infection in different mouse macrophage lineages in vivo[107] (FIG. 4b). Mice were infected with a panel of *M. tuberculosis* fitness reporters, including a 'clock' plasmid [G][84,108], a chromosomal replication complex reporter [G] and an environmentally responsive promoter reporter [G][84] sensitive to nitric oxide[88,109], which showed that alveolar macrophages were more permissive for bacterial replication than monocyte-derived interstitial macrophages. Selective depletion of the alveolar macrophage population by clodronate liposomes delivered to the airways led to a decreased bacterial burden in the lung, whereas depletion of the recruited interstitial macrophages, through intravenous administration of clodronate liposomes or the use of CC-chemokine ligand 2 (CCL2)-deficient mice, led to an increased bacterial burden in the lung. RNA-seq analysis of the infected populations of alveolar macrophages and interstitial macrophages indicated that the two host cell populations had acquired metabolically distinct states. Alveolar macrophages were enriched for transcripts associated with OXPHOS and fatty acid metabolism, whereas interstitial macrophages exhibited a marked Warburg shift. Treatment of *M. tuberculosis-infected* mice with the non-hydrolysable glucose analogue, 2-deoxyglucose, selectively decreased the size of the interstitial macrophage population, resulting in an increase in the bacterial burden. These findings were verified by in vitro experiments showing that treatment of *M. tuberculosis*-infected bone marrow-derived macrophages with 2-deoxyglucose promoted bacterial growth, whereas the FAO inhibitor etomoxir restricted bacterial growth. These data indicate that the different host macrophage lineages respond divergently to infection with *M. tuberculosis* with respect to their metabolic commitment, and that this divergence is relevant to infection control. These data are consistent with the recent report that the reduced number of recruited interstitial macrophages in CC-chemokine receptor 2 ($Ccr2^{-/-}$) mice upon infection with the highly virulent HN878 strain of *M. tuberculosis* explains the increased susceptibility of these mice[110]. The data are also supported by metabolic flux analysis of human macrophages, which shows that cigarette smoking reduces the glycolytic response of macrophages upon *M. tuberculosis* infection and that this increases bacterial survival[111].

The development of vaccines against tuberculosis has been hampered by the lack of biomarkers for immune protection[112] and the realization that IFNγ production, while clearly required for protection, is not sufficient to prevent disease progression. It has been observed that *M. tuberculosis* infection drives bystander macrophage populations at the site of infection into cell division[107]. This provides a possible alternative explanation for disease development and posits that, even in the face of an effective, controlling T helper 1 (T_H1) cell-mediated immune response, the selective expansion of alveolar macrophage populations, given their apparent commitment to OXPHOS, could support bacterial growth.

The observation that tissue-resident macrophages can be driven into replication by infection or inflammatory insult is not unique to *M. tuberculosis* infection. Similar observations have been made for skin macrophages in a murine cutaneous leishmaniasis model[113], whereby infection with *Leishmania* parasites was maintained by the presence of replicating, M2 macrophage-like resident dermal macrophages, even in the presence of a robust local T_H1 cell-mediated response.

These studies indicate that the M2 macrophage-like characteristics of alveolar macrophages provide an environment that is more permissive for the growth of *M. tuberculosis*, directly associated with the metabolic state of these macrophages and their bias towards FAO. By contrast, and comparable to S. Typhimurium, the M1 macrophage-like characteristics of interstitial macrophages are associated with control of *M. tuberculosis* growth, but also with the induction of a drug-tolerant phenotype.

Macrophage ontogeny and metabolism

The data from the mouse *M. tuberculosis* challenge model have broader implications with respect to macrophage biology in infectious disease control. Transcriptional profiling has shown that there are major differences between the embryonically derived, resident alveolar macrophages and the haematopoietically derived interstitial macrophages in the mouse lung[114,115]. All interstitial macrophages expressed transcripts encoding the tyrosine kinase MERTK, CD64, CD11b and CX3CR1, whereas all alveolar macrophages expressed the macrophage receptor MARCO[114,115]. The differences between the macrophage lineages were increased markedly in an acute lung injury model[116]. After treatment of the lungs with LPS to induce inflammation, functional analysis of the resident and recruited lung macrophages was carried out, looking at both transcript abundance and the concentrations of key metabolic intermediates. The resident alveolar macrophages proliferated locally and showed commitment to increased TCA cycle and OXPHOS. By contrast, the recruited macrophages were more inflammatory in phenotype and had increased glycolytic activity and arginine metabolism. These data are consistent with the murine tuberculosis model and indicate that the developmental origin of macrophages rather than the inflammatory immune environment is the dominant factor in determining macrophage phenotype and metabolism.

Helminth infections are known to strongly induce alternatively activated macrophages, and they are recognized drivers of the localized proliferation of resident macrophages[117,118]. The functional plasticity of macrophage populations has been studied in a co-infection model combining helminth challenge (with *Heligmosomoides polygyrus)* with *S.* Typhimurium infection. *H. polygyrus* is a naturally occurring gastrointestinal nematode of mice that induces a strong type 2 immune response that drives the proliferative expansion and M2-type activation of resident peritoneal macrophages[119]. Upon subsequent intraperitoneal injection of *S.* Typhimurium, there was an influx of monocyte-derived macrophages and neutrophils to the peritoneal cavity. This led to a reduction in size of the $F4/80^{hi}$ nematode-expanded, resident macrophage population. The $F4/80^{low}$ inflammatory macrophages recruited by *S.* Typhimurium were exclusively haematopoietic in origin and were not derived through conversion of the $F4/80^{hi}$ macrophages. The decreased numbers of $F4/80^{hi}$ resident macrophages that remained were reprogrammed by *S.* Typhimurium to acquire antimicrobial

properties, such as expression of NOS2 and increased MHC class II expression, although the extent of macrophage re-polarization was restricted and these cells retained many characteristics that reflected their resident macrophage origin.

Finally, it was interesting to note that the recruitment of the inflammatory monocyte-derived macrophages seemed to be linked to the disappearance of the F4/80^{hi} resident macrophages[119]. It is thought that this disappearance occurred as a result of induced apoptosis and could be linked to niche competition as a means of regulating the phenotypic characteristics of phagocyte populations in the presence of infectious stimuli.

In addition to the traditional cytokines that are associated with the polarization of macrophages and with driving functional phenotypes (FIG. 1), the transcription factor peroxisome proliferator-activated receptor-γ (PPARγ) is being increasingly recognized as a modulator of lipid metabolism linked to inflammation and immunity, particularly in tissue-resident macrophages. Early evidence indicated that PPARs inhibit proinflammatory responses in macrophages[120], but the role of PPARγ seems to be much more nuanced and is influenced by the target macrophage lineage[121]. PPARγ is required for tissue macrophage-mediated clearance of *S. aureus* skin infections and for control of *Pseudomonas aeruginosa* in the lung[122], and it is implicated in both the control and progression of *M. tuberculosis* infection, dependent on the model[123–126]. Further study of the role of PPARγ in macrophage polarization is required; however, it is clear that this nuclear receptor protein has an important role in the modulation of tissue-resident macrophage function.

Conclusions and future directions

Most of the studies that have been published on the control of microbial infections by macrophages have focused on classical killing pathways, such as the production of ROS and reactive nitrogen intermediates, the up-regulation of expression of antimicrobial peptides, the triggering of autophagy and the sequestration of co-factors. All of these mechanisms are highly relevant to infection control but we believe that these pathways overlay a heterogeneity in macrophage lineages that is developmentally defined, and that these macrophage lineages, through their metabolic commitment, determine how the different phagocyte populations respond to activating cytokines. Understanding how macrophage metabolism interfaces with microbial metabolism, and how this affects the control or progression of infection promises to be fascinating. Existing data from in vitro and in vivo infections with *S.* Typhimurium and *M. tuberculosis* link the phenotypes of tissue resident (M2-like) macrophages with bacterial growth, and the phenotypes of recruited inflammatory (M1-like) macrophages with control of bacterial growth, but also with the induction of a drug tolerance programme in the bacteria that is linked to bacterial persistence.

Currently, there is a disconnect between the research community studying the metabolism of intracellular pathogens and those immunologists who appreciate the relevance of macrophage ontogeny. It is important to understand the metabolic status of host cells and how this can be manipulated by pathogens trying to establish their optimum growth conditions. However, the homogeneous populations of transformed mammalian cell lines that are used routinely for these studies are not an accurate reflection of the in vivo situation.

Given the central role of macrophages in most microbial infections, it is important to appreciate the functions of the specific macrophage lineages and populations that determine the outcome of infection in vivo.

Our research on *M. tuberculosis* infections in tissue culture models and in mice has increased our appreciation of the value of certain tools (BOX 2) and our awareness of the information that needs to be generated to interrogate the macrophage-pathogen interface effectively. The iterative interactions of these tools and technical platforms are illustrated in FIG. 5. Integration of these tools will be required to cement the emerging links between host macrophage ontogeny and metabolism and the growth and replication states of intracellular pathogens. Tools and techniques — such as fluorescent fitness reporters, dual RNA-seq of host and pathogen mRNA and appropriate animal models — are already available for several microbial pathogens, and it is likely that data from these infection models will continue to accumulate. Such an abundance of tools and models has never before been available to study biochemistry and cellular metabolism, nor with such intriguing problems to address. It is likely that an increased appreciation of macrophage diversity will be crucial to the development of more effective vaccines, and more efficacious drug combinations that might target both host and pathogen.

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Glossary

Foamy macrophages
Macrophages with cytosolic lipid droplets containing cholesterol, cholesterol ester and triacylgycerol, which are frequently induced by chronic pro-inflammatory stimuli

Ml or M2 macrophages
Ml' and 'M2' are classifications historically used to define macrophages activated in vitro as pro-inflammatory (when 'classically' activated with IFNγ and LPS) or anti-inflammatory (when 'alternatively' activated with IL-4 or IL-10), respectively. However, in vivo, macrophages are highly specialized, transcriptomically dynamic and extremely heterogeneous with regards to their phenotypes and functions, which are continuously shaped by their tissue microenvironment. Therefore, the Ml or M2 classification is too simplistic to explain the true nature of in vivo macrophages, although these terms are still often used to indicate whether the macrophages in question are more pro- or anti-inflammatory

Dual RNA-sequencing
(Dual RNA-seq). A transcriptional profiling technique that enables simultaneous acquisition of the expression levels of mRNA transcripts in both the host cell and the pathogen

Tricarboxylic acid cycle

(TCA cycle). Also known as the citric acid cycle or Krebs cycle. This is a series of enzymatic reactions used in aerobic metabolism to release energy through the oxidation of acetyl-CoA to yield ATP and carbon dioxide

Succinate dehydrogenase complex
(SDH complex). An enzyme complex found in bacterial cells and in the inner mitochondrial membrane of eukaryotic mitochondria that is active in both the TCA cycle and the electron transport chain

Bipartite metabolism
A metabolic programme whereby one carbon source is used exclusively as an energy supply, while another carbon source, or sources, are used for anabolic processes

Auxotrophic
Organisms that are unable to synthesis all of the compounds required for growth are auxotrophic, meaning that they are dependent on their hosts to supply those compounds they cannot synthesize

Oxygen consumption rate
The total oxygen utilization capacity of a biological system under examination against time

Spare respiratory capacity
(SRC). The difference in the amount of ATP generated by oxidative phosphorylation at basal rate and at maximal respiratory capacity

Aerobic glycolysis
The conversion of glucose to lactate under conditions where oxygen is present at nonlimiting concentrations

Type IV secretion system
An ATP-dependent bacterial transporter complex that is frequently used to inject bacterial effector proteins or bacterial DNA into eukaryotic and prokaryotic target cells

Mitochondrial fusion and fission
The OXPHOS capacity of eukaryotic cells can be regulated in part through the fusion or fragmentation of mitochondria in a highly controlled manner

Fitness reporter organisms
Bacterial reporter strains, usually encoding a fluorescent protein-based readout, that are used to assess bacterial fitness with respect to responsiveness to noxious stimuli and replicative capacity

Fluorescence dilution reporter strain
A bacterial strain that can be induced to transiently express a fluorescent protein, which can then be quantified as it becomes diluted when the bacteria divide to infer bacterial replication rates

TIMER

A re-engineered red fluorescent protein that undergoes a conformational shift, and hence a change in fluorescence emission wavelength, as it ages thus providing a correlate of replication rates

‘Clock’ plasmid
An episomal plasmid encoding an antibiotic-resistance marker that is lost from a bacterial population at a fixed rate directly proportional to the rate of replication

Chromosomal replication complex reporter
A single-stranded DNA-binding protein-GFP fusion complex that persists for the duration of chromosomal replication and can be used to assess the replication status of a bacterial population

Environmentally responsive promoter reporter
A dual-fluorescent bacterial reporter strain that expresses one fluorescent protein constitutively and the other fluorescent protein under the control of promoters that are responsive to specific environmental stimuli

Ribosomal RNA correlates
Sequences encoding a destabilized or short-lived GFP are inserted into ribosomal RNA loci to provide a correlate of ribosomal RNA activity and bacterial replication

pH-sensitive GFP
A green fluorescent protein derivative that exhibits a shift in fluorescence emission wavelength in a pH-dependent manner

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Box 1 |

Trained immunity

Recent data indicate that innate immune cells can develop memory-type responses that intensify their recall response following an initial exposure to a pathogen. This phenomenon, termed ‘trained immunity’, has been reported in myeloid cell lineages and is thought to result from epigenetic modification and cellular metabolic rewiring. Compared with the classical immunological memory that is a feature of the adaptive immune system, trained immunity has reduced specificity as re-stimulated cells respond more robustly to a broader range of secondary stimuli. Two well-documented stimuli that trigger a trained immune response in macrophages are β-glucan of *Candida albicans* and *Mycobacterium bovis* bacillus Calmette-Guérin (BCG). The trained immune response induced by both agonists activates the AKT-mTOR (mechanistic target of rapamycin) pathway, triggers a shift of cellular metabolism towards glycolysis and promotes genome-wide changes in histone modification[77,127]. More recently, mevalonate, which is an intermediate of the cholesterol synthesis pathway, has been shown to promote trained immunity through mTOR activation[128,129]. It is clear that both intrinsic cellular metabolic alteration and sustained epigenetic reprogramming are required to establish trained immunity. Furthermore, β-glucan and BCG both also stimulate myelopoiesis at the level of haematopoietic stem cells[129,130]. These results suggest that trained immune reprogramming endures and therefore has considerable potential as a novel route to the correction or realignment of ineffective immune responses. A more comprehensive understanding of trained immunity as the product of a complex interaction between epigenetics, cellular metabolism and signalling networks, may provide a novel avenue to improve disease therapeutics and vaccines.

Box 2 |

Tools to understand the metabolic crosstalk between host and pathogen

The integration of these tools and technical platforms is presented graphically in FIG. 5.

Microbial fitness reporter strains

There are many types of fluorescent fitness reporter that have been generated in bacterial strains. These include ribosomal RNA correlates [G][96], inducible GFP dilution[85,131] TIMER fluorescence[83,132], chromosomal replication complex reporters [88,107,133] environmentally responsive promoter reporters[88,107,109,134] and pH-sensitive GFP [G][135,136]. The value of these reagents is that they enable the status of a pathogen to be linked spatially to the host macrophage population. This allows analysis and quantitation by confocal microscopy, phenotypic analysis of surface markers on host cells and cell sorting by flow cytometry for RNA-sequencing (RNA-seq) analysis or metabolic profiling.

Animal models

An animal model should recapitulate as much of the biology of the infection site of human disease as is possible. All models have caveats but we believe that a model that generates functionally relevant heterogeneity of host cell populations in vivo has considerably greater biological relevance than the in vitro infection of homogeneous transformed mammalian cell lines.

Flow-sorting capacity

To interrogate specific host–pathogen pairings with respect to infection control and progression requires sufficient flow-sorting capacity under appropriate biosafety conditions to discriminate macrophage lineages and microbial fitness phenotypes.

Single-cell RNA-seq and dual RNA-seq

The limited coverage of single-cell sequencing can be experimentally challenging, but this is an advancing field and the technologies are improving with extraordinary rapidity. Some platforms, such as the Seq-Well platform, can readily be used under biosafety level 3 (BSL3) conditions and require no specialized equipment[137]. Dual RNA-seq has the added advantage that the transcriptional profiles of host cells and bacteria can be directly integrated, and this has been carried out at both population level and for single cells[89,90].

Metabolic profiling

Metabolic profiling through the quantitation of key intermediates is readily achievable, and live profiling of metabolic activity can be assessed using Agilent’s Seahorse platform[138].

Chemical inhibitors

There are several chemical inhibitors of metabolic pathways of immune cells such as macrophages that have been invaluable in the manipulation of cell physiology to probe

immune phenotype[15]. These compounds, together with new inhibitors of specific bacterial pathways[40], provide new tools for modulation of the host–pathogen interface.

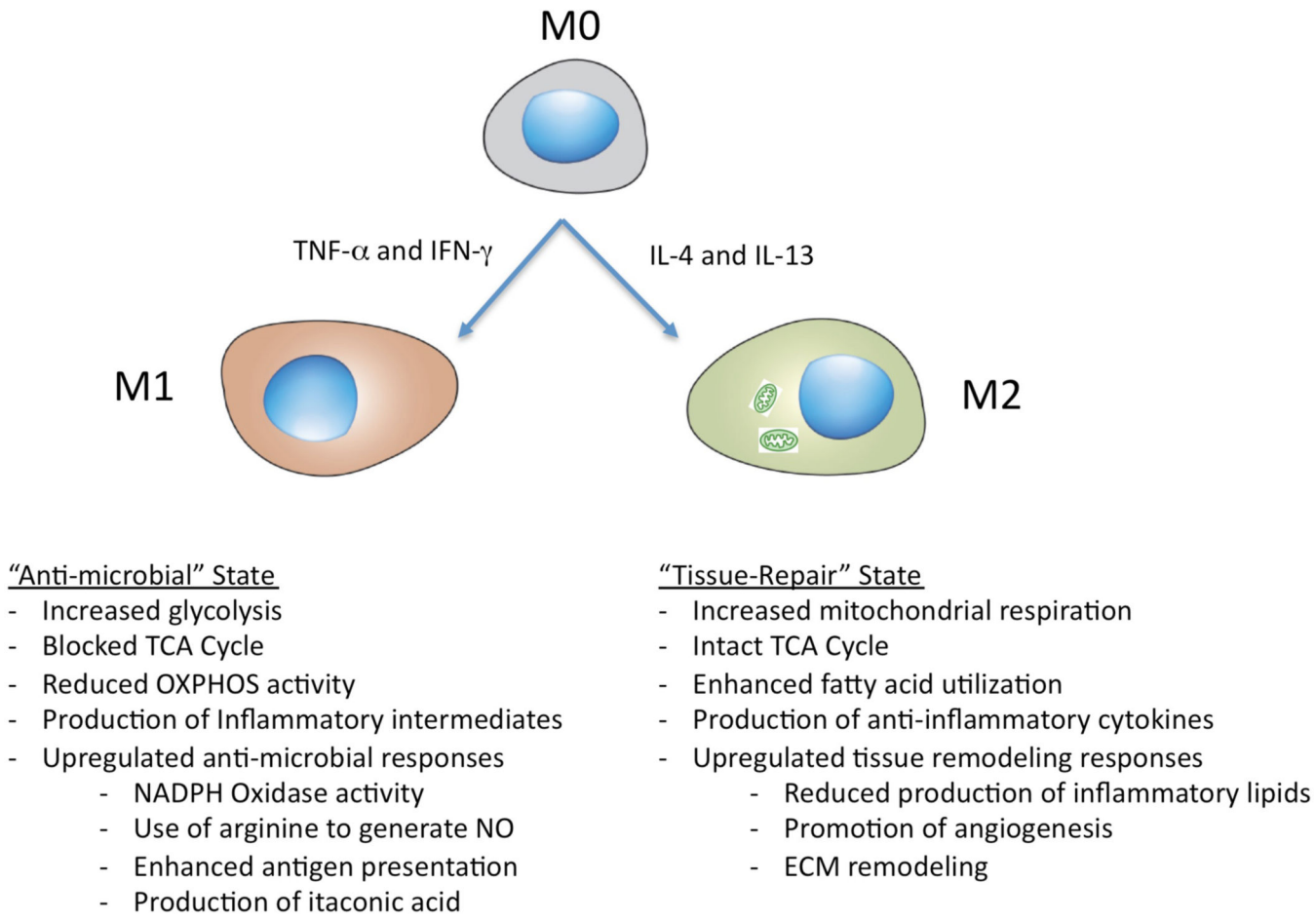

Figure 1 |. Phenotypic characteristics of the M1-like and M2-like macrophage polarization states.
The M1-like and M2-like macrophage polarization states are an in vitro paradigm that highlights the pro-inflammatory or anti-inflammatory phenotype of these cells but oversimplifies the highly specialized, transcriptomically dynamic and extremely heterogeneous nature of macrophages in vivo. In brief, M0 macrophages are activated to an M1-like macrophage status by exposure to IFNγ and/or tumour necrosis factor (TNF), or LPS, whereas M2-like macrophages are induced through treatment with IL-4 and/or IL-13. M1-like macrophages are more glycolytically active and markedly upregulate activities that are associated with microbicidal responses. By contrast, M2-like macrophages have increased mitochondrial respiration and enhanced fatty acid metabolism and upregulate activities that are associated with tissue remodeling or wound healing. ECM, extracellular matrix; NO, nitric oxide; OXPHOS, oxidative phosphorylation; TCA, tricarboxylic acid cycle.

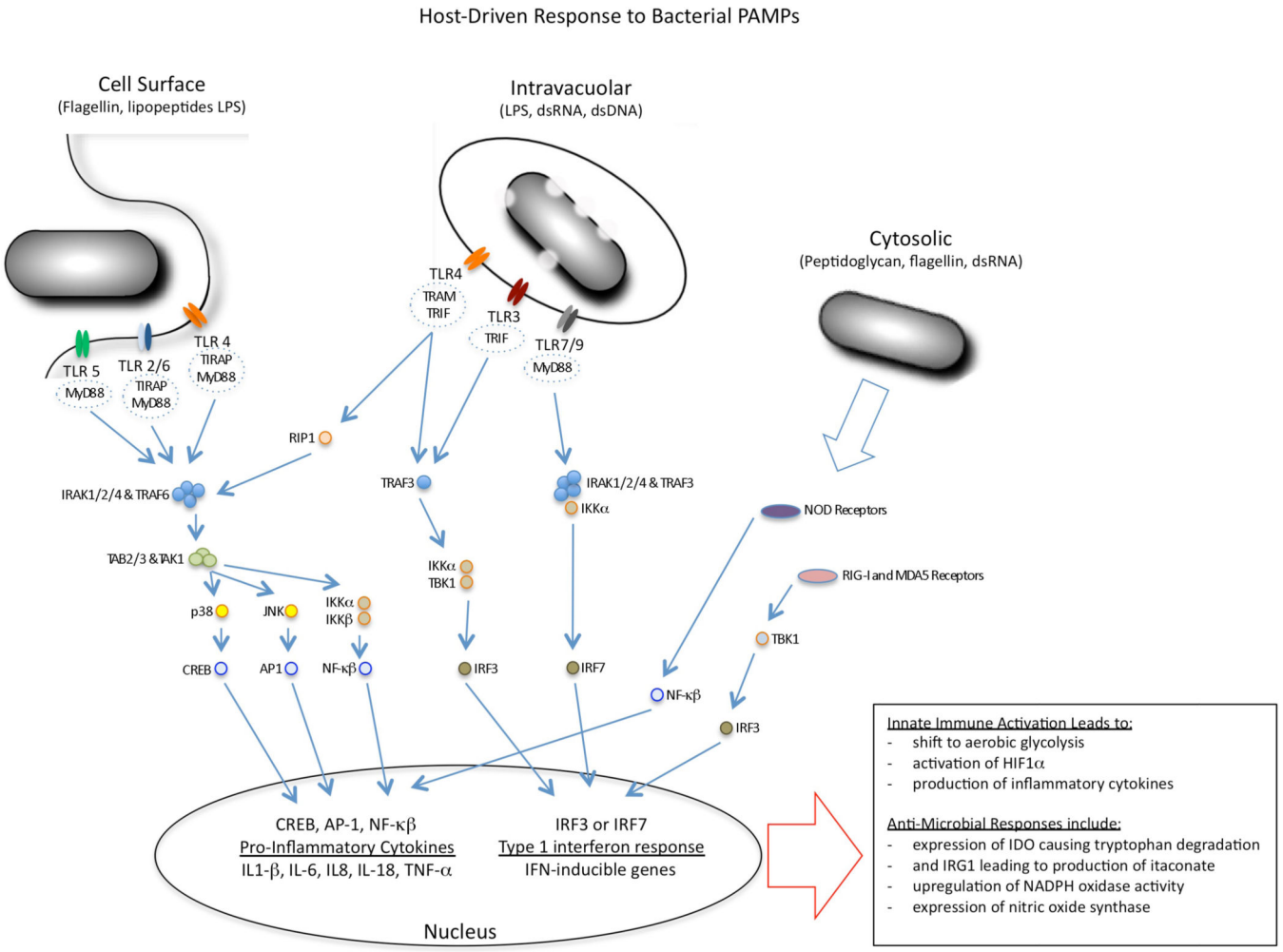

Figure 2 |. Modulation of macrophage metabolism by microbial mediators.

Microorganisms modulate the metabolic status of macrophages through the activation of innate immune signalling pathways or through the use of specific effectors, known as ‘virulence’ factors. The figure summarizes the major pattern recognition receptors (PRRs) found at the cell surface of macrophages and inside the cells, together with key components of their signalling pathways. Microorganisms activate Toll-like receptor (TLR) signalling pathways in host cells through their nucleic acids and cell wall constituents — known as pathogen-associated molecular patterns (PAMPs) — which drive macrophages into a state of enhanced glycolysis and increased production of lactate. The TLRs sense their ligands within the endosomal network as well as at the cell surface. They use different adaptor proteins that can modulate the outcome of TLR ligation, although the dominant responses are linked to either the production of pro-inflammatory cytokines through the activation of nuclear factor-κB (NF-κB) or the induction of a type I interferon response through interferon regulatory factor 3 (IRF3) and IRF7. PAMPs that are delivered into the cell cytosol by bacterial secretion systems activate signalling pathways through NOD-like receptors (NLRs), retinoic acid inducible gene I (RIG-I) and melanoma differentiation-associated gene 5 (MDA5), with similar metabolic and cellular outcomes. The metabolic shift in the macrophages is accompanied by the upregulation of antimicrobial responses,

including expression of indoleamine 2,3-dioxygenase (IDO) to limit tryptophan availability, production of itaconate through the activity of immunoresponsive gene 1 (IRG1) to intoxicate bacterial metabolism, and enhanced production of reactive oxygen and nitrogen species. ds, double-stranded; HIF1α, hypoxia inducible factor 1α; IKK, inhibitor of NF-κB kinase; IRAK, IL-1 receptor-associated kinase; LPS, lipopolysaccharide; MYD88, myeloid differentiation primary-response gene 88; NOS2, inducible nitric oxide synthase; TBK1, TANK-binding kinase 1; TIRAP, Toll/IL-1R (TIR)-domain-containing adaptor protein; TRAF, TNF receptor associated factor; TRAM, TRIF-related adaptor molecule; TRIF, TIR-domain-containing adaptor protein inducing IFNβ.

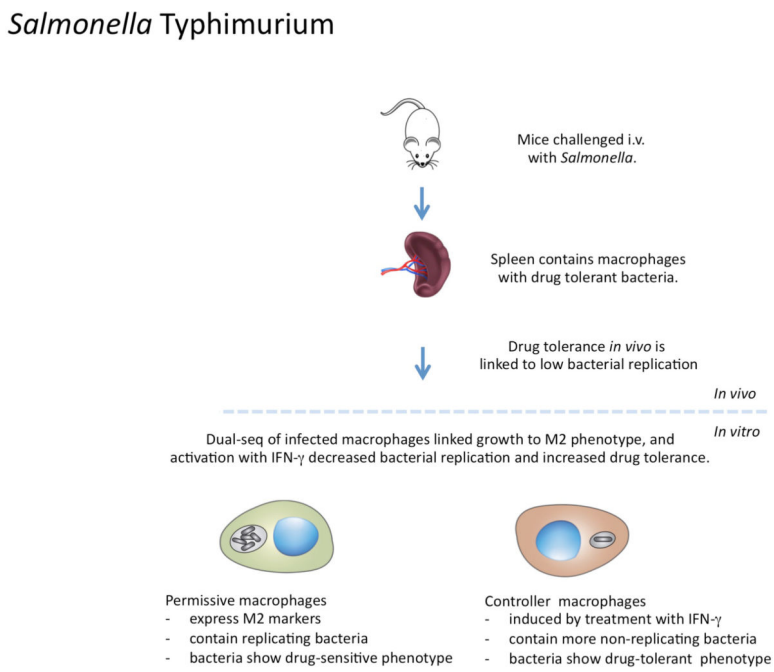

Figure 3 |. The links between macrophage phenotype and *Salmonella* Typhimurium physiology. In mice infected with *Salmonella enterica* serovar Typhimurium, the presence of 'persister' bacteria was shown in splenic macrophages[83,85]. These bacteria were non-replicating and had strong antibiotic tolerance. In vitro experiments established that the induction of a drug-tolerant phenotype was greatly enhanced in bacteria present in macrophages activated by IFNγ[85]. Single-cell RNA-sequencing of in vitro-infected, bone marrow-derived macrophages indicated that rapidly growing bacteria were found mainly in macrophages expressing markers of M2-like polarization[89]. These data suggested that the in vivo induction of drug tolerance in *S.* Typhimurium was most likely due to M1-like macrophages, and that support of bacterial growth was most likely due to macrophages with an M2-like phenotype.

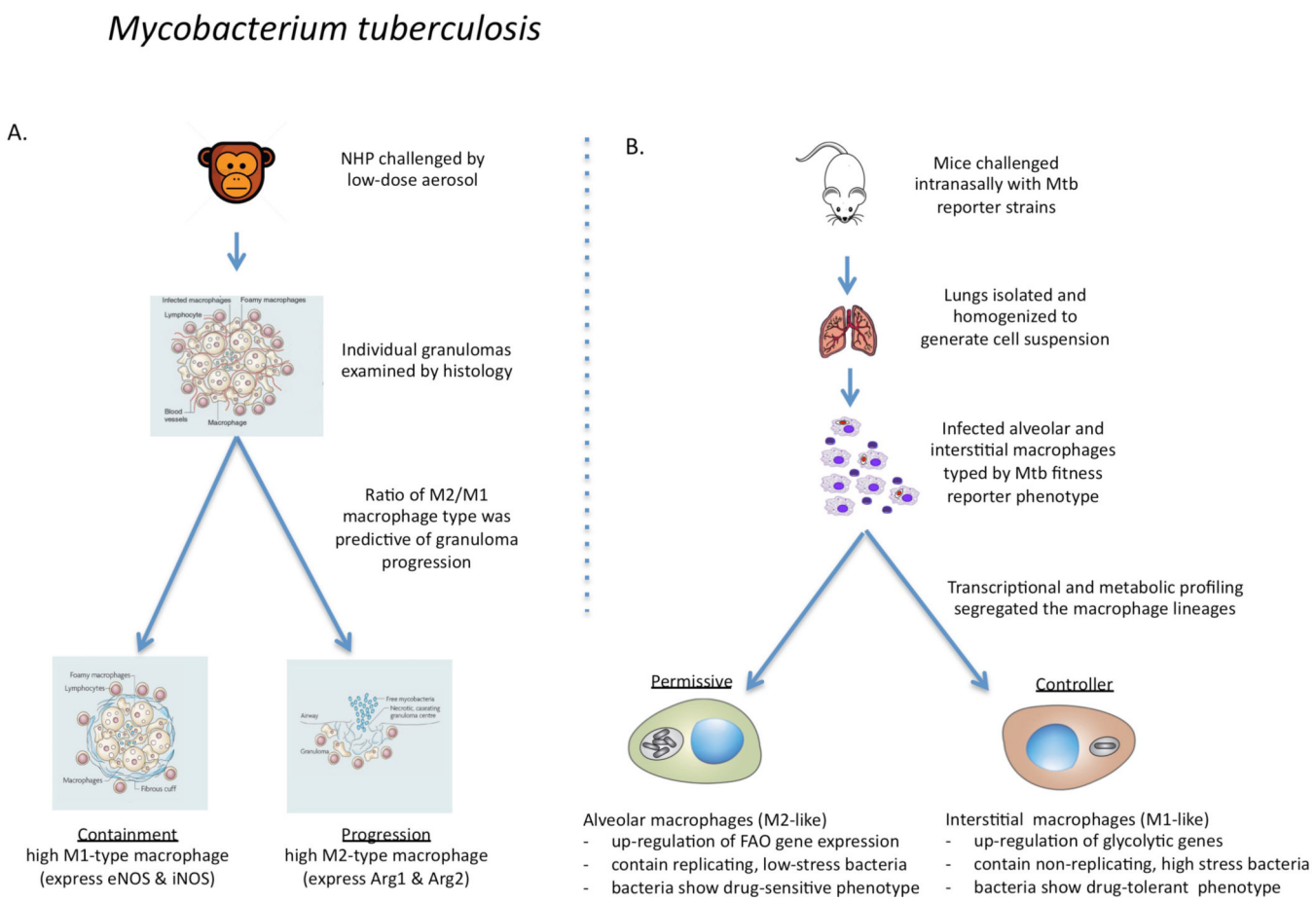

Figure 4 |. The links between macrophage phenotype and the progression of *Mycobacterium tuberculosis* infection in non-human primates and mice.

a | In macaque monkeys infected with *Mycobacterium tuberculosis,* the bacterial burden in individual granulomas and progression to active disease were found to correlate directly with an increased ratio of macrophages expressing M2 markers over Ml markers[105,106]. Progression was determined at the level of the individual granuloma without any consensus at the level of the host. **b** | In mice infected with fitness and replication reporter strains of *M. tuberculosis*, resident alveolar macrophages were more permissive of bacterial replication than were recruited interstitial macrophages[107]. RNA-sequencing showed that the alveolar macrophages were more committed to fatty acid oxidation (FAO) and oxidative phosphorylation, whereas the interstitial macrophages had higher levels of glycolysis. Selective depletion of the alveolar macrophage population reduced the bacterial burden, whereas depletion of the interstitial macrophage population led to an increase in bacterial burden. Therefore, in agreement with the data from non-human primates[105,106], the ratio of alveolar (M2-like) macrophages to interstitial (M1-like) macrophages affects bacterial growth and disease progression in mice. In addition, drug-tolerant *M. tuberculosis* were more abundant in vivo in macrophages expressing M1-like activation markers[95].

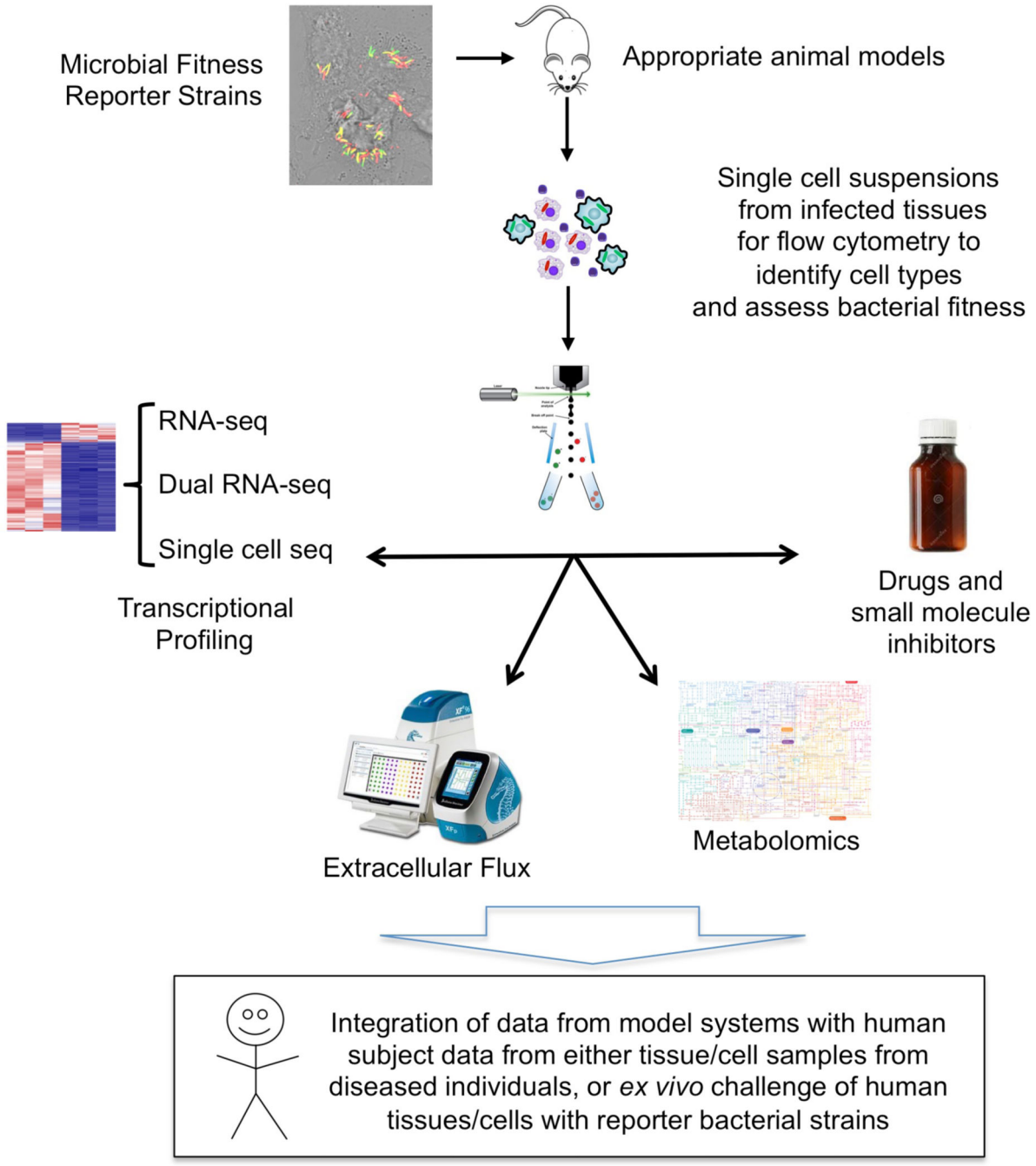

Figure 5 |. Technologies and tools for investigating the metabolic interplay between host and pathogen.

The figure illustrates how fluorescent microbial fitness and replication reporter strains (BOX 2) can be used to infect appropriate animal models to generate in vivo heterogeneity of relevance to disease progression or control. Infected tissues are analyzed by the generation of single cell suspensions for flow cytometry to yield cell populations defined by immune markers and bacterial fitness profiles. These cell suspensions can be sorted according to the host or microbial readout of relevance and then assessed further by various RNA-sequencing protocols to define the transcriptomes of both host and pathogen, and by metabolic profiling

through the analysis of intermediates or through metabolic flux studies. Small molecule inhibitors or drugs specific for host or microbial targets can be used to perturb the system and test hypotheses relevant to the metabolic interface between host cells and pathogens. Ultimately the data generated in the model systems need to be integrated with data from actual human disease to test its validity.

Table 1

Modulation of host macrophage metabolism through pathogen-associated molecular patterns and bacterial effector proteins

Pathogen	Intracellular niche	Primary intracellular carbon source	Modulation of host cell metabolism	Differential survival in macrophage lineages	Refs
Protozoa					
Leishmania spp.	Vacuolar	Glucose essential in vivo but can also use amino acids	Arginine deprivation response in host macrophages	Preferential growth in tissue-resident macrophages	113,139–141
Trypanosoma cruzi	Cytosolic	Strongly glucose dependent	Enhanced glycolysis	ND	138,142,143
Toxoplasma gondii	Vacuolar	Flexible, co-metabolizes glucose and glutamine	Innate immune activation through mTOR	Differential growth linked to macrophage polarization	144–147
Fungi					
Candida albicans	Vacuolar	Glucose, through glycolysis and gluconeogenesis	Innate immune activation	ND	148,149
Bacteria					
Listeria monocytogenes	Cytosolic	Glucose and glucose-6-phosphate	Innate immune activation and induction of specific effectors to reduce mitochondrial function	Type I interferon enhances pathogen spread	46,75,80,150
Shigella flexneri	Cytosolic	Pyruvate and glucose	Innate immune activation and induction of specific effectors to enhance glycolysis	ND	151–153
Salmonella enterica serovar Typhimurium	Vacuolar	Generalist	Innate immune activation and induction of specific effectors	Persister phenotype enhanced by type 1 immune responses	27,85,89,92
Coxiella burnetti	Vacuolar	Bipartite metabolism	Innate immune activation and induction of specific effectors to modulate lipid homeostasis	Tissue-resident macrophages highly susceptible to infection	154–157
Brucella spp.	Vacuolar	Glucose, through glycolysis	Enhanced glycolysis	ND	76
Legionella pneumophila	Vacuolar	Bipartite metabolism with serine preference	Innate immune activation and induction of specific effectors to reduce mitochondrial function	ND	54,79,158
Mycobacterium tuberculosis	Vacuolar	Cholesterol and fatty acids	Innate immune activation and induction of specific effectors to modulate host cell response	Growth sustained best in resident alveolar macrophages	40,42,107

mTOR, mechanistic target of rapamycin; ND, not determined.