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Evasion of innate immunity by *Mycobacterium tuberculosis*: is death an exit strategy?

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Abstract

Virulent *Mycobacterium tuberculosis* inhibits apoptosis and triggers necrosis of host macrophages to evade innate immunity and delay the initiation of adaptive immunity. By contrast, attenuated *M. tuberculosis* induces macrophage apoptosis, an innate defence mechanism that reduces bacterial viability. In this Opinion article, we describe how virulent *M. tuberculosis* blocks production of the eicosanoid lipid mediator prostaglandin E_2 (PGE_2). PGE_2 production by infected macrophages prevents mitochondrial damage and initiates plasma membrane repair, two processes that are crucial for preventing necrosis and inducing apoptosis. Thus, *M. tuberculosis*-mediated modulation of eicosanoid production determines the death modality of the infected macrophage, which in turn has a substantial impact on the outcome of infection.

As one of the most important bacterial pathogens, Mycobacterium tuberculosis infects and persists in normal healthy individuals[1]. The chronic nature of this infection implies that *M. tuberculosis* has developed strategies to avoid both the innate and adaptive immune responses[2,3]. Manipulation of macro phage death pathways is one of the complex mechanisms used by *M. tuberculosis* to evade these host defences.

Subversion of the death modality of macro phages, the principal host cells infected by *M. tuberculosis*, is a common feature of several other intracellular pathogens. Various bacteria and intracellular parasites induce apoptosis or necrosis following infection. Legionella pneumophila, Listeria monocytogenes, Shigella flexneri, Yersinia pestis and Salmonella enterica subsp. enterica serovar Typhimurium all induce pyroptosis, which is a form of necrosis that requires caspase 1 activation[4,5]. *S. enterica* induces pyroptosis through the recruitment of ice protease-activating factor (IPAF; also known as NLRC4), an apoptotic protease-activating factor 1 (APAF1)-related NOD-like receptor protein[4,6–9]. In general,

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Competing interests statement
The authors declare no competing financial interests.

DATABASES
Entrez Genome Project: http://www.ncbi.nlm.nih.gov/genomeprj
Coxiella burnetii | Danio rerio | Legionella pneumophila | Leishmania major | Listeria monocytogenes | Mycobacterium bovis bacille calmette–Guérin | Mycobacterium marinum | Mycobacterium tuberculosis | Salmonella enterica subsp. enterica serovar Typhimurium | Shigella flexneri | Yersinia pestis
UniProtKB: http://www.uniprot.org
caspase 1 | caspase 3 | caspase 9 | COX1 | COX2 | CPLA2γ | EP1 | EP2 | EP3 | EP4 | IL-1β | IL-18 | IPAF | Lta4h | Ncs1 | SYT7

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caspase 1-knockout mice are more susceptible to infection with these bacteria, suggesting that pyroptosis leads to pathogen clearance. However, as caspase 1 is crucial for the processing of mature interleukin-1β (IL-1β) and IL-18, two cytokines that are important for inflammation and host defence, the various consequences of caspase 1 activation are difficult to dissociate. Nonetheless, the detailed study of these different pathogens is instructive. For example, virulent *S.* Typhimurium induces apoptosis in gastrointestinal epithelial cells, which could prevent inflammation during bacterial penetration of the gut (Table I). Subsequently, infection of macrophages by *S.* Typhimurium induces pyroptosis, which leads to the release of pro-inflammatory mediators, including IL-1β, and activates host immunity. Crucially, the timing of macro phage pyroptosis seems to be modulated by bacterial virulence factors. By delaying macrophage pyroptosis, *S.* Typhimurium can parasitize the macrophage, replicate in a protected niche and use the cell to facilitate systemic dissemination[10]. Although stimulation of the host inflammatory response by pyroptosis is ultimately detrimental to the pathogen, these examples show that the pathogen's ability to manipulate the timing of host cell death is sufficient to create conditions that allow it to establish a systemic infection[8].

Pyroptosis might have a crucial role in macrophage defence against *M. tuberculosis* infection under certain circumstances, as it has been shown that *M. tuberculosis* prevents inflammasome activation and IL-1β processing, which normally lead to improved mycobacterial clearance and a lower bacterial burden in the lungs of aerosol-infected mice[11]. However, it should be noted that macrophages infected with *M. tuberculosis* produce IL-1β in a manner that is dependent on bacterial virulence[12,13]. Furthermore, a recent study shows that although a major role for IL-1β in host resistance to *M. tuberculosis* clearly exists, the production of IL-1β in mice infected with *M. tuberculosis* can occur by mechanisms other than caspase 1 and inflammasome activation[14].

By contrast, the obligate intracellular parasite Leishmania major, which is transmitted by an infected sand fly bite, is phagocytosed by neutrophils recruited to the skin. However, neutrophils are ineffectual at killing *L. major* and eventually undergo apoptosis. Myeloid cells, which are also recruited to the site of infection, phagocytose the infected apoptotic neutrophils[15] and, analogous to the Greeks hiding in the Trojan Horse, the parasite gains entry into the macrophage unannounced[16,17]. This enables the parasite to undergo intracellular replication without triggering an innate response. Thus, in this example, the pathogen takes advantage of apoptotic death to avoid inflammation and detection by the host's immune system.

Finally, like *M. tuberculosis*, several pathogens inhibit macrophage apoptosis, including *L. pneumophila*, Coxiella burnetii, *Brucella* spp., *Neisseria* spp. and *Streptococcus* spp. Although the microbial effectors and host targets have been defined in some cases, the assumption that the inhibition of apoptosis is a bacterial evasion mechanism has not always been clearly demonstrated. An exception to this is a study showing that the fates of macrophages and dendritic cells (DCs) differ following *L. pneumophila* infection[18]. Although *L. pneumophila* inhibits macrophage apoptosis, infected DCs undergo rapid apoptosis, which is less permissive for bacterial replication. Thus, similarly to *M. tuberculosis*, virulent *L. pneumophila* inhibits macrophage apoptosis and, instead, leads to necrosis.

Other investigations have shown that the type of macrophage death following infection with *M. tuberculosis* determines whether a successful antimycobacterial defence mechanism is activated. Infection with attenuated *M. tuberculosis* induces apoptosis[19–24]. Virulent *M. tuberculosis* induces necrosis of both human and mouse macrophages, a property that is not shared with non-pathogenic mycobacterial species[24–26]; this allows the bacteria to evade

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host defence mechanisms by inducing cellular lysis and spreading of the infection[24] (FIG. 1). The concept that virulent *M. tuberculosis* actively inhibits the induction of macrophage apoptosis[27] is supported by the identification of mutants that induce apoptosis instead of necrosis[28,29].

Thus, the death modality of infected macrophages should be considered an innate defence mechanism. Dissection of the cellular pathways that are altered by virulent *M. tuberculosis* has exposed a key role for host eicosanoid biosynthesis pathways in regulating the death modality of infected macrophages[24–26]. Here, we discuss the selective production of specific eicosanoids that have important functional consequences for the innate control of intracellular *M. tuberculosis* infection.

Host lipids modulate macrophage death

Apoptosis and the concomitant antimycobacterial activity of human macrophages can be triggered by *M. tuberculosis* through the activity of cytosolic phospholipase $A_2\gamma$ (CPLA2γ; also known as PLA2G4C), a group IV $CPLA_2$ that catalyses the release of arachidonic acid from the *sn-2* position of membrane phospholipids[20]. Arachidonic acid and its diverse products regulate death in several cell types[30]. This is thought to be due to the fact that arachidonic acid products are second messengers in tumour necrosis factor-induced apoptosis[31] and the fact that oxygen radicals, which are produced during lipoxygenation of arachidonic acid, induce the production of reactive oxygen species and are involved in the induction of cell death[32]. Arachidonic acid also activates sphingo myelinase, leading to ceramide production and apoptosis[33]. However, it is not clear which of these mechanisms are important *in vivo*[34].

One area of research in this field focuses on the roles of the eicosanoids prostaglandin E_2 (PGE_2) and lipoxin A_4 (LXA_4) in the regulation of programmed macrophage death[24–26]. The cyclooxygenases COX1 (also known as PTGS1) and COX2 (also known as PTGS2) convert arachidonic acid into the central intermediate PGH_2 [35], which is converted by specific synthases into a diverse range of prostanoids[36]. Interaction of these prostanoid species (which include the prostaglandins PGD_2, PGE_2, $PGF_2\alpha$, PGI_2 and thromboxane) with an array of specific prostanoid receptors plays a part in several cellular pathways. In the case of PGE_2, interactions with four PGE_2 receptors, EP1, EP2, EP3 and EP4 (also known as PTGER1, PTGER2, PTGER3 and PTGER4, respectively) trigger intracellular pathways that either promote or inhibit inflammation[37]. Importantly, the functional specificity of PGE_2 is largely determined by its interaction with these specific receptors[37]. EP1 mediates the elevation of intracellular Ca^{2+}. By contrast, EP2, which is involved in joint inflammation and neutrophil recruitment, and EP4, which induces cell migration in tumour invasion, are both involved in the upregulation of intracellular cyclic AMP levels. EP2 activates protein kinase A (PKA), and EP4 activates adenylyl cyclase and phospho inositide 3-kinase. Triggering EP3 downregulates cAMP concentrations and is known to mediate fever and angiogenesis.

Lipid bodies form at distinct cytoplasmic sites following infection of mouse macrophages with the attenuated strain Mycobacterium bovis bacille Calmette-Guérin (BCG); these lipid bodies are the sites of COX2 activity and PGE_2 generation[38]. Indeed, PGE_2 production has been a consistent finding following *M. bovis* BCG infection of mouse macrophages[39]. Macrophages infected with other attenuated *M. tuberculosis* strains also activate PGE_2 production, which prevents necrosis and leads to apoptosis instead[26] (FIG. 2a). By contrast, virulent *M. tuberculosis* strains, such as *M. tuberculosis* str. H37Rv or *M. tuberculosis* str. erdman, are much weaker inducers of PGE_2 production by macrophages[26]. This raises the possibility that virulent *M. tuberculosis* actively inhibits PGE_2 production. Thus, an

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important strategy that *M. tuberculosis* exploits to induce or avoid apoptotic cell death is the subversion of host eicosanoid biosynthesis pathways[25,26].

Lipoxins are also generated from arachidonic acid but require the action of different enzymes, including 5-lipoxygenases and 15-lipoxygenases[40]. Lipoxins are anti-inflammatory molecules and modulate chemokine and cytokine expression, monocyte trafficking and efferocytosis[41]. In contrast to the attenuated *M. tuberculosis* str. H37Ra, which is a weak LXA_4 inducer, virulent *M. tuberculosis* strongly induces the synthesis of LXA_4, which inhibits COX2 production and thus effectively shuts down PGE_2 biosynthesis[25,26] (FIG. 2b). In a PGE_2-poor microenvironment, the macrophage cannot prevent mitochondrial damage or enable the repair of plasma membrane disruptions effectively[24–26], and both of these processes are required to prevent necrosis and induce apoptosis[25,26]. Virulent *M. tuberculosis* in pre-necrotic macrophages continues to replicate and, after the cells are lysed, propagates the infection by spreading to uninfected macrophages. Thus, the balance of PGE_2 and LXA_4 production by the infected macrophage regulates the relative amount of apoptosis and necrosis following *M. tuberculosis* infection and has important functional consequences for innate control of intracellular *M. tuberculosis* infection.

The idea that lipoxin production by *M. tuberculosis*-infected macrophages is associated with increased bacterial replication and greater virulence was strengthened by the recent genetic analysis of zebrafish (Danio rerio) susceptibility to Mycobacterium marinum infection[42]. Multiple mutant classes of *D. rerio* with different innate susceptibilities to *M. marinum* were identified[42], and a hypersusceptible zebrafish mutant was found with a mutation that mapped to the *lta4h* locus, which encodes leukotriene A_4 hydrolase (Lta4h), an enzyme that is required for the final step of leukotriene B_4 (LTB_4) synthesis. Although Lta4h deficiency results in the loss of LTB_4 production, the addition of LTB_4 did not complement the genetic defect nor increase host resistance. In the absence of Lta4h, its substrate (LTA_4) accumulates and can lead to redirected eicosanoid synthesis and increased lipoxin synthesis. Therefore, it has been suggested that the increased susceptibility of the zebrafish *lta4h* mutant is due to an increase in lipoxin production. The same study presents human genetic data showing that polymorphisms in the *LTA4H* gene are associated with susceptibility to pulmonary and meningeal tuberculosis[42]. Thus, from fish to humans, the eicosanoids seem to have an unexpected role in susceptibility to tuberculosis.

Mitochondrial damage and death

The induction of LXA_4 by virulent *M. tuberculosis* inhibits PGE_2 production and triggers the mitochondrial permeability transition (MPT), leading to irreversible mitochondrial damage[26] (FIG. 3c). By triggering LXA_4 production in the host macrophage, virulent *M. tuberculosis* inhibits prostanoid production by blocking *COX2* mRNA accumulation. By contrast, attenuated *M. tuberculosis* induces the synthesis of only minimal amounts of LXA_4 and, instead, causes the production of substantial amounts of PGE_2. When macrophages are infected with attenuated *M. tuberculosis*, PGE_2 actively suppresses perturbations of the mitochondrial inner membrane[26] (FIG. 3b).

The MPT shuts down oxidative phosphorylation, leading to a loss of mitochondrial ATP production and the accumulation of reactive oxygen species, which in turn results in necrosis of the infected macrophage[43,44] (FIG. 3c). The MPT is accompanied by permeabilization of the mitochondrial inner membrane and dissipation of the mitochondrial membrane potential ($\Delta\Psi_m$)[44,45]. This event is usually irreversible and is frequently associated with mitochondrial outer-membrane permeabilization (MOMP). After macrophage infection with attenuated *M. tuberculosis*, a reversible MOMP is triggered,

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leaving the mitochondrial inner membrane intact[24,26]. This leads to the transient release of cytochrome *c*, which results in only moderate activation of caspase 3 and caspase 9, leading to the induction of apoptosis (FIG. 3b). If macrophages infected with virulent *M. tuberculosis* are treated with cyclosporin A, an inhibitor of the MPT, cytochrome *c* release is substantially reduced[46]. As cytochrome *c* release is a consequence of MOMP, this indicates that during infection with virulent *M. tuberculosis* excessive MOMP is driven by the MPT.

Cumulatively, these studies suggest that the infection of macrophages with virulent *M. tuberculosis* causes mitochondrial inner membrane disruption and irreversible MOMP, leading to necrosis. By contrast, the transient MOMP caused by avirulent *M. tuberculosis* results in apoptosis.

Blocking plasma membrane repair

The interaction of a mycobacterium with a host macrophage results in plasma membrane microdisruptions. Microdisruptions induced by attenuated *M. tuberculosis* are rapidly resealed by plasma membrane repair mechanisms that include recruitment of lysosomal and Golgi-derived vesicles to the lesions on the macrophage surface[25,47,48]. Recruitment of these vesicles to the plasma membrane can be assessed by measuring lysosome-associated membrane glycoprotein 1 (LAMP1) or α-mannosidase II translocation to the macrophage surface[49,50]. Active membrane repair prevents necrosis and is required for the induction of apoptosis. By contrast, if resealing of the plasma membrane microdisruptions is inhibited, as is the case with virulent *M. tuberculosis* infection, necrosis ensues.

Ca^{2+} sensors are of crucial importance for the recruitment of both lysosomes and Golgi vesicles to the membrane lesions (FIG. 3b). Gene silencing of the lysosomal Ca^{2+} sensor synaptotagmin 7 (SYT7) impairs the recruitment of lysosomes, but not Golgi-derived vesicles, to the cell surface[25,51]. The recruitment of Golgi-derived vesicles, which occurs independently of lysosome recruitment, requires the expression of neuronal calcium sensor 1 (NCS1), a protein that is particularly abundant in the Golgi[25,52]. Silencing *NCS1* expression or adding brefeldin A, a Golgi-specific transport inhibitor, inhibits translocation of Golgi vesicles. These data show that both lysosomal and Golgi-derived membranes are involved in plasma membrane repair and that they are recruited independently to plasma membrane lesions in infected macrophages (FIG. 3b).

Plasma membrane resealing is cAMP dependent[53], and the addition of forskolin, an activator of adenylyl cyclase, results in greater translocation of lysosomal membranes to the cell surface[25]. The protective effect of PGE_2 on mitochondrial stability is mediated through the PGE_2 receptor EP2[26], and PGE_2 binding to either EP2 or EP4 causes increased cAMP accumulation[54]. Consistent with this, PGE_2 treatment of human macrophages infected with virulent *M. tuberculosis* str. H37Rv reconstitutes plasma membrane repair mediated by lysosomal membranes. By contrast, PGE_2 does not affect Golgi-mediated repair[25]. Although the protective effects of PGE_2 on mitochondria require EP2, PGE_2-dependent lysosomal membrane translocation also requires phosphoinositide 3-kinase activation, which indicates that signalling through EP4 is involved[25].

These findings have important functional consequences for the control of intracellular mycobacterial replication. It was found that arachidonate 5-lipoxygenase-knockout (*Alox5*$^{-/-}$) mice, which are unable to produce LXA_4 and other ALOX5-dependent products, survive longer than wild-type mice after low-dose aerosol infection with virulent *M. tuberculosis*[55]. Conversely, PGE synthase-knockout (*Ptges*$^{-/-}$) mice, which are unable to produce PGE_2, succumb earlier than wild-type mice (S.M.B., M.D. and H.R., unpublished observations). However, as many cell types produce eicosanoids, these results do not provide information about the role of eicosanoids during innate immunity. In experiments

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using macrophages from *Ptges*$^{-/-}$ and *Alox5*$^{-/-}$ mice, it was found that *Ptges*$^{-/-}$ macro phages were unable to control intracellular *M. tuberculosis* infection, whereas *Alox5*$^{-/-}$ macrophages limited *M. tuberculosis* replication better than wild-type macrophages[25]. This phenotype was replicated *in vivo* when *Ptges*$^{-/-}$, *Alox5*$^{-/-}$ and wild-type macrophages infected with *M. tuberculosis* were adoptively transferred into the lungs of V(D)J recombination-activating protein 1-deficient (*Rag1*$^{-/-}$) mice. Mice that received infected *Alox5*$^{-/-}$ macrophages had a substantially lower mycobacterial lung burden than recipients that received infected *Ptges*$^{-/-}$ or wild-type macrophages. As *Rag1*$^{-/-}$ mice lack B cells and T cells, the greater capacity of *Rag1*$^{-/-}$ mice to control pulmonary infection following the transfer of *M. tuberculosis*-infected *Alox5*$^{-/-}$ macrophages must be attributed to either an intrinsic property of *Alox5*$^{-/-}$ macrophages or a unique interaction between *Alox5*$^{-/-}$ macrophages and the innate immune system[25].

One conceivable explanation for the role of PGE_2 in fostering membrane repair is that PGE_2 is required for the generation of SYT7, the lysosomal Ca^{2+} sensor essential for plasma membrane repair. As discussed, virulent *M. tuberculosis* stimulates LXA_4 production in macrophages, which inhibits PGE_2 production by downregulating *COX2* mRNA accumulation[26]. Indeed, it was found that, unlike transcription of *Lamp1*, transcription of *SYT7* mRNA is specifically induced by PGE_2 [25]. Likewise, *Alox5*$^{-/-}$ macrophages infected with virulent *M. tuberculosis* express more SYT7 than wild-type or *Ptges*$^{-/-}$ macrophages[25]. Collectively, these data indicate that PGE_2 is an essential modulator of SYT7 expression — although it is not known how this modulation occurs — and is therefore of crucial importance for the prevention of necrosis and induction of apoptosis. Cumulatively, these studies show that the balance of PGE_2 and LXA_4 production by infected macrophages affects the outcome of infection in the microenvironment of the lung.

A new model for pathogenesis

These findings establish causal relationships between the ability of the infected macrophage to restrict mycobacterial growth and both the protection of mitochondria and the resealing of plasma membrane lesions. The protection that is promoted by macrophage apoptosis seems to be based on sequestration of the pathogens in apoptotic bodies. Subsequent phagocytosis of apoptotic infected macrophages by other uninfected phagocytes could contribute to innate control of infection; however, the role of efferocytosis in bacterial killing is not well understood. An additional property of apoptotic macrophages is that the bacterial antigens that they contain are efficiently cross-presented by DCs, thus promoting a protective T cell response[56] (FIG. 1a). Conversely, by inducing LXA_4 production, which blocks PGE_2 synthesis, virulent *M. tuberculosis* causes irreversible mitochondrial damage and inhibits plasma membrane repair[25]. Both of these factors contribute to necrosis, which releases *M. tuberculosis* from the lysed macrophages into the surrounding tissue, fosters *de novo* infection of uninfected bystander macrophages and spreads the infection (FIG. 3c). Furthermore, pre-necrotic infected macrophages and necrotic ghosts do not engender DC cross-priming of antigen-specific T cells, which leads to a substantially delayed T cell response and impairment of antimycobacterial defence mechanisms.

Recent work has identified *M. tuberculosis* genes that inhibit apoptosis, which supports our hypothesis that apoptosis is an innate defence mechanism that is actively suppressed by virulent *M. tuberculosis*[28]. However, the induction of necrosis must be a regulated process, as it would not be advantageous for the bacterium to destroy its niche before it has the chance to replicate. Therefore, we propose a new model of host–pathogen interaction for *M. tuberculosis* infection. We postulate that virulent *M. tuberculosis* postpones programmed cell death during the initial phase of intracellular replication to maintain an advantageous environment for growth. We further suggest that when intracellular conditions are no longer

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conducive to bacterial replication, virulent *M. tuberculosis* uses an active mechanism to induce necrosis and exit the phagosome and the macrophage (FIG. 3c). Under these conditions it is crucially important for *M. tuberculosis* to spread and infect other cells. Virulent *M. tuberculosis* induces the production of LXA_4 by infected macrophages, which inhibits COX2 and prostanoid production, leading to a decline in the levels of PGE_2 and a decrease in the expression of PGE_2-regulated genes (for example, *Syt7*), thus impairing membrane repair by lysosomes. It has been demonstrated that this process leads to plasma membrane damage, but we propose that the same process also damages the phagosomal membrane and facilitates bacterial translocation from the phagosome into the cytosol. Bacterial translocation from the phagosome into the cytosol is a late event occurring 4 days after infection in human cells[57] and even later in mouse macrophages (S.M.B., P. Peters, M. Skold and N. van der wel, unpublished observations). Virulent *M. tuberculosis* has been shown to escape from the phagosome into the cytosol, and we suggest that this is only the initial step in bacterial exit from the macrophage[57].

Although *M. tuberculosis* has been reported to replicate in the cytosol during the late phase of infection[57], the stage has already been set for the induction of cellular necrosis at this point. In the absence of PGE_2, mitochondrial damage occurs and inhibits the recruitment of lysosomes to the plasma membrane, leading ultimately to necrosis. As recruitment of Golgi-derived vesicles to the cell surface is associated with phosphatidylserine flopping and annexin 1 deposition, two events that are necessary to form the apoptotic envelope, blockade of the Golgi-mediated repair pathway by virulent *M. tuberculosis* impairs the formation of the apoptotic envelope and leads to necrosis. Following necrosis, extracellular *M. tuberculosis* can infect other cells to initiate another cycle of replication and necrosis (a ‘boom and bust’ cycle). Although bacterial translocation into the cytosol has been proposed as an immune evasion strategy that prevents entry of *M. tuberculosis* antigens into the major histocompatibility complex (MHC) class II processing pathway[57,58], *M. tuberculosis* antigens that were translocated into the cytosol would then be more prone to presentation by MHC class I molecules. We think that, instead of modulating antigen presentation, the translocation of *M. tuberculosis* into the cytosol induces macrophage necrosis, functions as an exit mechanism for the bacterium and ultimately allows *M. tuberculosis* to infect other cells.

Our model integrates many experimental findings, but it also raises additional questions. Although we have concentrated on the early stages of innate immunity, ultimately *M. tuberculosis* induces granuloma formation, which is the pathological hallmark of tuberculosis. During *M. marinum* infection in zebrafish, early granuloma formation was shown to be beneficial to bacterial replication[42,59]. It remains to be determined whether eicosanoids affect this process, but their pleiotropic effects, including functioning as chemokines, make it likely that the answer will be yes. By contrast, the formation of pulmonary granulomas in *M. tuberculosis*-infected mice is closely associated with the initiation of T cell immunity[60], and mammalian granulomas are widely thought to be associated with containment of the infection, even if sterilization does not occur. The role of eicosanoids during these later stages of infection requires elucidation. A better understanding of their role is important, as it is known that prostaglandins can have both pro-inflammatory and anti-inflammatory effects, and some investigators have found that prostaglandins can impair antimicrobial immunity[61,62]. In this context, it will be particularly interesting to determine how eicosanoids affect acquired immunity and whether their effect on innate immunity influences the development of T cell immunity.

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Glossary

Apoptosis	A type of programmed cell death defined by chromatin condensation (pyknosis) and fragmentation, blebbing of the plasma membrane and formation of apoptotic bodies. The plasma membrane of an apoptotic cell remains intact and contains proteins that are cross-linked by transglutaminases such as annexin 1.
Efferocytosis	The uptake of apoptotic cells or apoptotic bodies by phagocytic cells.
Eicosanoid	A lipid mediator that is derived from arachidonic acid. Eicosanoids include prostaglandins, lipoxins, leukotrienes, prostacyclins, thromboxanes and hydroxyeicosatetraenoic acid compounds.
Mitochondrial membrane potential	The electrochemical gradient across the mitochondrial membranes, given the symbol $\Delta\Psi_m$. Complexes I, III and IV of the electron transport system in the inner mitochondrial membrane pump protons against their concentration gradient from the mitochondrial matrix into the inter-membrane space, making the matrix more negative.
Mitochondrial permeability transition	An increase in the permeability of the mitochondrial membranes to molecules of less than 1,500 daltons.
Necrosis	A form of cell death that is characterized by swelling of cytoplasmic organelles, including the mitochondria, and a loss of plasma membrane integrity.
Plasma membrane microdisruption	A pore formed by damage of the plasma membrane, as determined by measuring the diffusion of fluorescent dextran, an inert impermeant molecule.
Prostanoid	A lipid metabolite of arachidonic acid that is a product of the cyclooxygenase cascade and of specific prostanoid synthases.
***sn-2* position**	The second (that is, middle) carbon atom in the glycerol backbone of phospholipids, providing a link for fatty acids.

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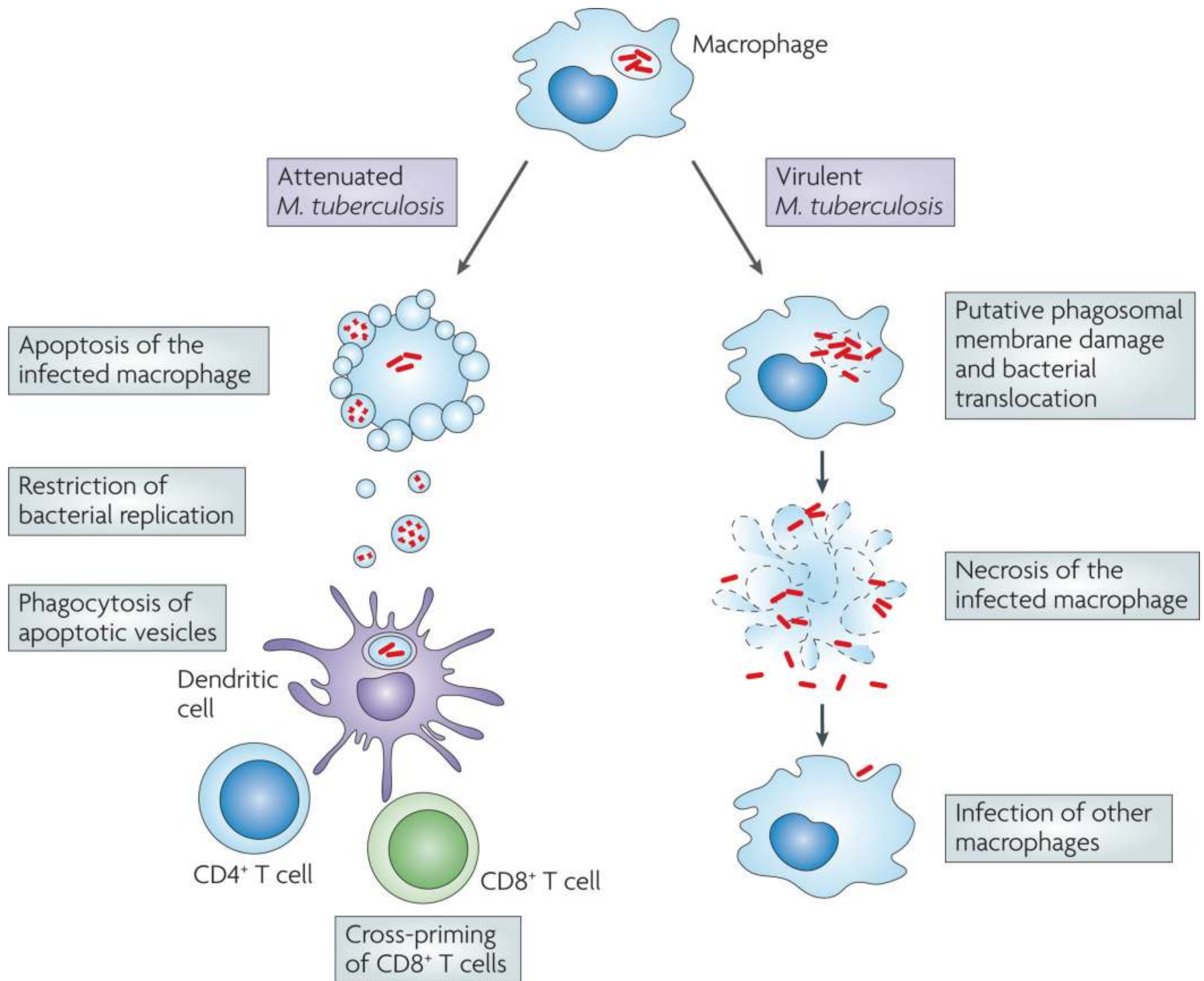

Figure 1. The fate of infected macrophages affects host resistance to *Mycobacterium tuberculosis* infection

Mycobacterium tuberculosis infects macrophages and then survives and replicates in the phagosome. Macrophages infected with attenuated strains of *M. tuberculosis* undergo apoptosis, a death modality that impairs bacterial replication. Apoptosis of infected macrophages provides an important link to adaptive immunity, as apoptotic vesicles containing bacterial antigens are taken up by dendritic cells. The dendritic cells can efficiently present these antigens to naive T cells, leading to their activation. By contrast, virulent *M. tuberculosis* inhibits apoptosis and, instead, induces necrosis. We propose that damage to the phagosomal membrane facilitates bacterial translocation into the cytosol and is a precursor to the full-scale induction of macrophage necrosis. Necrosis leads to intercellular dissemination of *M. tuberculosis*, as extracellular *M. tuberculosis* can infect other macrophages that have been recruited to the lung.

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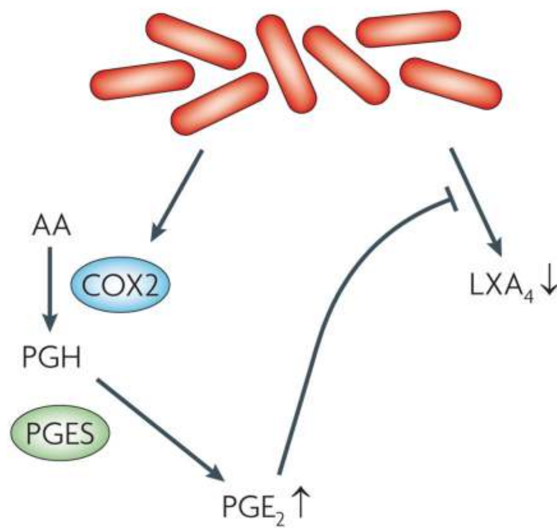

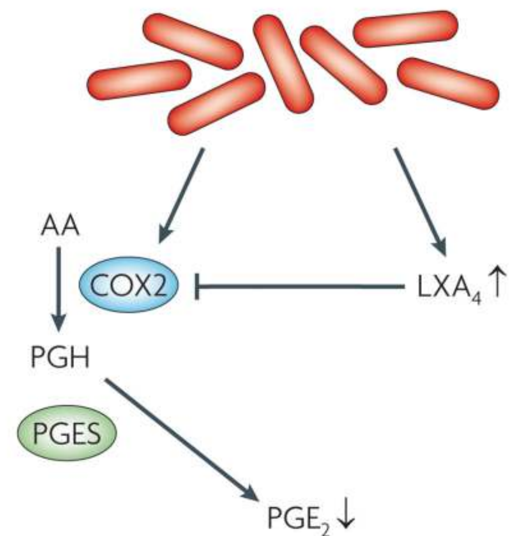

Figure 2. The balance of prostaglandin E_2 and lipoxin A_4 determine the cellular fate of macrophages infected with *Mycobacterium tuberculosis*

a | Infection with attenuated *Mycobacterium tuberculosis* strongly induces cyclooxygenase 2 (COX2) synthesis, which leads to prostaglandin E_2 (PGE_2) production. PGE_2 has at least three actions: signalling through PGE_2 receptor 2 (EP2; also known as PTGER2) protects mitochondria from inner-membrane damage and prevents loss of mitochondrial membrane potential; it also enables the repair of plasma membrane damage, an activity that is probably mediated by EP4; and it inhibits the production of lipoxin A_4 (LXA_4). These actions all prevent necrosis of the infected macrophage and, instead, trigger apoptosis, an innate defence mechanism. **b |** Virulent *M. tuberculosis* strongly induces the production of LXA_4, which prevents the accumulation of *COX2* mRNA. Consequently, prostaglandin biosynthesis falls. Without the protective actions of PGE_2, the infected macrophage is more likely to undergo necrosis, a form of cell death that allows the bacterium to evade innate immunity and T cell-mediated immunity. AA, arachidonic acid; PGES, PGE synthase (also known as PTGES). Figure is modified, with permission, from REF. 26 © (2008) The Rockefeller University Press.

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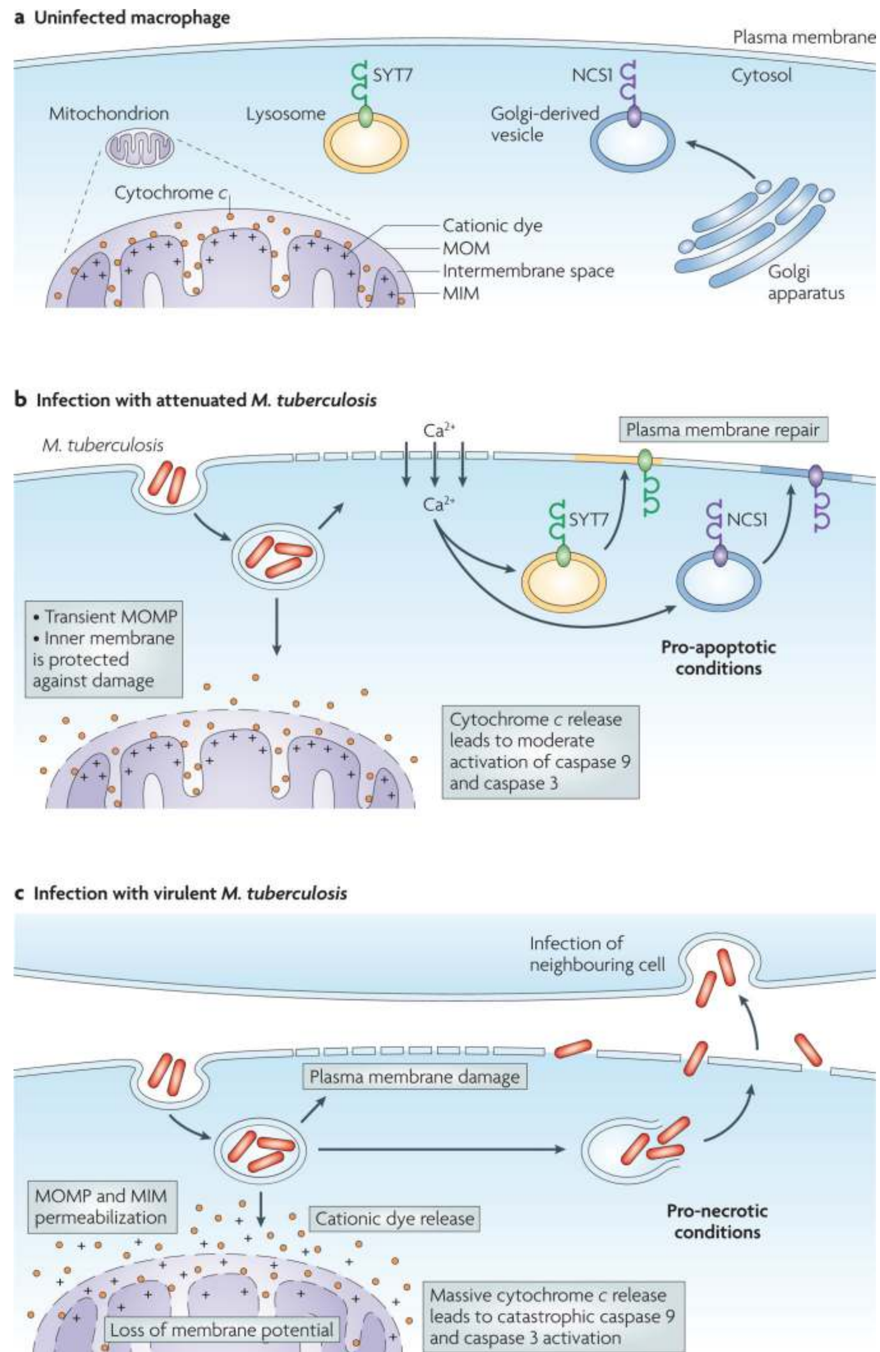

Figure 3. The antinecrotic action of prostaglandin E_2 is mediated through the induction of membrane repair and the protection of mitochondria

a | In an uninfected macrophage, different Ca^{2+}-regulated sensors are expressed in different vesicular compartments. Thus, the lysosome contains synaptotagmin 7 (SYT7), and the Golgi-derived vesicles contain neuronal calcium sensor 1 (NCS1). The mitochondrion has a double membrane consisting of the mitochondrial inner membrane (MIM) and mitochondrial outer membrane (MOM). The mitochondrial intermembrane space contains pro-apoptotic components, including cytochrome *c*. The inner leaflet of the MIM is negatively charged, which leads to specific binding of cationic dyes. **b |** Membrane microdisruptions develop during infection with attenuated *Mycobacterium tuberculosis*, and

the resulting entry of Ca^{2+} into the cell triggers the recruitment of lysosomes and Golgi-derived vesicles, both of which act as membrane donors for the repair of membrane damage. Prostaglandin E_2 (PGE_2) is required for membrane repair, because synthesis of the lysosomal Ca^{2+} sensor SYT7, which is essential for the recruitment of lysosomes to the membrane lesions, depends on PGE_2. Attenuated *M. tuberculosis* leads to MOM permeabilization (referred to as MOMP) but leaves the MIM intact. This allows cytochrome *c* to leak into the cytosol and leads to the activation of caspase 9, which contributes to the activation of caspase 3 and causes apoptosis. **c |** Infection with virulent *M. tuberculosis* induces the production of lipoxin A_4, which inhibits the production of cylooxygenase 2 and effectively prevents prostaglandin biosynthesis. In the absence of PGE_2, plasma membrane microdisruptions remain unrepaired. Concurrently, virulent *M. tuberculosis* induces MOMP and permeabilization of the MIM, which parallels the loss of mitochondrial membrane potential that occurs during necrosis. These conditions trigger macrophage necrosis. We propose that virulent *M. tuberculosis* also disrupts the phagosomal membrane and, by inhibiting membrane repair, facilitates bacterial translocation from the phagosome into the cytosol, which is a prerequisite for exit from the dying macrophage. Thus, bacterial inhibition of prostaglandin production is an immune evasion strategy that allows *M. tuberculosis* to avoid the detrimental consequences of apoptosis and to exit the macrophage and propagate the infection.

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Table 1

Benefits of cell death following a host–pathogen interaction

	Benefits for the host	Benefits for the pathogen
Apoptosis	• Contains the infection • Minimizes tissue injury • Can promote cross-priming • Can lead to pathogen killing	• Does not provoke inflammation • Allows the cell to act as a Trojan Horse for the pathogen, which can lead to dissemination of the pathogen or evasion of the immune response
Necrosis (including pyroptosis)	• Provokes an inflammatory response • Exposes the pathogen to humoral immunity (antibody and complement) • Exposes the pathogen to activated phagocytes • Promotes T cell priming • Leads to the maturation of antigen-presenting cells through danger signals	• Allows the pathogen to escape intracellular defence mechanisms • Allows the pathogen to disseminate
Inhibition of cell death	• T cell recognition of pathogen-derived antigens presented by infected macrophages leads to pathogen clearance • Action of cytokines and other mediators on the infected cell can lead to immune activation and control of the pathogen	• Allows the pathogen to evade humoral immunity • Provides time for the pathogen to complete its life cycle and replicate