Chapter 34 : Mycobacterium

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Mycobacterium

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aIntroduction

The *Mycobacterium* genus includes diverse pathogenic and non-pathogenic species (Table 1). Non-tuberculous mycobacteria (NTM) are prevalent in the environment and can cause opportunistic tuberculosis-like infections in many animals, including humans. NTM infections are important as they can cause false-positive test results due to cross immune reactions and may not be distinguished from obligate pathogenic mycobacteria. The *Mycobacterium tuberculosis* complex (MTC) responsible for tuberculosis (TB) includes *M. tuberculosis* (Mtb), *M. africanum*, *M. canettii*, *M. bovis* (Mb), *M. caprae*, *M. pinnipedii* and *M. microti*. While Mtb is largely responsible for TB in humans and 1.7 million deaths/year, Mb, responsible for bovine TB (bTB) is present in cattle and wildlife. Mb displays zoonotic potential; human TB cases acquired from infected animals are underestimated due to lack of resources to distinguish these genetically closely related species. Control of TB is a real challenge today and requires "One health" approaches, considering that the health of animals, humans and the environment is connected. The vaccine Bacillus Calmette Guérin (BCG) was obtained in 1921 by attenuation of a virulent Mb isolate. Today this vaccine has been administered to more than 3 billion people and is recommended by WHO in countries with high incidence of TB, to protect children from the most severe forms of TB. Cattle and wildlife are also affected by *M. avium* subsp. *paratuberculosis* (Map) responsible for paratuberculosis (paraTB) or Johne's disease; this is

widespread globally and represents an important source of animal suffering and economic loss[1].

aCharacteristics of the organism and source of infection
bMycobacteria represent a large family with few successful pathogens

The mycobacteria are a very large and diverse group of predominantly environmental organisms, a small proportion of which have evolved opportunistic pathogenic tendencies and still fewer have become dedicated, obligate, pathogens of humans and animals. Technically the mycobacteria are Gram-positive because of their cell wall architecture and peptidoglycan chemistry, but more importantly they are characterized by an *in vitro* characteristic: even when exposed to acid and heat, mycobacterial cell walls do not allow penetration of organic solvent. This enables the retention of a pink dye, carbol fuchsin, which is revealed through light microscopy. This commonly used taxonomic feature makes them "acid fast". In medicine and veterinary science, this resistant cell wall makes many disinfectants ineffective, allows ingested organisms to withstand gastric acid and, through complex interplays with innate immune mechanisms, allows pathogenic mycobacteria to persist within tissues. Perhaps because of their environmental origins in diverse niches, the pathogenic mycobacteria have evolved a range of strategies to perpetuate their lineages in animal hosts: environmental persistence, resistance to lysis within phagocytes, avoidance or perturbation of adaptive immunity, long incubation periods before the health of the host is severely compromised, and exit from the host by a range of routes.

bGenome peculiarities

[1] Common abbreviations used in this chapter: Antigen Presenting Cell **APC**; bovine tuberculosis **bTB** ; macrophage **MP** ; *Mycobacterium avium* spp *paratuberculosis* **Map** ; *Mycobacterium bovis* **Mb** ; M. tuberculosis **Mtb** ; *M. tuberculosis* complex **MTC ;** paratuberculosis **paraTB** ; tuberculosis **TB**.

Mycobacterial genomes characteristically have a high GC content and a small number of ribosomal RNA genes in relation to the size of the genome. Mb and Map have genomes of 4.32 Mb and 5.2 Mb with 66% GC and 69% GC, respectively. Mycobacterial genomes contain numerous repeat sequences that present a challenge for sequencing and genome assembly. These include mobile genetic elements such as prophages and insertion sequences (IS) belonging to various families. Some IS have been targeted for diagnosis and molecular typing of mycobacterial species. The most important of these are IS*1110* for the MTC, IS*1245* and IS*1311* for the *Mycobacterium avium* complex, IS*901* for *M.a. avium*, IS*902* for *M.a.silvaticum* and IS*900* and IS*1311* for Map. Mycobacterial genomes encode a large number of genes involved in lipid metabolism, consistent with the presence of cell walls and membranes rich in lipids, glycolipids, lipoglycans and polyketides. Also present are many genes encoding enzymes involved in lipid oxidation pathways used for metabolizing putative degradation products of host cell membranes.

aSources of infection

Both Mb and Map are perpetuated through an obligate parasitic relationship with animals, but both species can persist in the environment after they have been shed from an infected host. In most cases, the source of obligate pathogenic mycobacterial infection is an infected host, either through direct contact or by indirect contact in a contaminated environment. Spread of mycobacterial infections over larger distances is generally through trade in livestock, facilitated by the cryptic nature of both infections during lengthy subclinical stages. The relative contributions of direct contact versus environmentally acquired infections to persistence of these pathogens in a population of animals will depend on the husbandry system. For example, environmentally

acquired infections may be important in barn-raised calves (Eisenberg et al., 2010).

Mb and Map have spilled over into sympatric wildlife populations from their endemic domestic animal life cycles. Mb wildlife reservoirs include white-tailed deer (*Odocoileus virginianus*) in North America, the European badger (*Meles meles*) in Ireland and the UK, brush-tailed possum (*Trichosurus vulpecula*) in New Zealand, wild boar (*Sus scrofa*) and red deer (*Cervus elaphus*) in the Iberian peninsula and buffalo (*Syncerus caffer*) and lechwe antelope (*Kobus leche*) in Africa. Map circulates in wild deer populations in many parts of the world, and it is generally believed that this has arisen as a spill-over infections from domestic livestock. For example, rabbits are a reservoir of Map in regions of Scotland. Evidence for interspecies transmission has been demonstrated by molecular typing and experimental infection. Furthermore, wildlife infections potentially compromise disease control in domestic species because of spill-back into livestock. The best-known examples of this are Mb infection in brush-tailed possums in New Zealand and badgers in England. Rabbits excrete high numbers of Map bacilli in faecal pellets that can be ingested by grazing livestock. This, combined with a relatively high prevalence of infection, the high population density of rabbits with access to livestock pastures and the lack of avoidance of rabbit faeces by grazing livestock presents a risk of Map infection for sympatric livestock. Map can persist on pasture and in soil or water and sediment for months, protection from incident solar radiation favoring longer term survival (Whittington et al. 2004; Eppleston et al. 2014). Mb also can survive in soil and water for months particularly in cooler temperatures (Barbier et al. 2017). Mycobacteria may have acquired parasitic and pathogenic lifestyles by coevolution in the environment with phagocytic amoebae, and both MTC and Map

have been found in amoebae in the environment (Drancourt 2014; Samba-Louaka et al. 2018).

aSource of infection:Evolution and epidemiology

Whole genome sequencing (WGS) has facilitated construction of the most reliable phylogenetic trees of *Mycobacterium* strains to date. The evolutionary rate of pathogenic mycobacteria is very slow, ranging from 0.15 to 0.53 substitutions/genome/year for Mb (Crispell et al. 2019) and less than 0.5 for Map (Bryant et al. 2016). Due to these slow evolutionary rates, just a few Single Nucleotide Polymorphism (SNP) differences could represent decades of evolution. The MTC is thought to have evolved by clonal expansion from a smooth tubercule bacillus population within *M. canettii* (Galagan 2014). Several major lineages of human adapted pathogenic Mtb (designated L1-L4, L7) and *M. africanum* (L5, L6, L9) and a recently discovered earlier lineage (L8) evolved, each primarily associated with a distinct geographical distribution and lineage-associated differences in virulence, transmission and acquisition of drug resistance. The animal-adapted members of the MTC, often referred to as ecotypes and of which there are at least nine, are thought to have derived from an L6 ancestor with the characteristic chromosomal region of difference (RD) deletions RD7 to RD10 and are paraphyletic with some members more closely related to L6 than other animal-adapted strains. Despite their similar host distribution, Mb and *M. caprae* ecotypes correspond to two monophyletic groups. The Mb lineage is itself divided into 12 monophyletic groups; four corresponding to the previously described clonal complexes Eu1, Eu2, Af1 and Af2 and eight unclassified groups. Other divergence events occurred from the Mb ancestral strains, but all strains remain classified as Mb. Phylogeography of Mb strains suggests an origin in East Africa and while some groups appear to have remained in East or West Africa, others have spread across the globe (Loiseau et al. 2020). The evolutionary

success of Mb is due to its capacity to infect and transmit effectively in cattle, resulting in global spread through cattle trading.

Phylogenetic analysis of the *M. avium* subspecies has shown that Map and *M. avium. avium* evolved independently from *M. avium. hominissuis*. WGS studies have clarified the phylogeny of Map strains. The Map lineage is subdivided into two major sub-lineages designated S (sheep-type; *MAP-S*) and C (cattle-type; *MAP-C*) (Bryant et al. 2016). Members of these two groups can be distinguished with respect to their cultural characteristics, virulence and pathogenicity (Stevenson 2015). *MAP-S* strains have been isolated from sheep, goats and camels whereas *MAP-C* strains have a broader host range encompassing all ruminant types, humans and some wildlife. Strain type is not exclusive to host provenance as interspecies transmission can occur where prevalence of disease is high and where different species are in close proximity. *MAP-S* can be further subdivided into Type I and Type III strains and *MAP-C* has a subdivision encompassing the 'Bison-type' strains. *MAP-S* and *MAP-C* can be differentiated by genetic polymorphisms, particularly by the presence/absence of large sequence polymorphism that can be the result of insertions or deletions (Stevenson and Ahlstrom 2020) but also by highly conserved single nucleotide polymorphisms (SNPs) such as in IS*1311*. *MAP-S* strains appear to have retained more ancestral genetic sequences and are more diverse than *MAP-C* strains. Like Mb, *MAP-C* strains appear to have evolved via a process of deletion of genetic regions, indels and SNPs. They are the predominant strains isolated from cattle, also very efficient in infecting and transmitting between cattle and have probably been spread around the globe through cattle trading.

The high discriminatory power of WGS has revealed greater diversity among virulent mycobacterial strains than previously recognised and has facilitated detection and differentiation of mixed strain infections (concomitant or sequential infections

of a single host by genetically distinct strains) and microvariation (where an isolate undergoes intra-host evolution) (Byrne et al. 2020). The presence of multiple strains with different genotypes can result in altered phenotypes such as antibiotic resistance and virulence and affect clinical outcome and transmission dynamics.

WGS data has revealed the close genetic relatedness of cattle and badger isolates in sympatric populations and persistence of bacterial lineages on or near farms for several years despite interim clear herd tests (Biek et al. 2012). Combining Bayesian phylogenetic and machine learning approaches to analyze WGS data, Crispell et al. (2019) were able to infer that transmission of Mb occurred more frequently from badgers to cattle than *vice versa* and that intraspecies transmission occurred at higher rates than interspecies transmission for both, suggesting that both cattle and badgers need to be targeted in control programs.

aVirulence factors and pathogeneomics

Current knowledge of virulence factors is derived from mining mycobacterial genomes (Mtb genomes mostly) for homologues of genes encoding factors known to be virulent in other organisms, as well as the construction and analysis of mutant libraries associated with appropriate screens in cellular and animal models. It is not within the scope of this chapter to give a comprehensive account of mycobacterial virulence factors but key factors pertinent to Mb and Map virulence will be briefly described.

bThe cell wall

The pathogenesis of mycobacterial disease largely depends on the immunoregulatory cell envelope. This highly structured and complex entity is a virulence factor *per se* that comprises unique lipids and glycolipids. The cell envelope also confers to mycobacteria their hydrophobicity and natural resistance to antibiotics such as β-lactamases. The cytoplasmic membrane

comprises phosphatidylinositol mannosides (PIMs) and lipomannans and lipoarabinomannans (LAMs) which both derive from PIMs. LAM from slow-growing pathogenic mycobacteria, modified by mannose residues, exquisitely manipulate the immune system. LAM inhibits maturation of the phagosome upon entry into macrophages (MPs) by interfering with intracellular calcium concentrations; LAM antagonizes apoptosis of infected MPs to the benefit of mycobacterial multiplication and downregulates MHC-II antigen presentation to favor mycobacterial escape from the immune response; LAM targets the C-type lectin receptor DC-SIGN on the surface of dendritic cells (DCs) to downmodulate their maturation and capacity to activate the adaptive immune response (Koul et al. 2004; Dulberger et al. 2020). The external face of the cell envelope is composed of mycolic acids that are essential to the growth of mycobacteria. They can be free or attached to trehalose sugar to give trehalose dimycolate (TDM), also called "cord factor" in reference to the capacity of mycobacteria to grow as serpentine cords, which relies on TDM production. TDM is highly inflammatory and is involved in granuloma evolution and caseation. Free lipids at the surface of the cell envelope include phthiocerol dimycocerosate (PDIM) that is crucial for infection. PDIM masks pathogen-associated molecular-patterns (PAMPs) present in the mycobacterial envelope to avoid recognition by PAMP-receptors and immune signaling (Cambier et al. 2014). The cell wall of *Rhodococcus equi* (Chapter 34) has similar characteristics and roles in its pathogenesis.

bESAT-6/CFP-10

ESAT-6 (Early Secreted Antigenic Target of 6 kDa) and CFP-10 (Culture Filtrate Protein of 10 kDa) are secreted by virulent mycobacteria from the MTB complex. The history of discovery of these key virulence proteins goes back to the late 1990's when genomic comparison of all BCG vaccine strains used in different countries revealed deletion of one common

chromosomal region called RD1. It appeared that RD1 loss was the principal cause of BCG attenuation (Behr et al. 1999). The RD1 locus encodes the ESX-1 Type VII secretion system that allows the ESAT-6/CFP-10 heterodimeric complex to be secreted. Both Mtb and Mb genomes encode 5 ESX systems in total but only ESX-1 and 5 are involved in virulence. ESX-1 is key to virulence: mutant strains deleted of the system are highly attenuated in mice and other animal models. Together with the cell wall compound PDIM (Augenstreich et al. 2017) ESAT-6 breaks the phagosomal membrane of the MP to allow escape of bacteria into the cystosol. The ESAT-6/CFP-10 heterodimeric protein also drives necrosis of infected cells allowing progression of the disease, interferes with signaling pathways in infected MPs, and downregulates the immune response of the infected host. Since the ESX-1 system is absent from *M. avium* and Map, which are also successful pathogens, why the ESX-1 secretion system contributes to virulence in only some species remains unknown.

bPE and PPE proteins

Mycobacterial genomes contain numerous *pe* and *ppe* genes encoding the PE and PPE protein families, named after the conserved proline (P) and glutamic acid (E) residues in their N terminal domains. In Mtb, *pe/ppe* genes cover up to 10% of the genome. PE/PPE proteins may be membrane nutrient proteins or involved in pathogenesis and are transported across the inner mycobacterial membrane by the Type VII secretion systems ESX-1, ESX-3 and ESX-5. PE/PPE proteins can be membrane or cell wall associated, or secreted heterodimers. The PPE protein has three highly conserved N-terminal alpha helices that bind to its cognate PE protein via hydrophobic interactions, which form a composite Type VII secretion signal. The C-terminals of the proteins are highly variable and have been used to classify the PE and PPE proteins each into five subgroups. They are involved in a number of mycobacterial virulence mechanisms: they inhibit

phagocytosis by MPs and prevent acidification and/or maturation of the phagosome; interact with TLR2 and mediate cell apoptosis, cytokine secretion and necrosis as well as maturation and activation of dendritic cells; inhibit reactive nitrogen species production. Enzymatic functions have been attributed to a few PE proteins; for example, they can function as lipases during starvation. They scavenge and cleave triacylglycerol as a nutrient source and have a role in iron homeostasis through mycobactin-mediated and heme-iron acquisition. Different members of the PE/PPE family express this large range of functions. Finally, all PE and PPE proteins possess many predicted and experimentally validated immunogenic epitopes that are of interest for development of diagnostics and vaccines. Of particular note are $PPE18_{IV}$ and $PPE42_{V}$, which form part of the M72/AS01E and ID93 subunit vaccines against human TB vaccines, in development (Schrager et al. 2020).
bMce proteins

The number of mammalian cell entry (Mce) proteins in *Mycobacterium spp.* varies from 6-66. The genes encoding these proteins are organized into operons usually comprised of two *yrb*E genes and six *mce* genes (*mce*A-*mce*F). The number of operons varies but all mycobacteria possess the mce1operon. *M. tuberculosis* has four (mce1-mce4), Mb has three (mce1, mce2 and mce4) and Map has eight (mce1-mce4 and two copies each of mce5 and mce7). The operons do not appear to be co-regulated and may function at different stages of infection. The Mce1, Mce2 and Mce4 proteins show 99.6-100% homology between Mtb and Mb. The homology between *M.avium* Mce proteins and the respective individual Mtb Mce proteins ranges from 56.2 to 85.5%. Alignment of Mtb and *M.smegmatis* Mce proteins reveals 58.5 to 68.5%. Since Mce proteins are present in non-pathogenic mycobacteria and deletion mutants of some *mce* genes are still pathogenic, Mce proteins are also likely to have roles in mechanisms other than virulence. Our knowledge of the function

of Mce proteins is derived from studies with Mtb. Mce1 proteins have been most studied for their role in mammalian cell invasion. They are functionally and structurally similar to ABC transporters and are involved in the transportation of fatty acids and mycolic acids. Deletion of the mce1 operon leads to a decrease in the expression of genes required for lipid transport and metabolism, affecting the cell wall. Mce2 proteins may be involved in the metabolism and import of sulfolipids and deletion mutants of the mce2 operon in Mb are attenuated. Mce3 proteins also appear to be involved in adhesion and penetration of cells, are immunogenic and mutations have been associated with drug resistance. Mce4 proteins are involved in the catabolism of cholesterol, cell invasion and formation of granulomas. Mce proteins also play a role in modulating host cell signaling (Fenn et al. 2020).
aRegulation of virulence

Successful infection by pathogenic mycobacteria relies on their ability to adapt to their extracellular and intracellular living conditions. They need to adapt to harsh environments such as exposure to Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNI) that are abundantly produced by the host cell, low pH, hypoxia in the granuloma, nutrient starvation or change in carbon sources. Moreover, these conditions vary during the lengthy infectious process and so mycobacteria constantly adapt, doing so through several transcription factors and two-component systems that sense the environment and upregulate or downregulate specific genetic programs.
bThe PhoPR two-component system

This two-component system, composed of the PhoP sensor that activates the PhoR transcriptional regulator, is central to Mtb and Mb virulence. PhoP controls production of key virulence factors such as ESAT-6 (Frigui et al. 2008) and the lipids from the cell wall (Walters et al. 2006). Deletion of the PhoPR system in Mb also attenuates virulence because

bacteria become less resistant to acidic stress and display reduced capacity to arrest phagosomal maturation in the MP (García et al. 2018). Interestingly, the PhoPR system allows fine tuning of virulence depending on the targeted host: in animal-adapted Mb and *M. africanum* strains, the PhoPR system induces low secretion of ESAT-6 and lipids which is compensated for by other mutations in the genome to allow productive infection. Humans are normally spill-over hosts for Mb infections. However, there was an exceptional outbreak of Mb infections transmitted between humans in Spain in the 1990's which was due to overexpression of the PhoPR two-component system (Gonzalo-Asensio et al. 2014).

bThe Dos R/S/T dormancy regulon

Redox reactions are a normal component of life that help maintain the balance between the oxidants and reactive species and the antioxidants inside the cell. In the lung, Mtb is exposed to a range of environments and so must constantly adapt its redox balance, which it does through two main mechanisms. The Dos/R/S/T dormancy regulon is a system where the two sensors DosT and DosS relay to the DosR response regulator information about the redox environment. DosR upregulates a set of 47 genes in the DosR regulon that helps Mtb adapt to the hypoxic environment of the granuloma. Mtb enters into dormancy, a non-replicative state characterized by metabolic quiescence. In this reversible state, Mtb can long-survive in a harsh intracellular environment.

bSigma factors

Sigma factors, some of which are involved in virulence and pathogenesis, form part of a tightly regulated network. Mb has 11 sigma transcription factors and Map has 19. A Mb SigA mutant is attenuated in a guinea pigs but not in the Australian brush-tail possum, which supports the hypothesis that mycobacteria employ different mechanisms to replicate and survive in different hosts. SigH and SigL both have key roles in survival

in MPs and Map SigH and SigL deletion mutants are significantly attenuated in mice. A Mtb SigC mutant is unable to induce early immune responses and form necrotic granulomas in lungs and spleens of guinea pigs. SigE is one of the major regulators involved in mycobacterial stress responses. SigE regulates genes involved in maintaining cell envelope integrity, lipid metabolism and the methyl citrate cycle. SigF is induced by cold shock, hypoxia and oxidative stress in BCG, but not in Mtb. Furthermore, using some sigma factors mutants as live attenuated vaccines confers significant immunity to challenge with virulent mycobacteria in mice and goats.

bIron regulation

Acquisition and storage of iron is essential for intracellular survival of pathogens. In mycobacteria, iron acquisition is mediated by small, soluble iron chelators called siderophores. These include mycobactins and exochelins, which are produced specifically in response to iron starvation. In some mycobacteria both cell-associated and secreted siderophores are often present. Mb produces only mycobactins and is unable to utilize water-soluble exochelins for iron uptake. Map cannot synthesize mycobactin when grown *in vitro* and it therefore has to be added to the culture medium for growth to occur. Mutations have been identified within the genes required for mycobactin biosynthesis, most notably in *mbtA* and *mbtE*, that could explain this deficiency. However, transcriptomic studies have shown that, despite these mutations, Map transcribes the mycobactin genes inside macrophages and infected tissues. In the host, Map may also acquire iron by the mycobactin-independent and iron dependent mechanism. The IdeR regulator upregulates *mbtB* (iron acquisition) and *bfrA (*iron storage). Polymorphisms in the promoter of *bfrA* in Map-S relative to Map-C strains results in differential gene regulation.

aTypes of disease

Most mycobacterial diseases are characterized by chronic inflammation typified by an accumulation of MPs and sometimes multinucleate giant cells, together with lymphocytes and variable degrees of fibrosis. Acute inflammatory cells may also be present in pyogranulomas, depending on the host species. Typical acid-fast bacilli (AFB) are visible in light microscopy within MPs, often in very low numbers (e.g., only one or a few per high power field), except in so-called multibacillary conditions (e.g., *M. avium* infection in birds and pigs, lepromatous paratuberculosis) where vast numbers of AFB are visible.

The location of these lesions varies with the pathogen. Predilection sites for Mb are the lung and lymph nodes of the head and thorax, while for Map they are the small intestine and mesenteric lymph nodes. The clinical diseases caused by these organisms are related to the organ systems involved: typically, there are respiratory signs for Mb and intestinal dysfunction for Map. However, weight loss despite adequate nutrition is a sign common of advanced stages in both infections, consistent with systemic effects on metabolism.

In most individuals, infection with virulent mycobacteria leads to a chronic disease state that is termed “latent infection” in human TB or bTB (individuals are skin test or IFN-gamma responders but may have no detectable lesions) or subclinical disease in paraTB. This is a silent stage of infection during which inflammatory processes are initiated and progress, and in which the bacterial burden eventually increases in individuals that succumb to clinical disease. In other individuals, infection may persist for life, and reactivate at some stage, a situation referred to as “latency”. The phenomenon is the subject of intense research focused on the granuloma and persistence of the mycobacterium within MPs. Assumptions about life long infection, latency and its importance in human TB have been questioned (Behr et al.

2019). In the case of Map, dormancy of the organism occurs in the environment, suggesting that latency may occur *in vivo,* but a wide range of pathogenic pathways including recovery from infection and disease have been documented (Whittington et al. 2017). The magnitude, type and individual variations of the host immune response are a key part of the divergent clinical outcomes of mycobacterial infections. Some animals progress to a clinical form of the disease and die whereas others apparently clear the infection or remain subclinically infected.

aPathogenesis

bInitial uptake, intracellular persistence and role of innate cells in early physiopathology

cInteraction with Pathogen-Associated-Molecular-Patterns Receptors

Glycolipids and glycopeptidolipids from the cell wall are recognized by pattern recognition receptors (PRRs) of targeted cells. The main PRRs for mycobacteria are: (i) the C-type lectins receptors that include the Mannose Receptors recognizing LAM and mannosylated LAM from the cell wall; DC-SIGN also interacting with LAM and other ligands; MINCLE that recognizes TDM (cord factor) and Dectin 1 although the mycobacterial ligand remains elusive; (ii) the Toll-Like Receptors are mainly surface TLR1, 2 and 4 recognizing cell wall glycolipids and endosomal TLR9 recognizing bacterial DNA, and (iii) the Nod-Like Receptors including NOD1, NOD2 which detect peptidoglycan and muramyl dipeptide. Early interactions taking place between the range of mycobacterial components and PRRs allow non-opsonic entry into target cells such as MPs and DCs and orchestrate the immune response by production of cytokines and chemokines.

cEpithelial cells

Mycobacteria are able to enter and to live in various cell types including non-professional phagocytes. It is largely

accepted that upon entry into the lung, alveolar MPs phagocytose Mtb and traverse the alveolar barrier via diapedesis, a mechanism referred to as the "Trojan Horse" (Nguyen and Pieters 2005), to establish infection. A role for Alveolar Epithelial Cells (AECs, also called pneumocytes) in primary infection is also accepted. Mtb infects type 2 AECs in humans and in an AEC type cell line, Mtb replicates ~15–20-fold more than in MP (Mehta et al. 1996). Several mechanisms are proposed for Mtb internalization in type 2 AEC, including the ESAT-6 virulence factor that both lyses AEC membranes and acts as an adhesin for laminin expressed at the basolateral surface of pneumocytes (Kinhikar et al. 2010). Another adhesin, Heparin-binding hemagglutinin (HBHA) produced by Mtb, specifically targets AEC and allows Mtb dissemination (Pethe et al. 2001). During replication in AEC, Mtb seems to increase in virulence. paraTB in ruminants begins in the small intestine and follows ingestion of Map-contaminated milk or fecal material. In the small intestine Map invades M cells that cover the dome of Peyer's patches (Momotani et al., 1988). Map also uses enterocytes to cross the epithelial barrier and access underlying MP. Interestingly, Map infection in the intestinal mucosa down regulates inflammatory genes such as IL-1 and TNF which may help the bacterium to establish a niche.

Alveolar macrophages (AMP) in the lung (Mb) or intestinal MPs in the submucosa (Map) are central to pathogenesis. MTC complex bacilli enter the MP via a variety of receptors including the complement receptors (CR1, CR3, and CR4), the immunoglobulin receptors (FcR), the scavenger receptors or the C-type lectin receptors (mannose receptor (MR), dectin 1, dectin 2, Mincle, DC-SIGN), some of which being also PRRs. Mb and Map have evolved numerous strategies to subvert MP defense. Uptake via complement receptors or the MR limits phagosome maturation and may represent a strategy to evade microbicidal activity. Phagolysosome acidification, that normally occurs

through action of the vacuolar H+ proton pump V-ATPase, is blocked by pathogenic mycobacteria that inhibit recruitment of the pump to the vacuole. The maturing phagosome acquires and loses molecules, and the maturation speed depends on the receptor used for entry. CR- or MR-mediated entry favors slow maturation to the advantage of the mycobacterium. Lipids and glycolipids tailor phagocytosis by MP: for example, LAM targets the MR to route mycobacteria to the early phagosome. Both LAM (Fratti et al. 2003) and PIM (Vergne et al. 2004) interfere with acquisition of the lysosome cargo to block phagosome maturation.

Exclusive localization of mycobacteria inside the phagosome has long been the dogma, established by seminal work from Armstrong and Hart (Armstrong and Hart 1971). However, this scenario was later debated where virulent strains of Mtb, but not avirulent Mtb or the BCG vaccine, were shown using electronic microscopy to escape into the cytoplasm of the MP (McDonough et al. 1993; van der Wel et al. 2007). The major Type VII secretion system ESX-1 is involved in this mechanism and evidence of this happening *in vivo* has been obtained in the mouse model (Simeone et al. 2015). Escape to the cytosol may explain why Mtb (Stanley et al. 2007) or Mb (Malone et al. 2018) induces a strong type 1 interferon (IFN) response, a feature long thought to be restricted to viral infections. Mtb DNA is detected by the cytosolic sensor cGAS and triggers the type 1 IFN response to the benefit of virulent mycobacteria (Collins et al. 2015; Wassermann et al. 2015; Watson et al. 2015). Map does not express the type VII secretion system ESX-1 and there is no report of type 1 IFN produced in response to this infection in calves or animal models.

MP death is induced by different pathways. Under physiological conditions, apoptosis and autophagy respectively dispose of malfunctioning cells and organelles to maintain cell homeostasis. Necrotic cell death occurs in infected tissues to

eliminate the assaulting pathogen. These different forms of MP cell-death are observed during Mtb infection and are either pro-host or pro-pathogen. Autophagy is one of the most efficient anti- Mtb cell death programs and the bacterium has numerous strategies to avoid apoptosis among which ESAT-6 role is prominent (Mohareer et al. 2018).

Neutrophils are the first cells recruited in response to a danger signal. They are Ying and Yang in mycobacterial infections. For humans as for cattle, some individuals in close contact with active TB transmitters remain free of every sign of infection, i.e., negative in the Tuberculin-Skin-Test (TST). This is strong indication that their innate immune system is efficient at eliminating mycobacteria. Neutrophils are probably involved in such defense: in humans there is a positive correlation between the number of neutrophils circulating in blood and resistance to Mtb infection (Martineau et al. 2007) and are actively involved in early protective inflammation and granuloma formation. However, neutrophils are also over-represented cells during active TB in humans (Eum et al. 2010) and in susceptible mouse models (Kroon et al. 2018) and their highly toxic arsenal is destructive for the lung. In laboratory models, neutrophils are recruited to the lung in several waves following infection with Mtb (Lombard et al. 2016). In experimentally infected cattle, they reach the granuloma together with T cells coincidentally with necrosis development (Palmer et al. 2019). In Map infected cattle there is upregulation of IL-8, a master cytokine involved in neutrophil recruitment. Although the role played by neutrophils in Map control or paraTB pathophysiology remains less defined, an *in vitro* study suggests that neutrophils are effective in killing Map but encounter with MPs later during the infection could dampen this effective control (Ladero-Auñon et al. 2021). To complicate the situation, it is now established that, like MPs

or dendritic cells (DCs), neutrophils are not a homogenous population. Some subsets, akin to granulocytic suppressive myeloid-derived suppressor cells, are recruited in numbers during active TB in humans and mouse models where, by suppressing T cells, they may help the pathogen to multiply (Magcwebeba et al. 2019). In cattle, suppressive neutrophils similar in phenotype and function to mouse suppressive neutrophils have recently been discovered (Rambault et al. 2021).

Dendritic cells (DCs) are professional antigen presenting cells (APCs) that bridge innate and adaptive host immune responses. Their key function is in antigen presentation and in directing the downstream immune response. The C-type lectin receptor DC-SIGN expressed by DCs recognizes the cell-wall LAM to downmodulate DC maturation and the adaptive immune response (Koul et al. 2004; Dulberger et al. 2020). Mucosal DCs have an anti-inflammatory phenotype that aids mycobacterial survival. DCs may also serve as shelters for persistence of mycobacteria as demonstrated in animal models (Jiao et al. 2002) and human cells (Tailleux et al. 2003).

aAdaptive immunity and Mycobacterial pathogenesis

The outcome of a mycobacterial infection results from a complex interplay between the immune response of the host and the ability of the mycobacteria to persist and subvert these responses. The adaptive immune response has two distinct arms: cell-mediated immunity involving T cells and humoral immunity producing antibodies. The first is of major importance to clear intracellular pathogens while the second is the most important to eliminate extracellular pathogens. Both arms are triggered in response to mycobacterial infections. However, the intracellular lifestyle of virulent mycobacteria points to T-cell-mediated responses as the most critical.

bAdaptive immunity and the granuloma

The core function of the granuloma that is formed in response to mycobacterial tissue infection is to contain the bacteria. It is the focus of the immune response, with lymphocytes, monocytes and neutrophils, recruited from blood to the tissue, to form this cellular lesion, (Krüger et al.; 2015Palmer et al. 2019). However, mycobacteria have strategies to survive intracellularly and persist within the granuloma, hence complete eradication of infection does not usually occur. This leads to the establishment of an "ongoing battleground" aimed at the control of the proliferation of the mycobacteria, which involves a balanced cellular immune and cytokine response to limit tissue damage as well as mycobacterial dissemination. Mycobacteria subvert these defences in a dormant state, waiting until they are able to proliferate and break through the local immune response to spread the infection both within the animal and to other individuals. The granuloma is a dynamic lesion. The site of granuloma formation varies depending on the tissue tropism of the mycobacterial species, but there are similarities in the cellular composition and dynamics regardless of location. In naturally and experimentally Mb infected cattle, $CD4^+$ and $CD8^+$ T cells and (WC1+) γδ T cells are present in early-stage lesions, with activated $CD8^+$ T cells on the margins, whereas $CD4^+$ and γδ T cells are located both on the periphery and in the MP-rich central part of the lesions. At the later stage, bTB lesions evolve and are characterised by a fibrotic capsule and a central necrotic core. Both $CD4^+$ and $CD8^+$ T cells are present (Liebana et al. 2007). A recent study highlighted the heterogeneity of granulomatous lesions both over time and within individual animals, with a somewhat self-governing nature of individual lesions (Palmer et al. 2021). This heterogeneity and apparent autonomy for each granuloma has also been described in human TB.

In paraTB the granuloma contains $CD4^+$ T cells as the predominant T cell population interspersed with MPs in the

granulomatous lesions in sub clinically infected goats. Smaller numbers of $CD8^+$ T cells and γδ T cells were also seen (Valheim et al. 2004). In sheep with paraTB, there are two distinct lesion types: multibacillary and paucibacillary. Paucibacillary lesions in the ileum have high numbers of $CD4^+$ and γδ T cells, in contrast to the more severe multibacillary lesions (Little et al. 1996).

Interleukin (IL)-8 or C-X-C motif chemokine ligand 8 (CXCL8) binds to its receptors CXCR1 and CXCR2 that are highly expressed on neutrophils as well as T cells. IL-8 is expressed by Mb infected MPs, suggesting that it may be involved in recruiting cells to the granuloma. A study of early granulomas in Mb identified high levels of CXCL9 and MCP-1/CCL2 (Palmer et al. 2019). CXCL9 binds to CXCR3, which is expressed on effector $CD4^+$ and $CD8^+$ T cells, natural killer (NK) and NK T cells. This chemokine would therefore lead to recruitment of $CD4^+$ T cells to the lesion. MCP-1 is one of the main chemokines involved in migration and infiltration of monocytes/macrophages. Similarly, CXCL10, also known as interferon-induced protein-10 (IP-10), mediates T cell recruitment to inflammatory sites. This chemokine is produced in cattle granulomas after aerosolised Mb infection (Palmer et al. 2015) and by peripheral blood cells and in ileocaecal valve tissue, the primary site of infection with Map (Alonso-Hearn et al. 2019).

bCell-mediated immune responses

Upon encountering antigen presented by an APC, naive $CD4^+$ T cells differentiate into various cell lineages, including the classical effector Th1 and Th2 subsets, and more recently identified Th17, Th9, T follicular helper and induced T regulatory cell subsets. Th1 cells support pro-inflammatory, protective immune responses against mycobacterial infections whereas Th2 cell activation supports B cell maturation and the production of anti-inflammatory cytokines. During the early stages of infection with both Mb and Map, a pro-inflammatory

Th1 immune response predominates but wanes as animals progress in disease severity or to the clinical stage. Th1 cells produce a range of cytokines, but the key to mycobacterial protective responses is the production of interferon-gamma (IFN-γ). IFN-γ is a vital mediator of MP activation for bacterial clearance by promoting phagolysosomal maturation (Arsenault et al. 2012). Patients genetically deficient in the IFN-γ signalling cascade are highly susceptible to mycobacterial infections (Bustamante et al. 2014), as are gene knock out mice (Flynn et al. 1993; Mogues et al. 2001). Th1 cytokines are also important in MP pro-inflammatory activation and to stimulate protective reactive oxygen and nitrogen species that aid mycobacterial killing. IFN-γ and TNF-α mRNA levels are elevated within the granulomas in the lungs of Mb infected animals and in the ileal lesions of Map infected animals (Palmer et al. 2015; Fernández et al. 2017). IL-17, a pro-inflammatory cytokine associated with Th17 cells, is also induced in granulomas of cattle with bTB (Shu et al. 2014).

bHumoral immunity to Mycobacteria

In response to infection with Mb or Map, elevated serum antibody levels are associated with the later stages of infection and severity of the disease. The main role of B cells is to secrete antibodies, but they can also act as APCs and present antigen to T cells. Th2 cells are important for the activation of B cell responses, however Th2-dominant immune responses and the cytokines they produce, such as IL-10, IL-4 and IL-13, are considered non-protective against mycobacterial infections and support anti-inflammatory MP polarisation (Biswas et al. 2012). More recent studies have provided supporting evidence for a greater role of B cells in immune control of mycobacterial infections. In bTB cattle, B cells are present within the granuloma and develop into IgM, IgG, and IgA expressing memory B cells (Lyashchenko et al. 2020). In human TB patients, *in vitro* killing capacity of peripheral blood

cells is correlated with the proportion of activated and atypical memory B cells and IgG1 responses (O'Shea et al. 2018). Elevated Map-specific faecal IgA is associated with resilient animals (Begg et al. 2015). Together, these findings support a positive role of humoral immunity in protective responses in bTB and paraTB.

bSubversion of adaptive immune responses

Mycobacteria subvert or modulate many adaptive immune pathways to counteract the host immune protection mechanisms. During antigen presentation by APCs, the co-stimulatory molecules present on the T cell or APC surface interact to modulate activation and T cell differentiation. They include CD28 that binds to CD80/CD86, CD40 that binds to CD40 ligand (or CD154) and ICOS that binds to ICOSL. Mycobacteria can downmodulate MP expression of CD80/CD86 as well as CD40 signalling such that there is a deficit of pro-inflammatory cytokines and iNOS. In turn, the killing capacity of the MP is decreased. Similarly, limitation of IL-12 expression is beneficial to survival of the mycobacterium since it negatively impacts the protective Th1 cell response. Lipoproteins secreted by Mb and Map, such as LprG, are among virulence factors that inhibit MHC class II expression on the surface of APCs to decrease antigen presentation and enhance *in vivo* survival of Mb and Map (Harding and Boom 2010).

Sa$Control

In countries with well-developed surveillance programs, bTB is a regulated disease in cattle. bTB case detection relies on the TST where a poorly defined mixture of antigens from Mb is injected in the skin of the animal to reveal delayed-type hypersensitivity cell-mediated immune responses developed in response to previous exposure to mycobacteria. However, in the field, animals are also exposed to environmental mycobacteria that share many antigens with virulent Mb. Thus, this *in vivo*

test can be complemented by an *in vitro* Interferon-Gamma Release Assay where peripheral blood cells are stimulated with molecularly defined antigens such as ESAT-6 and CFP10 that are present in virulent Mb strains (Smith et al. 2021). If the animal has been in contact with Mb, CD4 T-cells proliferate and release IFN-γ. However, the detection of immune responses systemically is only part of the picture since the true battlefield is at the site of infection. Unfortunately, control of bTB and paraTB is hampered by a perceived lack of diagnostic tests that can identify early cases to enable their removal before shedding of pathogens and transmission to other individuals.

WGS can be employed for epidemiological tracing, identifying additional links between herds and cases not revealed by other standard genotyping methods, and thus suggest other sources of infection. These benefits and the reducing cost of WGS have led to some countries integrating WGS into control or surveillance programs for bTB. The USA official bTB eradication program has employed WGS for tracing new cases since 2013, which has reduced both time and costs associated with epidemiological investigations (Orloski et al. 2018).

There is no commercial vaccine currently available for bTB. Experimental vaccination with BCG shows good levels of protection and decrease in the shedding of Mb in the environment. However, because of interference with diagnostic tests, BCG vaccination is not allowed in countries that have based their bTB surveillance programs on detection and slaughter of infected animals. While efficacious commercial vaccines exist for paraTB they tend not to be used in cattle in most countries because they cause cross reactions in immunological tests for bTB, thus adversely impacting trade in livestock. Perversely the regulations in place to limit spread of bovine TB, which are mandated by OIE and therefore impact

most countries, hamper the control of paratuberculosis (Whittington et al. 2019).

aGaps in knowledge and anticipated directions

First, it is important to emphasize that most of the knowledge on the mechanisms of virulence of pathogenic mycobacteria has been gained from studies in humans, cell lines or mouse models. For example, regarding the MP as the key cell for early mycobacterial uptake and evolution of the infection, the picture may be different in cattle alveolar MP, as compared to the mouse, encountering Mb or to human alveolar MP encountering Mtb. More knowledge needs to be gained from the natural host of each pathogen, experimentally or naturally infected, and from physiological models such as cattle Precision Cut Lung Slices for example (Remot et al. 2021).

There are knowledge gaps regarding the distribution and frequency of different pathogen genotypes and how these relate to transmission, virulence, pathogenicity, immunogenicity, and persistence. More WGS studies could help address these knowledge gaps and help inform the choice of strains for vaccine development. Additionally, more needs to be known about the effects of mixed genotype infections and superinfection in co-infection of hosts with different mycobacteria and how this influences disease outcomes, diagnosis and control.

Regarding human TB, it is estimated that one-third of the world population is latently infected with Mtb, representing a huge challenge to control and eradication of the disease. In animals infected with Mb or Map, this latent phase of the disease is also challenging in control programs. For bTB, control programs generally remove contaminated animals before they progress to clinical disease. However, these costly programs can only be run on a yearly basis at best and shedding from contaminated animals may occur before the animal is removed. For paraTB, animals in a latent or subclinical phase also have low level, intermittent, shedding of mycobacteria in

the faeces and thus remain a risk for disease spread in the herd. The ability to predict individuals that will progress and become highly infectious and/or succumb to the disease would be of great help to divert or prevent this disease progression. The diagnosis of subclinical or latent infection and the mechanism(s) of progression from latent to active disease are areas of intensive research in human and veterinary medicine (Plain et al. 2011; Carranza et al. 2020). Systems biology approaches, such as transcriptomic studies that are now being conducted for both Mb and Map to assess host responses to infection are a highly promising avenue. They should help to identify new biomarkers detectable in blood cells that will help to predict bacilli shedding in correlation with the status of the immune system. Some potential biomarkers such as chemokines CXCL10 and CXCL8 (IL-8) have already been identified (Alonso-Hearn et al. 2019; Fang et al. 2020). Others could also be associated with lipid metabolism and angiogenesis (Purdie et al. 2019).

Vaccination of cattle with BCG would certainly help to control bTB disease in cattle. This will require the development of strategies allowing to **d**ifferentiate **i**nfected from **va**ccinated animals, the so-called DIVA approach. This approach relies on molecularly defined diagnostic tools, based on proteins or peptides that are present in Mb strains but absent from BCG, to replace the poorly discriminating TST currently in use in control programs. Remarkable progress has been made along those lines and some countries such as the United Kingdom are considering using such tools in their national program (Srinivasan et al. 2020). There is a similar need for paraTB since, although vaccines are commercially available for sheep and cattle, vaccine uptake in cattle is limited by cross-reactivity impacting bTB surveillance programs and a lack of DIVA. Epidemiological tracing by WGS, clearly shows the important role of the wildlife reservoir for

interspecies transmission of Mb. A complementary approach is to vaccinate the wildlife reservoir with BCG to reduce transmission to cattle and some countries are undertaking this strategy (Gormley and Corner 2013). However uncertainties remain about routes of transmission from wildlife reservoirs to cattle and confound approaches to control bTB in regions where there is high prevalence (Allen et al. 2021). More research is needed on transmission dynamics between Mb, Map and wildlife and WGS studies could provide a way forward in this respect.

Mb and Map remain very complex and widespread organisms and to control their diseases we need to understand more about these pathogens, the physiopathology of the diseases they cause, and how best to achieve life-long immune protection in the host. Avenues to better control of these diseases are numerous and not mutually exclusive, since Mb and Map must be tackled from several directions and can infect the same animal species. This will require time and investment in research and development as well as coordination between the different international and national policy agencies and livestock industry leaders who have the final decision on the deployment of such measures in the field.

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Table 1 Pathogenic *Mycobacterium* infections of animals

***Mycobacterium* species**	**Natural host***	**Infection/disease characteristics**
M.avium avium/M.avium silvaticum	Birds Ruminants Cats	Avian tuberculosis Enteric disease similar to paratuberculosis Panniculitis, skin lesions/ulcers, disseminated infection
M.avium hominissuis	Pigs Humans	Granulomatous lesions in lymph nodes (neck) and gastrointestinal tract. Pulmonary infections, submandibular adenopathies, disseminated infections in patients suffering from acquired immunodeficiency syndrome
M.avium paratuberculosis	Ruminants, Camelids, Lagomorphs Humans?	Johne's disease/paratuberculosis; chronic granulomatous enteritis Crohn's disease in humans?
M.tuberculosis/M.africanum/M.canettii	Humans	Tuberculosis, primarily respiratory infection but can disseminate to other organs.
M.bovis	Ruminants, wild boar, Mustelids, possums, cats, dogs, humans	Bovine tuberculosis: chronic granulomatous caseous-necrotising inflammation affecting primarily lymph nodes and lungs but can disseminate
M.caprae	Ruminants, pigs, humans	As for bovine tuberculosis
M.microti	Rodents, cats	Tuberculous skin lesions
M.pinnipedii	Fin-footed mammals	Tuberculous lesions in lymph nodes, lungs, mediastinum, pleura, liver and spleen
M.orygis	Antelope, humans	As for bovine tuberculosis
"Dassie bacillus"	Rock hyrax	Granulomatous lesions in the lungs, liver and spleen
M.mungi	Mongoose	Tuberculous lesions in nose, nasal cavity, skin and lungs
"Chimpanzee bacillus"	Chimpanzees	Granulomas in liver and spleen
M.suricattae	Meerkats	Granulomatous lesions in spleen, liver, head lymph nodes and lungs
M.leprae/M.lepromatosis	Humans Eurasian red squirrels, armadillos (*M.leprae*)	Hansen's disease in humans characterized by skin lesions and infection of peripheral nerves. Leprosy-like skin lesions in Eurasian red squirrels; skin ulcers and infection of peripheral nerves in armadillos
M.lepraemurium	Rodents, cats	Leprosy-like skin lesions

Non-tuberculous mycobacteria (NTM)		
M.abscessus, M.celatum, M.chelonae, M.fortuitum, M.gordonae, M.intracellulare, M.kansasii, M.marinum, M.nonchromogenicum, M.phlei, M.scrofulaceum, M.szulgai, M.terrae, M.triviale	Environment, various animals	NTM are prevalent in the environment but cause opportunistic tuberculosis-like infections of many animals, including humans

*Full host range unknown for many *Mycobacterium* species

Figure 1

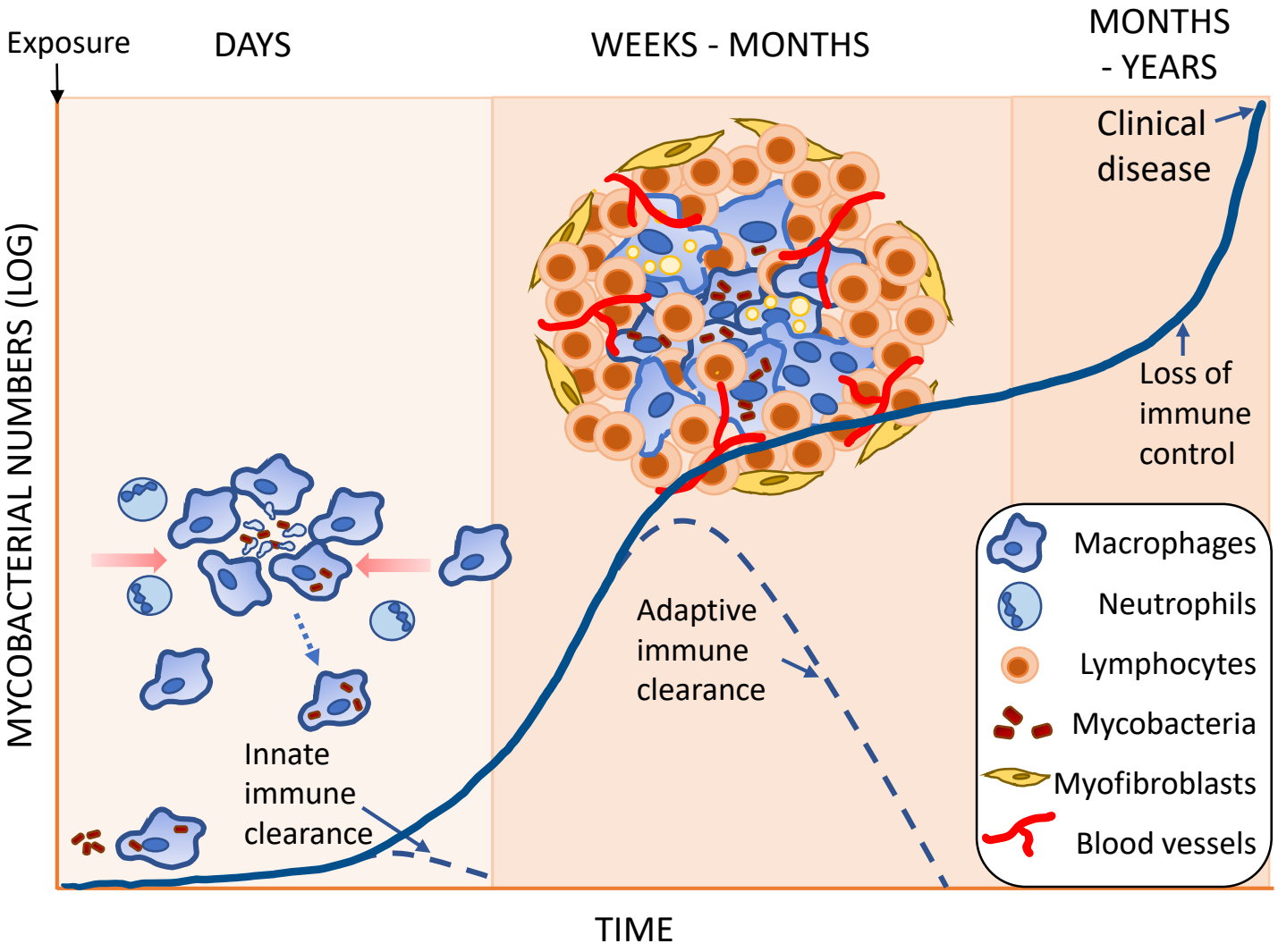
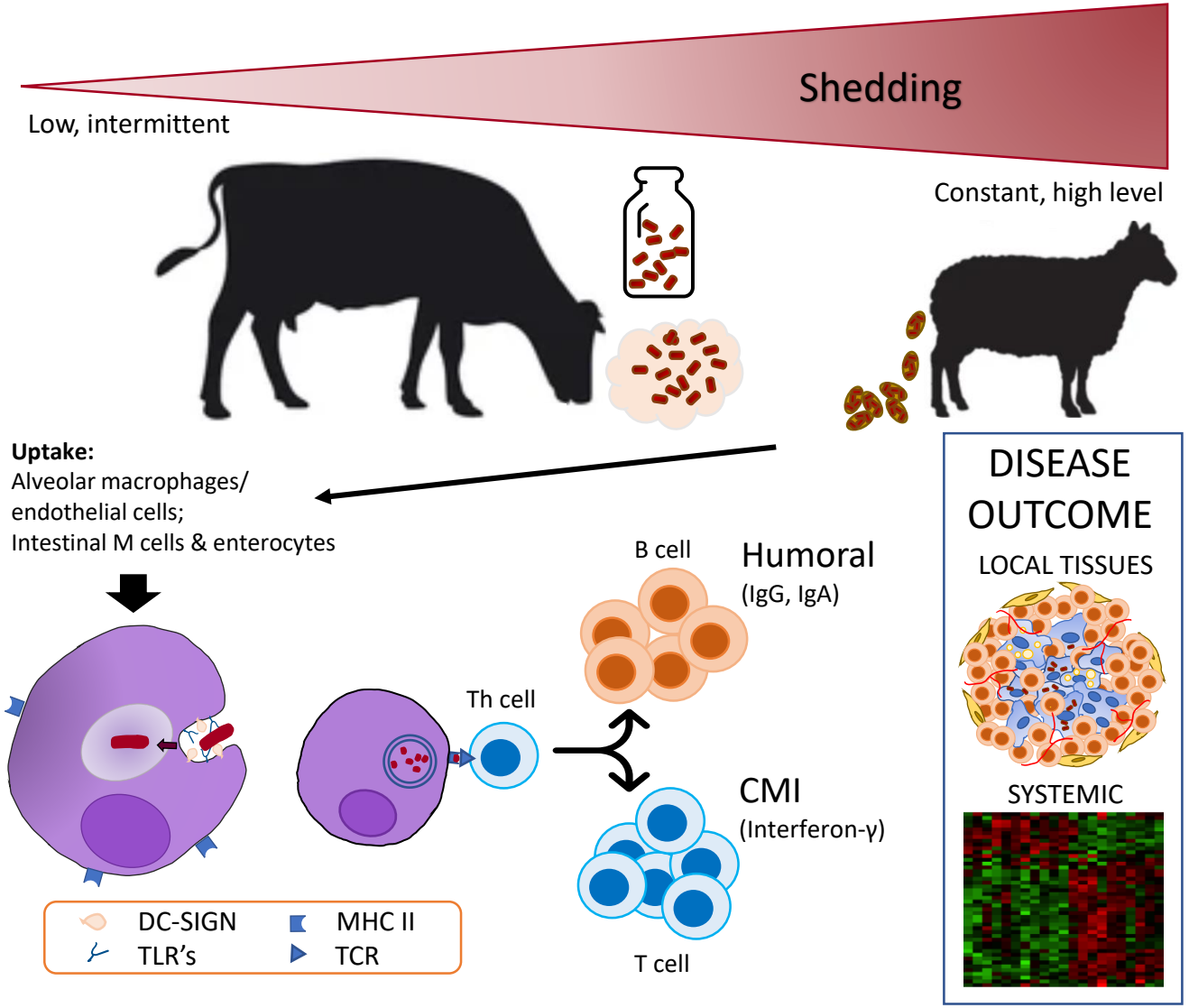

Exposure
DAYS
WEEKS - MONTHS
MONTHS - YEARS
Clinical disease
Loss of immune control
MYCOBACTERIAL NUMBERS (LOG)
Innate immune clearance
Adaptive immune clearance
Macrophages
Neutrophils
Lymphocytes
Mycobacteria
Myofibroblasts
Blood vessels
TIME

Figure 2


Shedding
Low, intermittent
Constant, high level
Uptake:
Alveolar macrophages/
endothelial cells;
Intestinal M cells & enterocytes
B cell
Humoral
(IgG, IgA)
Th cell
CMI
(Interferon-γ)
T cell
DC-SIGN
TLR's
MHC II
TCR
DISEASE
OUTCOME
LOCAL TISSUES
SYSTEMIC

Legends to figures

Figure 1: The mycobacterial granuloma. Immediately after entry into the body at mucosal sites -mainly the lung for Mb and the intestine for Map- bacilli are engulfed by resident macrophages. In the early hours and days following this first event, innate cells are recruited to the infection site to destroy bacilli. If not successful, the adaptive immune response is initiated and T lymphocytes that have been primed in the lymph node draining the site of infection are recruited to form a highly organized cellular structure: the mature granuloma. This circumvents infection and either clears bacilli or establishes a lesion where mycobacteria enter into a dormant state. This latency phase can last for months or years until an immunosuppressive event takes place, which allows bacilli to actively replicate again. Destruction of the granuloma leads to bacilli expelling in the airways or shedding in feces and contact animals can be infected.

Figure 2: Overview of the pathogenesis of virulent mycobacteria (Mb and Map). Infection with virulent mycobacteria leads to a chronic disease state with progression in the shedding of mycobacteria from low, intermittent shedding in the early and subclinical phases to continuous, high levels of shedding associated with clinical disease. The key routes of exposure are via direct aerosol transmission and shedding into the milk for Mb and via faecal shedding and oral transmission for Map. Other exposure routes have also been reported, including environmental and wildlife reservoirs. Initial uptake is via alveolar macrophages and alveolar epithelial cells for Mb, or via M cells in the intestinal Peyer's patches for Map. Binding of glycolipids and glycopeptidolipids from the mycobacterial cell wall to pattern recognition receptors including DC-SIGN and TLRs on macrophages enables non-opsonic cell entry, persistence in the phagosome and inhibition of the maturation of the phagolysosome for the benefit of the mycobacterium. However, infected animals generally mount both a humoral and a cell-mediated immune (CMI) response of varying efficacy, where interferon-gamma (IFN-γ) is key to protection. Granulomas are central to mycobacterial control. However, granulomas within the same individual are heterogeneous and autonomous. Peripheral blood cell transcriptomics are also offering novel insights into the complexity of the systemic immune response to mycobacterial infection.