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Spotlight on mycobacteria and dendritic cells: will novel targets to fight tuberculosis emerge?

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Over thousands of years microbes and mammals have co-evolved, resulting in extraordinarily sophisticated molecular mechanisms permitting the organisms to survive together. *Mycobacterium tuberculosis* is one of the best examples of successful co-evolution, since the bacilli have infected one third of the human population, but in 90% of the cases without causing overt disease. Despite this, increasing incidence of Human Immunodeficiency Virus (HIV) infection and the emergence of drug-resistant strains means that tuberculosis is in fact an extremely serious emerging threat to global health. Decades of work have focused on the interaction of this pathogen with its established cellular host, the macrophage, but still novel therapeautics remain elusive. While the macrophage is clearly important, recent evidence suggests that understanding the role of dendritic cells, which are key regulators of immunity, may be a crucial step in identifying new means of controlling this disease. Novel technologies, in particular genome-wide transcriptome analyses, are advancing our ability to dissect the complex dynamic relationships between dendritic cells and mycobacteria, highlighting new areas for study that have not been previously explored.

Introduction

Tuberculosis (TB) is an infectious disease caused by the pathogenic micro-organism *Mycobacterium tuberculosis* (*M.tb.*), which shares a long history with the human population (Figure 1). The disease commonly affects the lungs, but may spread to almost any organ of the body. Transmission is mainly *via* the inhalation of aerosolized droplets from the lungs of people with active respiratory disease during close contact. It may take many months from the time of infection until symptoms develop, making early diagnosis difficult. Two major patterns of disease then emerge. Primary TB is seen as an initial infection, usually in children, which results in the formation of small granulomas that generally spontaneously resolve without further spread of the infection. The secondary manifestation of TB occurs mostly in adults as a reactivation of previous infection (or re-infection), often when the individual's health status declines. The granulomatous inflammation is much more florid in this case and can become widespread, leading to significant cavitation in some instances.

With over one-third of the world's population infected with *M.tb.* and three million people killed by the disease per year, TB is an emerging global problem. However, in the presence of an effective immune response, only 5–10% of the infected population will develop active TB in their lifetime. Until recently it was thought that current antibiotic treatments were sufficient to control the disease on a global scale, considering their high efficacy in optimal treatment regimes and the low level of conversion from latent to active disease in the population as a whole. However, the emergence of multi-drug-resistant and extensively drug-resistant TB strains,

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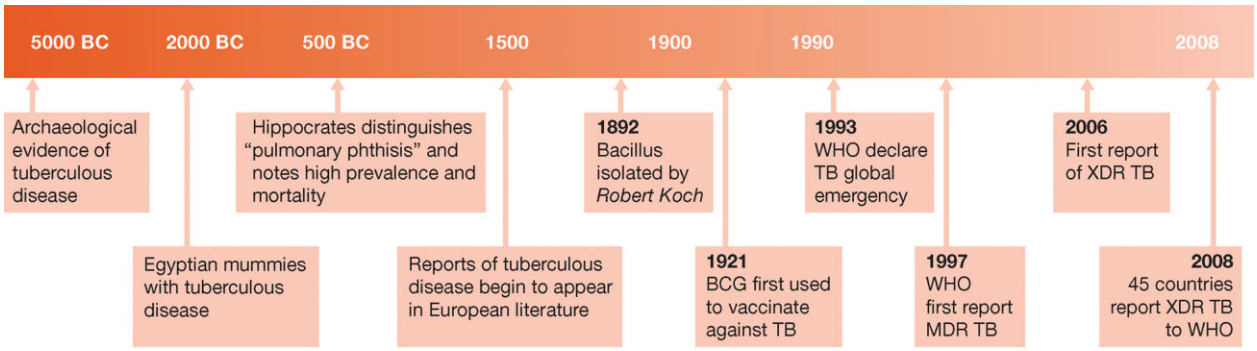

Figure 1. Timeline of landmark events in TB history with mankind since 5000BC. MDR TB, multi-drug resistant tuberculosis; XDR TB, extremely drug-resistant TB.

coupled with increasing incidence of HIV co-infection threatens to return us to an era without any effective way of controlling this disease.

A better understanding of the biology of *M.tb.* is critical in order to define novel therapeautic targets and strategies to fight TB. Following inhalation, the *M.tb.* target cell for replication has long been established as the alveolar/interstitial MF (Cunningham et al, 1925), and to date the vast majority of TB literature has focused on the interactions of the pathogen with this cell type. However, although the MF is clearly important in TB, this cell type is unable to initiate or shape the adaptive immune responses, which are so important during *M.tb.* infection, without the guiding influence of the DC.

DCs occupy a unique position in the vertebrate immune system and in this review we will summarize how they are involved in immunity to TB, and importantly how they serve a role that is both critical and distinct from that of MFs. We will review current knowledge of the cross-talk between *M.tb.* and the human host in the context of both MFs and DCs, and finally will discuss new data that begins to elucidate the special relationship between DCs and *M.tb.* during infection.

Glossary

Cavitation
Cavities form in the lung tissue where granulomas develop a necrotic core adjacent to the wall of a large air passage. This is advantageous for bacterial spread both within the host and to other individuals.

Draining lymph node (LN)
The lymph node is the site of initiation of an adaptive immune response. It is a structure containing the specialized microenvironment for lymphocyte priming; DCs and antigen drain from the periphery into the node, where naïve T cells and B-cells can be activated to proliferate and become immune-effector cells (a process called 'priming').

Steady-state DCs
In the 'steady state', *i.e.* the absence of infection or other inflammatory stimuli, DCs in an immature form patrol the body's tissues taking up samples of the environment and migrating to the lymph nodes to ensure that the homeostasis of the immune system is maintained.

Phagocytosis
Phagocytosis is a mechanism that cells use to take up solid particles. In the immune system this is carried out by a family of cells including monocytes, MFs, DCs and granulocytes, and is an important part of the defence against pathogens.

Role of DC in immunity during *M.tb.* infection

The immune response to *M.tb.* is multifaceted and complex, involving both DCs and MFs. These two related populations are both members of the myeloid lineage derived antigen-presenting cell (APC) family. Whilst they are related, they exhibit different functional characteristics and have distinct roles in TB. DCs are the unique immune cells possessing the ability to lead either immunity or tolerance, and to determine the characteristics (and ultimately the efficacy) of the immune response. Upon mycobacterial encounter, DCs effectively phagocytose live bacilli at the site of infection. Studies in MFs have shown that *M.tb.* is able to utilize a range of cell surface molecules to initiate its uptake, many of which are also expressed by DCs (Table 1).

The relative importance of the different receptors during natural infection has been difficult to establish, and in fact points to a significant role for DCs. Complement receptor 3 (CR3) had been thought to be required until studies in a knock-out mouse model failed to show a difference in infection compared to wild-type mice following intravenous inoculation of *M.tb.* (Hu et al, 2000), whereas it seems that the significance of DC-specific ICAM-3 (intercellular adhesion molecule 3) grabbing non-integrin (DC-SIGN) may have been underestimated. One hypothesis is that different receptors may dominate the interaction of *M.tb.* with different host cells or at different stages of infection. The mannose receptor (MR) could be most important early in infection, as it is highly expressed in particular by alveolar MFs in the steady state, but its expression is down-regulated by interferon (IFN)-γ that is efficient in producing successful immune response to TB (Schreiber et al, 1993). In contrast, despite negligible expression of DC-SIGN on alveolar MFs in the steady state, it seems that expression is induced during active infection with TB and moreover that MFs expressing DC-SIGN may be preferential targets for infection in humans (Tailleux et al, 2005). The emerging role of DC-SIGN should also be considered in the light of known mycobacterial interactions with DCs *via* this molecule, resulting in efficient

Table 1. DC and MF expression of cell surface receptors capable of mediating phagocytosis of mycobacteria *in vitro* and/or *in vivo*

Receptor	Synonyms	Proven relevance *in vivo*	DC expression	MF expression	References of interest
CD14	—	−	+/−	+	(Khanna et al, 1996; Peterson et al, 1995)
Complement receptor 1	CD35, C3b/C4b receptor	−	+/−	+	(Nauta et al, 2004; Schlesinger, 1993)
Complement receptor 3	CD11b/CD18, C3b receptor, MF 1 antigen	−	+	+	(Ben Nasr et al, 2006; Hu et al, 2000; Villeneuve et al, 2005)
Complement receptor 4	CD11c/CD18	+	+	+	(Bermudez et al, 1999; Schlesinger, 1993)
DC-SIGN	CD209, CLEC4L	+	+	+/−	(Geijtenbeek et al, 2003; Tailleux et al, 2005, 2003b)
MR	CD206	−	+	+	(Engering et al, 1997; Lohmann-Matthes et al, 1994)
Scavenger receptor (class A)	—	−	+	+	(Kurzai et al, 2005; Zimmerli et al, 1996)
Tranferrin receptor	CD71, p90	−	+	+	(Brinkmann et al, 2007; Roecklein et al, 1992)

uptake of bacteria and the induction of an immunosuppressive, interleukin-10 (IL-10)-secreting phenotype (Geijtenbeek et al, 2003).

In addition to the DCs present in the lung in the steady state, monocyte-derived DCs are recruited to the lung interstitium from the bloodstream and take up live bacilli within 48 h of intranasal infection with Bacillus Calmette-Guérin (BCG) expressing green fluorescent protein (GFP) (Reljic et al, 2005). The number of DCs in the lung containing BCG was found to peak at 48 h post-infection followed by decline at 72 h, while the proportion of infected MFs remained steady during the same period (Reljic et al, 2005). Uptake of mycobacteria by DCs results in cell activation and maturation characterized by a high level expression of major histocompatibility complex (MHC) class II and co-stimulatory molecules including CD40, CD80 and CD86. These are supplied to T cells in the presence of mycobacterial antigens following migration to the draining lymph node (LN).

In this specialized microenvironment the presentation of antigenic peptides by migratory DCs induces the activation and differentiation of naïve $CD4^+$ αβ T cells into effector T cells (Jiao et al, 2002; Tian et al, 2005). During T cell priming, several cytokines produced by DCs are essential for the appropriate stimulation of T cells. In particular, during *M.tb.* infection, the Type 1 cytokines IL-12, IL-1 and IL-18 are secreted, leading to differentiation of T-helper 1 (Th1) cells (Salgame, 2005). The Th1 response initiated by DCs is of great importance as people with genetic mutations in the genes encoding proteins important for Th1 polarization suffer from recurrent or fatal mycobacterial infections (Ottenhoff et al, 1998). Recently, it has been shown that DCs exposed to *M. tb.* also secrete IL-23, which can contribute towards polarization of T cells towards the novel IL-17-producing Th17 phenotype (Stockinger & Veldhoen, 2007). IL-17 enhances T cell priming and potentiates the inflammatory response, including the induction of nitric oxide synthase (NOS)-2. This results in enhanced neutrophil recruitment and the accumulation of MFs at the site of infection (Khader & Cooper, 2008), but the exact role of DC-produced IL-23 during *M.tb.* infection remains to be resolved.

In addition to T cell polarizing cytokines, DCs also produce the pro-inflammatory cytokines, tumour necrosis factor (TNF)-α and IL-6, which act to enhance the IFN-γ and TNF-α produced by the stimulated T cells, subsequently resulting in increased MF-mediated antimicrobial activity (Giacomini et al, 2001; Henderson et al, 1997; Hickman et al, 2002; Kim et al, 1999).

With their unique role in T cell priming, DCs and not MFs will be the most important cell type for initiating immunity to TB. Following *in vivo* depletion of the $CD11c^{hi}$ DC populations in mice, the T cell response to TB is significantly delayed and disease is exacerbated (Tian et al, 2005). However, studies in mice using GFP-tagged BCG have shown that mycobacterial dissemination from the lung is also mediated by the infected migratory DCs draining to the LN (Humphreys et al, 2006), alluding to a role for DCs in the pathology as well as the resolution of TB.

DCs are important targets for *M.tb.* immune evasion

A characteristic of TB is the formation of a granuloma (see Box 1). This is a crucial site for communication between the host immune system and *M.tb.* and also for survival of the bacillus. Although DCs are known to be present in the TB granuloma, their role in this environment is uncharacterized but is likely to be critical if we are to fully understand the interactions of the pathogen and the immune system in this environment. Unfortunately, few models exist in which latent *M.tb.* infection can be accurately studied. In the most commonly used system, the mouse, latency must be induced by the use of drug therapy and does not accurately represent the human situation. Alternative approaches to, and validation of, models that closely mimic human latent infection are needed.

The infection status of DCs in the granuloma is unknown, but the high level of MF infection and death results in the development of necrosis in the core of the tubercles, which may progress to cavitation and spread of disease (Tufariello et al, 2003). Recently, for the first time, Gan et al (2008) have identified a molecular mechanism used by virulent mycobacteria at this late stage to induce efficient MF death but escape MF-mediated killing and maintain their ability to replicate within the granuloma. In contrast to attenuated *M.tb.* strains such as BCG, virulent mycobacteria block MF apoptosis and

Box 1: The granuloma

A granuloma is an organized collection of immune cells, specifically including a large proportion of MFs, which occurs in a tissue as a result of chronic unresolved inflammation. The inflammatory stimulus may or may not be infectious, and a number of conditions apart from TB also exhibit granulomas including cryptococcosis, sarcoidosis, leprosy and chronic granulomatous disease.

The purpose of the granuloma for the host in an infectious disease is two-fold. Firstly, through the formation of a fibrous capsule it may be able to physically contain the infected area and prevent pathogen spread. Second, by bringing together an array of immune effector cells including APCs, lymphocytes and granulocytes, the granulomatous area becomes important for the development of effective immunity.

The tuberculous granuloma was first defined by Ghon in 1912, but until recently a lack of accurate and detailed examination of primary human granulomas hampered the understanding of their precise role in TB. Interestingly, it seems that while the interior of the granuloma harbours few APC containing mycobacterial antigens, the areas immediately surrounding the granuloma exhibit abundant, organized aggregates of APC and proliferating lymphocytes and are therefore the likely site of active immunity (Ulrichs & Kaufmann, 2006). The centre of the TB granuloma frequently contains an area of necrosis, thought to result from previous extensive MF infection and killing, but the granuloma also allows the chronic maintenance of *M.tb.* in infected MFs. Therefore, the TB granuloma may be considered not only as a crucial part of the protective immune response to the disease, but also as a facilitator in the development of latent infection, which is hard for the immune system to tackle and is notoriously difficult to treat by conventional methods.

instead induce cell death by necrosis, preventing bacterial killing by effector molecules associated with programmed cell death. A further advantage for the mycobacteria is that the loss of membrane integrity during necrosis allows viable bacilli to escape from host cells, after which they can infect new cells thus propagating the cycle of infection (Gan et al, 2008).

Alongside the immediate advantage of enhancing intracellular survival, a MF apoptosis blockade could possibly contribute to immune evasion. Schaible et al (2003) infected human MFs derived from monocyte-enriched peripheral blood mononuclear cells with BCG *in vitro* and in agreement with the Gan (2008) study, detected apoptosis in cells infected by this attenuated strain. The authors demonstrated that the apoptotic vesicles released by the dying cells contain mycobacterial lipids and proteins and were taken up by uninfected DCs during co-culture (Schaible et al, 2003). However, this study also detected vesicle release following virulent *M.tb.* infection, a difference that is not easily accounted for by the protocols used. Murine MFs infected with BCG engineered to express an ovalbumin peptide also undergo apoptosis, accompanied by apoptotic vesicle production (Winau et al, 2006). The injection of these vesicles into mice allows their uptake by DCs resulting in the priming of $CD8^+$ T cells specific for the encoded peptide. Vaccination of naïve mice using the vesicle preparation was able to confer protection against challenge with virulent *M.tb.* equally as well as the conventional BCG vaccine (Winau et al, 2006). Overall, these studies support the conclusion that the inhibition of apoptosis by virulent *M.tb.* as demonstrated by Gan et al (2008) is likely to have significant advantages for the bacterium *in vivo*.

The study by Gan et al (2008) also emphasized how *M.tb.* manipulation of the host cell at a molecular level can have dramatic effects on the course of disease in the whole organism, and hints at potential new targets for therapeautic intervention. Another area of ongoing research is how *M.tb.* is able to actively interfere with phagocyte function, and so how we might be able to block this process to enable bacterial destruction by the target cell.

M.tb. and BCG both possess genes encoding proteins capable of interfering with the anti-mycobacterial properties of MF nitrogen oxide (NO). Analysis of *M.tb.* mutants with defects in proteasome-associated genes has illustrated the importance of this pathway. In particular, a mycobacterial mutant deficient in a proteasomal adenosine triphosphatase was attenuated in mice, and when wild-type mycobacteria were exposed to proteasomal phosphatase inhibitors they became markedly sensitized to reactive nitrogen species produced by MFs (Cole et al, 1998; Darwin et al, 2003). If this effect could be reproduced *in vivo*, the interaction of *M.tb.* with MFs might be turned more to the host's advantage and result in more effective bacterial killing.

As in the MF, mycobacteria also manipulate important functions of DC, which is not surprising considering their importance in the anti-mycobacterial immune response. In mice infected with GFP-expressing *M.tb.*, it has been demonstrated that the DC subpopulation most efficacious in stimulating $CD4^+$ T cells in the LN is the one that carries a lower burden of mycobacteria (Wolf et al, 2007). There is also evidence that live *M.tb.* can inhibit MHC class II antigen presentation without a decrease in the surface expression of MHC class II and co-stimulatory molecules (Hava et al, 2008). The poor antigen presentation observed in *M. tb.*-infected DCs could be explained by a mis-timing of antigen availability and the maturation process, as proposed by Hava et al (2008). DCs are extremely responsive and can undergo the process of maturation rapidly following stimulation. Particularly in the case of slow-growing intracellular pathogens, or arrest of phagosome maturation, it may be that the activation program proceeds ahead, before abundant mycobacterial antigens can be acquired by the cell. This apparently simple case of poor timing may represent a novel way for pathogens to manipulate DCs and thus the immune response to their own advantage.

M.tb. possesses several ligands for the toll-like receptor (TLR) family of molecules expressed by both MFs and DCs. Binding of ligands such as mannosylated-lipoarabinomannan (Man-LAM) and other lipoproteins to TLRs results in downstream signalling

events causing cellular activation (and maturation in the case of DC) and instructing the developing immune response. Mycobacterial Man-LAM and other lipoproteins act as potent TLR2 agonists. Activation of TLR2 modulates various MF and DC activities with two main, opposing effects. On one hand, a number of pro-inflammatory cytokines are produced (TNF-α, IL-12 and IL-1) and DC maturation is induced by the mycobacteria. On the other hand, TLR2 signalling has been associated with inhibition of the MF response to IFN-γ, which is fundamental in limiting the infection. TLR2 signalling also induces the production of IL-10, an important immunoregulatory cytokine with the ability to dampen DC function and the immune response in general. The significance of this cytokine is attested by the fact that IL-10 has been identified as a correlate of susceptibility for TB in both mice and humans (Bonecini-Almeida et al, 2004; Eum et al, 2008).

In addition, there is evidence that IL-10 is pivotal during the chronic/latent stage of pulmonary TB, with increased production, playing a potentially central role in promoting reactivation (Beamer et al, 2008; Turner et al, 2002). However, the role of IL-10 in acute infection (at least in animal models) is unclear, as IL-10 knock-out mice control virulent *M.tb.* as well as wild-type mice (North, 1998). IL-10 is also an important cytokine for the development and expansion of T-regulatory (Treg) cells. While the role of Treg cells in suppressing immunity to *M.tb.* is only beginning to be investigated, it is likely to be of importance. Several reports described that Treg cells are present at increased number in the blood, lungs and LN of infected mice as well as of TB patients (Chen et al, 2007; Guyot-Revol et al, 2006; Ribeiro-Rodrigues et al, 2006). Of particular interest is the finding that $CD11c^+$ DCs from TB-infected murine lungs during acute infection significantly up-regulated chemokine (C-C motif) ligand 17 (CCL17) gene expression (compared to MF) (Jang et al, 2008). In humans, the receptor for CCL17 is expressed by $CD4^+CD25^+$ Treg cells, suggesting a possible role for DCs in their recruitment to the site of primary infection (Iellem et al, 2003).

M.tb. occupies similar but distinct niches in MFs and DCs during infection

The ability of *M.tb.* to infect MFs is well-established, but its ability to infect DCs is a more recent discovery (Gonzalez-Juarrero & Orme, 2001). Following inhalation of the bacilli during primary exposure they are engulfed by alveolar MFs residing within or underlying the lung alveoli, whose role is to phagocytose and destroy invading pathogens. Subsequently mycobacteria are also phagocytosed by DCs.

M.tb. phagocytosis requires interaction with specific cellular receptors that can be expressed on MFs and DCs as mentioned earlier, but also involves host cell plasma membrane cholesterol. Phagocytosis of mycobacteria was abolished following cholesterol depletion from the host membrane, while uptake of non-mycobacterial cargoes was unaffected (Gatfield & Pieters, 2000). These findings led to the discovery of a mycobacterial cholesterol receptor, homologous to human Receptor-$C_{k,}$ that is required to mediate the interaction between MFs and the mycobacteria, but whether the same mechanism operates during DC infection is unknown (Kaul et al, 2004).

Following the formation of the *M.tb.*-containing vacuole and detachment from the plasma membrane, the early phagosome begins maturation through a series of intracellular fusion and fission events. For non-mycobacterial cargos this involves the gradual accumulation of lysosomal components and in activated MFs there is also recruitment and activation of inductible-NOS (iNOS) and the nicotinamide adenine dinucleotide phosphate (NAPDH) oxidase complex to the phagosomal membrane, resulting in the production of microbicidal reactive oxygen and nitrogen species (Bogdan et al, 2000; Garin et al, 2001). Together these mechanisms cause pathogen destruction and thereby generate antigenic peptides that are subsequently presented to the host immune system, posing a significant problem for any microbe wishing to establish a survival niche in these cells.

Whether taken up by a DC or a MF, *M.tb.* will initially face the same challenges. Tailleux et al (2003a) carried out the first detailed study of the cellular niches occupied in human DCs compared to MFs. They found that monocyte-derived DCs phagocytosed virulent *M.tb.* in a manner comparable to monocyte-derived MFs. The mycobacterial phagosome also avoided acidification and lysosomal fusion in DCs as in MFs, as detected by the lack of accumulation of an acid–tropic dye during 5 days of infection. In phagocytes, generally the process of complete phagosome maturation following FcR- or MR-mediated uptake takes under 30 min (Aderem & Underhill, 1999), with the pH dropping to below 5 within the first 10 min (Yates et al, 2005), *i.e.* too acidic for *M.tb.* replication. Based on the expression of early endosomal markers such as Rab5, an absence of late endosomal markers including Rab7 and a pH 6.8, the *M.tb.*-containing phagosome in MFs appears to have been arrested at about the 4 min stage (Rohde et al, 2007). Remarkably, this means that *M.tb.* must significantly interfere with the normal process of phagosome maturation within the first few minutes after internalization, and it has evolved a formidable array of strategies to achieve this.

A number of mechanisms are important for *M.tb.* arrest of phagosome maturation in MFs. These remain uncharacterized in DCs, but based on the similarities highlighted above at least some of them are likely to be equally relevant. Mycobacterial cell wall lipids are key effectors in this process; in particular Man-LAM is a highly pleiotropic molecule. Downstream processes of Man-LAM binding to the MR inhibit phagolysosomal fusion in human MFs *in vitro* (Kaul et al, 2004), which could be the critical step in the early establishment of infection. Man-LAM also interferes with the host calcium–calmodulin–phosphoinositide-3 kinase (PI3K) pathway, barring the generation of large quantities of phosphatidylinositol-3-phosphate (PI3P) (Vergne et al, 2004b), a lipid that is important in the correct regulation of intracellular trafficking including phagolysosomal fusion. Thirdly, the same molecule interferes with the accumulation of syntaxin 6, which mediates the delivery of lysosomal hydrolases and the vacuolar proton ATPase to the phagosome (Vergne et al, 2004a). This is further supported by the actions of additional cell wall components; trehalose 6,6′-dimycolate and phenolic glycolipid, which through unknown mechanisms are

both required for mycobacterial prevention of phagosome acidification (Indrigo et al, 2002; Robinson et al, 2008).

Alongside the actions of cell wall lipids, a newly identified protein in the mycobacterial cell wall, Rv3671c, seems to confer the ability to resist a level of phagosomal acidification by protecting mycobacterial intracellular pH and permitting survival (Vandal et al, 2008), further increasing *M.tb.*'s resilience in a hostile environment. *M.tb.* also secretes active mediators into the host cell cytoplasm. For example, the phosphatase SapM is able to hydrolyse any PI3P that has escaped the inhibitory effects of Man-LAM on its generation (Vergne et al, 2005). As well as subverting normal vesicular trafficking, the depletion of PI3P also inhibits autophagosome formation, which has the potential to override *M.tb.*-mediated phagosome arrest in MFs (Gutierrez et al, 2004). This further emphasizes the importance of disrupting the function of PI3P for *M.tb.* survival.

More detailed characterization of the *M.tb.*-containing phagosome in DCs revealed the presence of coronin 1 on the vesicular membrane (Tailleux et al, 2003a). Also known as tryptophan aspartate-containing coat protein, coronin 1 accumulates transiently on the surface of nascent phagosomes but is actively retained by the *M.tb.* vacuole in MFs, where it is critically required for mycobacterial survival (Jayachandran et al, 2007). This further supports the idea that at least during the early stages of phagosome remodelling, similar mechanisms are at work in the DC and MF environment.

Despite such similarities, the surprising finding was that *M.tb.* is unable to replicate in DCs. This is supported by evidence from *in vivo* studies of mycobacterial infection (Jiao et al, 2002). Whilst *M.tb.* is not killed or directed to lysosomes in DCs, it is also not multiplying within the cells, which implies a stark difference in the interaction of the mycobacterium-containing phagosome with DCs compared to MFs. Tailleux et al (2003a) investigated potential explanations for the lack of replication observed in DC and uncovered important differences in the way the two cell types respond following phagocytosis of *M.tb*. In addition to interfering with phagosome maturation, mycobacteria normally also manipulate intracellular trafficking in MFs to acquire nutrients needed for growth. The mycobacterial phagosome in MFs has access to transferrin-bound iron (Kelley & Schorey, 2003) and GM1 ganglioside (Russell et al, 1996). The early endosomal small GTPase Rab5 is actively retained by the *M.tb.* phagosome, and facilitates this iron acquisition by regulating the routing of recycling vesicles (Kelley & Schorey, 2003). Moreover, *M.tb.* is capable of manipulating other members of the Rab family, including Rab22a that prevents the Rab conversion between Rab5 and Rab7 on the nascent phagosome, which would otherwise herald the onset of phagosome maturation (Roberts et al, 2006). Tailleux et al (2003a) found the absence of Rab11 on *M.tb.*-containing phagosomes in DCs compared to MFs, illustrating a lack of interaction of the phagosome with recycling vesicles. Furthermore, in DCs the *M.tb.* phagosomes were largely negative for markers of the endoplasmic reticulum, showing that they were also cut-off from the host biosynthetic pathway that is a significant source of lipids in particular, which are so important for *M.tb*. Figure 2 illustrates how phagosome maturation is differentially affected by *M.tb.* in DCs and MFs.

Transcriptome analyses shed light on co-evolution strategies of *M.tb.*

Current approaches to detection, prevention and treatment of TB are inadequate, and rational development of new tools has been limited by poor understanding of the fundamental biology of TB, particularly during latent infection. The advent of transcriptomic technologies has revolutionized this field, allowing molecular dissection of the conditions encountered by mycobacteria during infection and of the mutual adaptation of this pathogen and its host cells.

Host APCs are extremely important in mediating the immune response that will eventually determine resistance *versus* susceptibility to disease, and there are a number of recent studies addressing the interaction of *M.tb.* with these cell types at the molecular level (Chaussabel et al, 2003; Fontan et al, 2008; Volpe et al, 2006). An important aspect of the DC role is the secretion of immuno-modulatory cytokines and chemokines that shape the local and systemic immune response. Understanding the response to pathogens in terms of cytokine and chemokine production may allow the identification of a profile associated with successful clearance of the pathogen (that should be the aim of immune therapies) and could also point to further mechanisms of immune evasion that would serve as targets for intervention. Consistent with the distinct functional specialization of DCs and MFs, there is accumulating evidence that *M.tb.* infection induces different responses from the two cell types. Jang and colleagues (2008) compared chemokine and cytokine gene transcription in $CD11c^+$DC and $CD11b^+$MF from TB-infected murine lung at 2 weeks post-infection, and showed that IL-12p40 and CCL17 were significantly upregulated in DC compared to MF, and that chemokine (C-X-C motif) ligand 10 (CXCL10) was higher in MF. This is particularly interesting as the receptor for CCL17 is expressed by $CD4^+CD25^+$ T-regulatory cells, at least in humans, suggesting a possible role for DC in their recruitment to the site of primary infection (Iellem et al, 2003). A similar study using human monocyte-derived DC and MF examined the early time points in infection *in vitro* and detected an increase in IL-12 gene transcription in DC, but not MF, where instead the transcription of the immunosupressive cytokine IL-10 was the dominant response from as early as 3 h post-infection (Giacomini et al, 2001).

The cytokine and chemokine response is not the only difference between DCs and MFs during *M.tb.* infection. As previously mentioned, Tailleux and colleagues (2003a) demonstrated that while *M.tb.* replication proceeds normally in human monocyte-derived MFs, it is controlled in DCs and viable mycobacteria are retained within these cells. Transcriptional profiles of *M.tb.*-infected human and murine MFs have been described, but far less has been reported about DCs (Jang et al, 2008; Li et al, 2008; Volpe et al, 2006). Recently, expanding on their previous work, Tailleux and colleagues carried out a study simultaneously investigating the global transcriptional profile of both infected MFs and DCs, and also of the mycobacteria isolated from each cell type during infection (Tailleux et al, 2008). From the first time point post-infection at 4 h, DCs and MFs exhibited both core and differential responses to *M.tb.*,

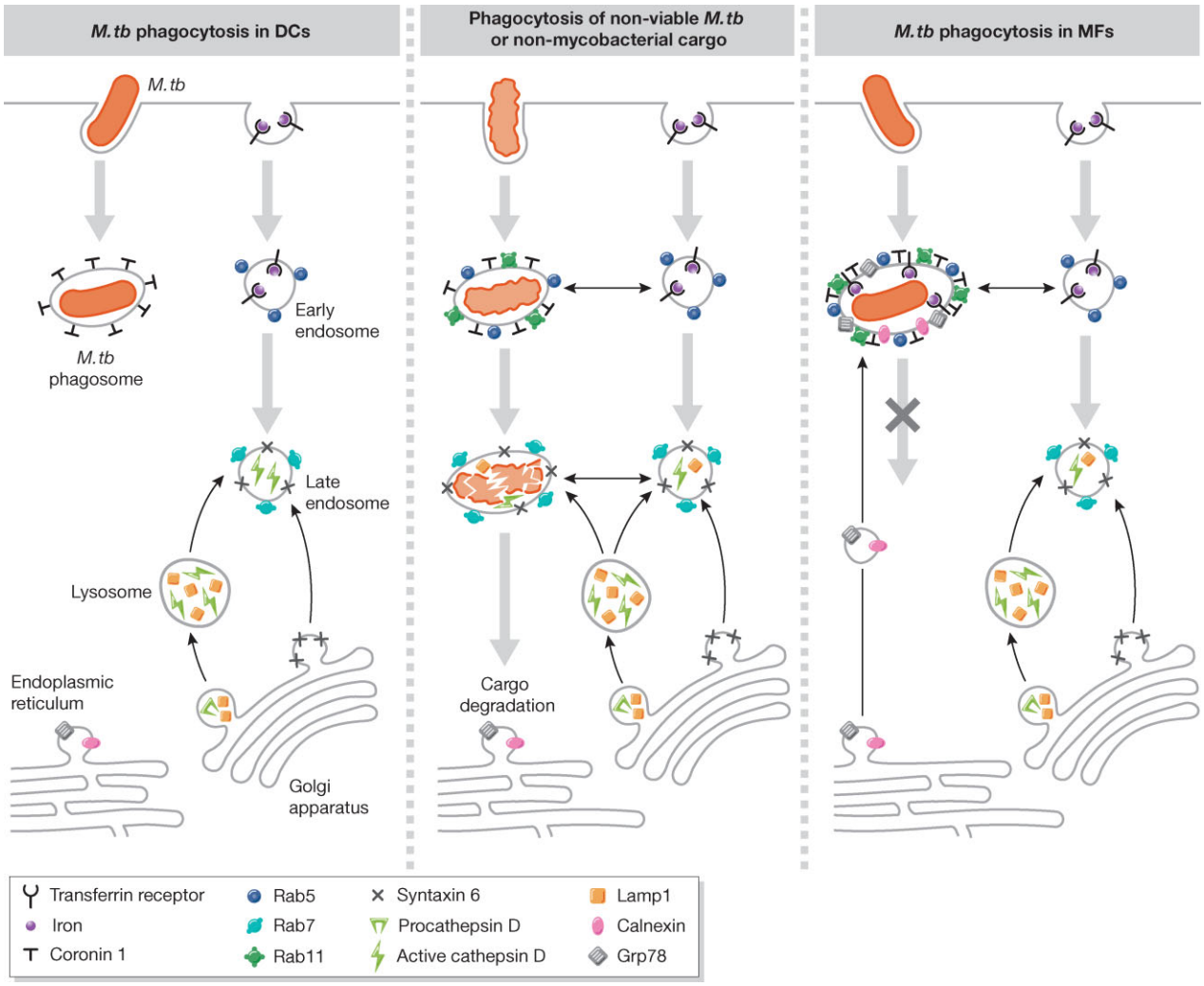

Figure 2. The process of normal phagosome maturation is differentially affected by *M.tb.* in DCs and MFs. The normal process of phagocytosis (for killed mycobacteria, or non-mycobacterial cargoes) involves gradual maturation of the nascent phagosome. Coronin 1 expression is transient, and the loss of this protein combined with the switch of Rab5 to Rab7 expression, and the acquisition of syntaxin 6, promotes maturation of the phagosome and finally lysosomal fusion. In MFs the *M.tb.* phagosome is arrested at an early stage of maturation, and retains the expression of coronin 1 with Rab5. Interaction with early/recycling endosomes is encouraged by the expression of Rab11, and this gives mycobacteria access to transferrin-bound iron. Lipids are also an important energy source for mycobacteria, and can be acquired through interaction with the endoplasmic reticulum/biosynthetic pathway, which is detected by the presence of calnexin and Grp78 on the phagosome membrane. In comparison to MFs, far less is known about the mycobacterial phagosome in DCs. What is understood is that the phagosome is similarly arrested and retains coronin 1 expression, but exhibits far fewer interactions with the host intracellular trafficking machinery (based on the data of Tailleux et al, 2003a).

showing a clear cell-type dependent transcriptional programme. The distinct roles of DCs and MFs in the immune response to TB were reflected accurately in the data. For example, the lipid–antigen- presentation molecules CD1a–c, and the p40 subunit of the Th1-polarizing cytokine IL-12 were modulated in DCs only. Conversely, the expression of the inflammatory cytokines IL-1b and IL-6 was induced mainly in MFs (Tailleux et al, 2008).

Following on from the findings of Tailleux et al (2003a) that the *M.tb.* phagosome in DCs behaved differently from that in MFs, it was particularly interesting that a number of gene families associated with intracellular vesicle trafficking, cytoskeleton remodelling and vesicle acidification were also differentially modulated in the two cell types. For example, the small GTP-binding proteins Rac isoform 1 and 2 showed opposite patterns of regulation: Rac1 was induced in DCs but not in MFs, whereas Rac2 was induced in MFs but not detected in DCs (Tailleux et al, 2008). As well as roles in the regulation of intracellular trafficking, the Rac 2 (but not the Rac 1) isoform has been associated with an increase in reactive nitrogen species in activated MFs, which is important in the control of *M.tb.* (Kuncewicz et al, 2001). MFs also expressed higher levels of the

Phox subunits p40, p67, and p91 that are involved in directing vesicular targeting, compared to DCs (Tailleux et al, 2008).

Overall DCs were more responsive to *M.tb.* infection than MFs, for example they expressed a higher number of genes involved in the IFN response. Of note, the very strong induction of the suppressor of cytokine signalling 2 exclusively in *M.tb.*-infected DCs could have important consequences for the different cytokines induced in the two cell types (Tailleux et al, 2008).

On the other side of the cross-talk, analysis of the *M.tb.* transcriptome in the two host cells also revealed interesting differences. The changing respiratory state of the bacilli inside the human phagocytes from aerobic to micro-aerobic or anaerobic was exemplified by the overall repression of gene transcription (relative to aerobic exponential-phase growth). As expected, *M.tb.* displayed a gene transcription profile consistent with active replication in MFs, for example the increased transcription of ribosomal genes. In contrast, the *M.tb.* transcriptome in DCs revealed a situation more akin to mycobacterial responses under conditions of nutrient limitation, oxygen deprivation or dormancy (Tailleux et al, 2008).

These studies are important because they offer for the first time the exciting possibility of linking the transcriptional responses of both mycobacteria and host cells to biological outcomes, and therefore the hope of understanding the factors controlling mycobacterial replication on the cellular level. If the cross-talk between the bacteria and the two cell types can be dissected in more detail, it might lead to novel mechanisms of inhibiting *M.tb.* replication in MFs using the same strategies that DCs naturally employ. The opposing possibility is also interesting, that *M.tb.*'s lack of replication in DCs is advantageous for the pathogen, and is specifically induced by *M.tb.* in this cell type. Perhaps replication in and destruction of DCs would induce too strong an inflammatory response, or result in an increased level of antigen presentation by bystander APC. Therefore understanding what makes MF alone so permissive to replication and using this information to reinstate replication in DCs could be equally informative.

Overcoming TB during active disease is likely to require strategies distinct from those needed to eliminate latent infection, which is notoriously difficult to treat. Although *in vitro* studies have obvious limitations, some useful information has been gained by subjecting *M.tb.* to conditions similar to those encountered in the lung during chronic infection and then examining the transcriptional response of the bacteria. The Wayne model (Wayne & Hayes, 1996) for example, uses gradual oxygen depletion to mimic the development of the granuloma, and was recently employed by Muttucumaru and colleagues (2004). The authors analysed mycobacterial gene expression at regular intervals during oxygen deprivation and demonstrated that entry of *M.tb.* into a non-replicating persistent state involved the significant upregulation of 178 genes compared to aerobic cultures. Of these, 88 were also regulated during further, extreme oxygen deprivation but within the remaining genes were a high proportion encoding unidentified proteins that are actively transcribed at the onset of dormancy (Muttucumaru et al, 2004). Identification of such proteins could well lead to the characterization of novel pathways involved in dormancy, and perhaps novel means to interfere with the bacterial capacity to establish a successful dormant state.

Pending issues

- Need for good models of *M.tb.* latency and reactivation
- Understanding the role of DCs in the TB granuloma
- Clarifying the function of IL-23 and IL-17 in TB

A study by Hampshire et al (2004) used an *in vitro* model of progressive nutrient depletion focussed on characterizing a small sub-population of mycobacteria that are able to survive and continue replication under extreme conditions *in vitro*. This work aimed to understand the distinct features of the sub-population of mycobacteria that are able to withstand such a hostile environment, so that the bacteria causing persistent infection in the granuloma *in vivo* might be better understood and therefore better targeted by therapies. The study identified a set of gene transcripts that are transiently upregulated during *M.tb.*'s process of active adaptation to nutrient limiting conditions, including, for example, the enzyme $Rel_{M.tb.}$ which is thought to mediate the downregulation of protein synthesis required for survival during dormancy (Hampshire et al, 2004). The same enzyme is also upregulated and required for survival under extreme hypoxic conditions (Sherman et al, 2001), and therefore might be of interest as a therapeautic target to prevent latent infection. The authors went on to characterize the transcriptome associated with the spontaneous reactivation of mycobacterial division in the culture, as a model for the emergence from latency in the granuloma. They successfully identified sets of genes involved in DNA repair and preparation for re-initiating cell wall synthesis, both potential new areas for intervention that could prevent emergence from latency in the human once the granuloma had developed (Hampshire et al, 2004).

Studies similar to the ones discussed here are extremely important. By understanding the host response to TB, and particularly how it is deficient or how it is manipulated by mycobacteria, we will be better informed in our strategies for vaccine design and immune-targeted therapies. For example, the study of Jang and colleagues (2008) hinted at a role of DC in the promotion of regulatory T cells during TB, proposing a mechanism for a biological effect reported previously in patients (Hougardy et al, 2007). This finding certainly warrants further investigation. The work of Tailleux et al (2003a, 2008) also began to link the biological observation of restricted *M.tb.* replication in DCs with the transcriptional response in these cells, and work is ongoing to identify and characterize the pathways responsible for this phenomenon. This offers the possibility of exploiting an apparently natural pathway of host cell resistance to TB, and opens the door for a new approach to control the infection. On the side of the pathogen, the studies of Hampshire and colleagues (2004) and Muttucumaru et al (2004) not only advance our understanding of *M.tb.* biology, but also allow the identification of potential novel mycobacterial targets to prevent successful establishment of latency, or to treat TB

during this notoriously difficult life stage. What remains to be seen is how these studies correlate with mycobacterial gene expression *in vivo* under similar conditions.

Conclusions

M.tb. is an emerging global threat, made more alarming by the increasing occurrence of multi-drug resistant and extremely-drug resistant forms of the disease. There is a desperate need for studies using novel approaches and new technologies to advance our understanding of this pathogen. Historically, research has focused heavily on the MF, and rightly so given the importance of this cell type, however new data are revealing that the interaction of *M.tb.* with host DCs could be equally important. We have illustrated here the interactions of this bacterium with its cellular hosts, focussing on DCs and how recent transcriptional studies are beginning to unravel the complex inter-relationship between *M.tb.* and APCs. There is a striking evidence to challenge the traditional view that DCs are mere bystanders during TB infection, as they actively take up *M.tb.* and are intimately involved in the initiation and shaping of the immune response. By better understanding the role of DCs in immunity to TB and how they are exploited by mycobacteria, we hope to elucidate new ways of controlling this disease.

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For more information

WHO Tuberculosis:
http://www.who.int/tb/en/

Extremely drug-resistant tuberculosis (XDR-TB) awareness site:
http://www.xdrtb.org/

TB-related clinical trials:
http://clinicaltrials.gov/ct2/results?term=tuberculosis

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