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Regulation of antigen presentation by *Mycobacterium tuberculosis*: a role for Toll-like receptors

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Abstract

Mycobacterium tuberculosis survives in antigen-presenting cells (APCs) such as macrophages and dendritic cells. APCs present antigens in association with major histocompatibility complex (MHC) class II molecules to stimulate CD4$^+$ T cells, and this process is essential to contain *M. tuberculosis* infection. Immune evasion allows *M. tuberculosis* to establish persistent or latent infection in macrophages and results in Toll-like receptor 2 (TLR2)-dependent inhibition of MHC class II transactivator expression, MHC class II molecule expression and antigen presentation. This reduction of antigen presentation might reflect a general mechanism of negative-feedback regulation that prevents excessive T cell-mediated inflammation and that *M. tuberculosis* has subverted to create a niche for survival in infected macrophages and evasion of recognition by CD4$^+$ T cells.

Mycobacterium tuberculosis is an intracellular pathogen that infects phagocytic antigen-presenting cells (APCs) in the lung, including alveolar macrophages, lung macrophages and dendritic cells (DCs). In these cells, *M. tuberculosis* survives in modified phagosomes and uses multiple mechanisms to evade both innate and adaptive host immunity, including inhibition of phagosome maturation, resistance to innate microbicidal mechanisms and cytokine-mediated host defences, and, as reviewed here, inhibition of antigen presentation.

CD4$^+$ T cells and the major histocompatibility complex (MHC) class II molecules that induce CD4$^+$ T cell responses are central to host resistance to *M. tuberculosis*[1–6]. In mice,

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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
Escherichia coli | *Mycobacterium bovis* bacille Calmette–Guérin | *Mycobacterium smegmatis* | *Mycobacterium tuberculosis*
UniProtKB: http://www.uniprot.org
CIITA | DC-SIGN | IFNγ | IL-6 | IL-10 | Lgt | LpqH LprA LprG | LRG47 | LspA | MYD88 | NOS2 | PhoS1 | TLR1 | TLR2 | TLR4 | TLR6 | TLR9 | TNF
FURTHER INFORMATION
Clifford V. Harding's homepage: http://www.case.edu/med/pathology/faculty/harding.html
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between 1 and 3 weeks after infection, *M. tuberculosis*-specific T cells appear in the lungs, interferon-γ (IFNγ) is expressed (mainly by CD4$^+$ T cells, but also by other cells) and the bacterial burden is controlled. In addition to IFNγ production, CD4$^+$ T cells can kill *M. tuberculosis*-infected target cells and provide help for other T cell subsets such as CD8$^+$ T cells and γδ T cells, which are important for control of *M. tuberculosis* infection. Thus, the mechanisms involved in MHC class II antigen processing and presentation, which are required for CD4$^+$ T cell activation, are crucial for controlling *M. tuberculosis* infection (FIG. 1).

In humans, 9 out of every 10 healthy individuals control *M. tuberculosis* in the lungs when infected with the bacterium. The pathogen is not eliminated, however, and a few mycobacteria can persist for years, residing inside macrophages in granulomas and evading elimination by the host immune response. This persistence allows progression to active tuberculosis (TB), either as the progressive primary disease or, years later, as reactivation TB when T cell immunity fails (FIG. 1).

Multiple mechanisms contribute to the ability of *M. tuberculosis* to survive in the host. In this Review, we focus on the mechanisms that allow *M. tuberculosis* to evade CD4$^+$ T cell immunity. Priming of *M. tuberculosis*-specific T cells is not inhibited by *M. tuberculosis*, although there is a poorly understood lag period before priming of CD4$^+$ T cells[7,8] and CD8$^+$ T cells[9], during which bacterial proliferation continues in the lung. In fact, the *M. tuberculosis* cell wall is rich in molecules with adjuvant activity (not all of which is mediated by Toll-like receptors (TLRs)) that readily induce immune responses in animal models and in humans. *M. tuberculosis* infection in humans results in conversion of the tuberculin skin test result from negative to positive, which is indicative of strong CD4$^+$ T cell responses to *M. tuberculosis* proteins *in vivo*. This *in vivo* CD4$^+$ T cell response is associated with substantial IFNγ production and is readily detected *ex vivo*. After priming, however, CD4$^+$ T cells target the lung but do not eradicate *M. tuberculosis*. This suggests that effector CD4$^+$ T cells fail to recognize *M. tuberculosis*-infected cells. One mechanism by which this can occur is through inhibition of antigen presentation by MHC class II molecules.

CD4$^+$ T cell

A T cell that expresses the CD4 receptor. These cells recognize antigens that are presented on the surface of host cells by MHC class II molecules.

This Review focuses on the mechanisms by which *M. tuberculosis* and TLR signalling inhibit MHC class II antigen presentation, allowing infected APCs to become niches for *M. tuberculosis* survival and evasion of T cell responses. TLRs are a family of receptors that detect a wide range of microbial molecules known as pathogen-associated molecular patterns (PAMPs) to activate innate immunity and enhance adaptive immunity. TLRs are expressed on many cells, but their roles on APCs are particularly important. TLRs are not the only receptors that recognize *M. tuberculosis*; for example, nucleotide-binding oligomerization domain (NOD) receptors might also have roles in host defences against *M. tuberculosis*[10], and DC-specific ICAM3-grabbing non-integrin (DC-SIGN) mediates recognition and internalization of *M. tuberculosis* by DCs and inhibits DC maturation, potentially contributing to immune evasion[11]. Among the TLRs, TLR2, TLR9 and, possibly, TLR4 are responsible for recognizing *M. tuberculosis* and are therefore largely responsible for the unique adjuvant activity of *M. tuberculosis*.

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IFNγ and *M. tuberculosis* infection

The interactions between *M. tuberculosis* and innate and adaptive immune cells result in the secretion of chemokines and cytokines, of which IFNγ and tumour necrosis factor (TNF) are particularly important in TB. Among its many effects, IFNγ has an important role in activating macrophages and enhancing their expression of MHC class II molecules, resulting in enhanced antigen presentation to T cells.

IFNγ, its signalling pathway and its effector mechanisms are crucial for containing mycobacterial infection[12–16]. $CD4^+$ T cells secrete IFNγ to activate infected macrophages and induce microbicidal functions[12–14]. $CD8^+$ T cells and natural killer cells also produce IFNγ in response to *M. tuberculosis* infection. IFNγ-knockout mice are extremely susceptible to infection with *M. tuberculosis*; these animals exhibit defective macrophage activation and decreased expression of inducible nitric oxide synthase 2 (NOS2)[12]. IFNγ also has a key role in humans, as defects in the genes encoding IFNγ or the IFNγ receptor predispose the host to serious mycobacterial infections[17]. In addition to the induction of molecules that contribute to innate antimicrobial mechanisms (for example, NOS2 and the IFN-inducible GTPase LRG47 (also known as IRGM))[16], IFNγ induces molecules, including MHC class II molecules, that are necessary for antigen processing and presentation. Its central role in regulating antigen processing and presentation by MHC class II molecules might be especially important when $CD4^+$ T cells are crucial for the control of an infectious pathogen such as *M. tuberculosis*.

M. tuberculosis and its components inhibit certain macro phage responses to IFNγ[18–28]. Although some reports suggest that mycobacteria inhibit Janus kinase–signal transducer and activator of transcription (JAK–STAT) signalling or IFNγ receptor expression[29,30], most studies have indicated that these proximal steps in IFNγ signalling are not inhibited by *M. tuberculosis*[20,22–24]. Consistent with the preservation of proximal IFNγ signalling, most IFNγ-induced genes are not inhibited by *M. tuberculosis* (for some, such as *NOS2*, *M. tuberculosis* and IFNγ act synergistically in inducing gene expression)[20,24,31], indicating that inhibitory mechanisms must instead affect the subsequent steps in signalling and transcriptional regulation. *M. tuberculosis* inhibits the induction of a subset of IFNγ-induced molecules, including molecules that contribute to antigen presentation[24], providing a mechanism for evasion of immune surveillance.

$CD8^+$ T cell

A T cell that expresses the CD8 receptor. These cells recognize antigens that are presented on the surface of host cells by MHC class I molecules, leading to host cell destruction, and are therefore also known as cytotoxic T cells.

γδ T cell

A T cell that expresses the γδ T cell receptor. Although the exact function of γδ T cells is unknown, it has been suggested that mucosal γδ T cells are involved in innate immune responses

Granuloma

An organized structure that comprises lymphocytes, macrophages, neutrophils and, sometimes, fibroblasts and that often has a necrotic centre, which arises in response to

continued antigenic stimulation in the presence of macrophages (as occurs, for example, in *M. tuberculosis* infection).

Toll-like receptor

A membrane-spanning protein that recognizes conserved ligands on pathogens, such as flagellin, lipopolysaccharide or DNA, and that is therefore a key recognition molecule in the host innate immune response.

Tuberculin skin test

A method of diagnosing *M. tuberculosis* infection by injecting TB antigens intradermally. A delayed-type hypersensitivity response, dependent on the presence of sensitized T cells, is seen in those infected with *M. tuberculosis*. This does not distinguish latent infection from active TB.

Pathogen-associated molecular pattern

A small molecular motif that is conserved across microbial species and engages innate immune receptors, in particular TLRs. Examples include lipopolysaccharide, peptidoglycan and flagellin.

Natural killer cell

A lymphocyte that does not express the T cell receptor or B cell receptor and that confers innate immunity.

M. tuberculosis and TLR signalling

Several studies have demonstrated that *M. tuberculosis*-infected macrophages have decreased MHC class II molecule expression and decreased antigen presentation, reducing $CD4^+$ T cell recognition of infected macrophages[20,22–24,30,32–38]. Comparison of the T cell responses to model antigens presented by *M. tuberculosis*-infected macrophages and to antigens presented by uninfected macrophages showed that *M. tuberculosis* reduced antigen presentation by macrophages 12–18 hours or more after infection[32,35].

Recent studies have provided insights into the molecular mechanisms involved in the inhibition of MHC class II antigen presentation by *M. tuberculosis*. Viable *M. tuberculosis* is not required for inhibition of macrophage MHC class II expression and antigen presentation, which can be achieved by exposure of macrophages to *M. tuberculosis* lysate[22,30,33–35,39]. Biochemical fractionation was used to identify *M. tuberculosis* components that inhibited MHC class II molecule expression, and several *M. tuberculosis* lipoproteins, including LpqH[32], LprG[40] and LprA[41], were found to be key inhibitors. These lipoproteins, as well as PhoS1 (also known as PstS1), are agonists of TLR2 (REFS [23,32,40–43]) (TABLE 1), and their inhibition of MHC class II molecule expression and antigen presentation is dependent on TLR2 and its adaptor, myeloid differentiation primary-response protein 88 (MYD88)[19,23,32,40]. Furthermore, MHC class II inhibition that is mediated by viable *M. tuberculosis* is itself also largely dependent on TLR2 (REFS [23,32]) and, to an even greater degree, on MYD88 (REF. [23]), although some MHC class II inhibition might be due to non-lipoprotein components of *M. tuberculosis* and could be MYD88 independent[18,19,21].

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Thus, prolonged TLR2 signalling induced by *M. tuberculosis* lipoproteins (and, potentially, by other TLR2 agonists expressed by *M. tuberculosis*[18]) results in inhibition of MHC class II molecule expression and antigen presentation by *M. tuberculosis*-infected macrophages.

M. tuberculosis *lipoproteins and TLR2*

TLR2 forms heterodimers with either TLR1 or TLR6 for recognition of triacylated or diacylated lipopeptides, respectively[44–48], or the corresponding bacterial lipoproteins. Lipopeptide-induced association of the TLR1 and TLR2 extracellular domains has been characterized by X-ray crystallography, which revealed that the cysteine side chain-linked diacylglycerol moiety of the lipoprotein ligand extends its acyl chains into a hydrophobic pocket in TLR2, and the *N*-linked acyl chain of the ligand interacts with TLR1 (REF. [44]).

Bacterial lipoproteins are made as pre-prolipoproteins with an amino-terminal signal sequence[49] (FIG. 2a). They are processed by a pre-prolipoprotein diacylglyceryl transferase (Lgt)[50], which catalyses the thioether linkage of a diacylglycerol moiety to the cysteine immediately following the signal sequence. Then, lipoprotein signal peptidase (LspA) cleaves the signal sequence[51,52], and apolipoprotein *N*-acyltransferase (Lnt) adds an *N*-linked acyl chain concurrently with transport across the inner membrane[52]. This pathway for synthesis has been demonstrated in a wide range of bacterial species, including *Escherichia coli*, and mycobacterial homologues of both Lgt and LspA have been identified[51,53]. Thus, mature mycobacterial lipoproteins have an amino-terminal, triacylated cysteine residue, although the exact biochemical structure of the acyl chains remains poorly defined.

Glycolipids and other mycobacterial agonists of TLR2

A second class of acylated mycobacterial TLR2 agonists consists of cell wall glycolipids[54–56], including lipoarabinomannan (LAM), lipomannan (LM) and phosphatidylinositol mannoside (PIM)[54,57–60] (FIG. 2b). All three contain a PIM core structure with 2–4 acyl chains[60–63]. These glycolipids, like triacylated lipopeptides, signal through TLR2–TLR1 heterodimers[45,64]. PIM, LM and LAM vary in their potency as TLR2 agonists, which is possibly influenced by both the carbohydrate structure and the acylation state of the glycolipid[59]; for example, triacylated LM is a TLR2 agonist, whereas diacylated LM is not pro-inflammatory[60]. The mannose-capped form of LAM that is expressed by *M. tuberculosis* (ManLAM) is less potent than the LAM that is expressed by other myco-bacteria, such as *Mycobacterium smegmatis*[55,65,66]. Other mycobacterial agonists of TLR2 could include trehalose dimycolate, which has been implicated by some studies in signalling through TLR2, in part[67].

T helper 2 cell

A type of activated T helper cell that participates in phagocytosis-independent responses and downregulates pro-inflammatory responses that are induced by T helper 1 cells. T helper 2 cells secrete IL-4, IL-5 and IL-6.

Consequences of TLR signalling

Although TLR signalling enhances both the innate and adaptive immune responses, it can also downregulate some immune functions. TLR2, in particular, has been implicated in the downregulation or deviation of the immune response through the induction of interleukin-10 (IL-10) and T helper 2 cell or regulatory T cell responses, for example[68]. Prolonged TLR signalling might provide homeostatic feedback regulation that limits the extent of the induced responses. Although TLR signalling in APCs induces microbicidal and inflammatory effectors, *de novo* MHC class II antigen processing and presentation is

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inhibited by prolonged signalling with agonists of TLR2 (REFS [32,40,41]), TLR9 and TLR4 (REFS [69–72]). Downregulation of antigen presentation is not specific to *M. tuberculosis*, as it can be induced by components from many microorganisms, but it could be especially pronounced during persistent infection with intravacuolar pathogens that survive microbicidal mechanisms and that can persistently colocalize with TLRs in phagosomes for prolonged TLR signalling (TLR2 can be recruited to phagosomes as well as reside on the plasma membrane[47,73]).

This signalling pathway could be particularly relevant for *M. tuberculosis*. The prolonged residence of *M. tuberculosis* in the phagosome and the abundance of cell wall ligands for TLR2 that are released from viable mycobacteria and that can traffic out of the phagosome (including lipoproteins and glycolipids) provide ample opportunity for TLR2 signalling in the phagosome or on the APC surface[74–76]. For example, LpqH is shed from live, intracellular mycobacteria[74], making it available to stimulate TLR2 (REFS [32,77,78]) in association with TLR1 (REF. [46]). Lipoproteins and the glycolipids that form the outer envelope of *M. tuberculosis* are also found in exosomes and can thus affect adjacent uninfected cells[75,79,80]. The intracellular persistence of *M. tuberculosis* and shedding of TLR2 agonists allow for chronic stimulation of TLR2, which leads to inhibition of MHC class II expression and antigen processing.

Balancing immunity and immune evasion

The role of prolonged TLR signalling in decreasing MHC class II antigen presentation must be interpreted in the context of the overall impact of TLR signalling on host immune responses. TLR2 is important in host defence against mycobacterial infection, as revealed by the increased progression of infection (as shown by increased organism burden in the lung) in TLR2-knockout mice that are infected with *M. tuberculosis* or *Mycobacterium bovis* bacille Calmette–Guérin (BCG) bacilli[81–84], although there is redundancy with other receptors (possibly TLR9 and TLR4)[81]. Other knockout mouse studies suggest that TLR4 (REF. [85]) and TLR9 (REF. [86]) also contribute to host resistance to *M. tuberculosis* infection. *Myd88*$^{-/-}$ mice display serious deficiencies in their resistance to *M. tuberculosis* infection, but this could be the result of the abrogation of multiple MYD88-dependent mechanisms, including signalling by the IL-1 receptor as well as by TLRs[87–89]. However, humans with a MYD88 deficiency have increased susceptibility to infections with pyogenic bacteria but not mycobacteria[90]. The complex nature of the role of TLRs in ‘protection’ against *M. tuberculosis* is underscored by observations from mouse infection models, including the observation that a TLR4 deficiency does not always result in increased susceptibility to *M. tuberculosis* infection[91] and a recent study questioning the roles of TLR2, TLR4, TLR9 and MYD88 in *M. tuberculosis* infection[92].

Mouse *M. tuberculosis* infection is used as a model for acute primary infection, but it is inadequate as a model for the asymptomatic persistent infection that is found in humans, making it difficult to assess the role of TLR2 during chronic infection using mouse models. However, several studies have noted that humans with certain TLR2 polymorphisms have increased susceptibility to mycobacterial disease[93–95], indicating that TLR2 has an important role in immune responses to *M. tuberculosis*.

TLR signalling by *M. tuberculosis* generally promotes host immunity, but it can also result in downregulation of antigen presentation in a subset of APCs. As discussed below, the effects of TLR signalling in mycobacterial infection can include both containment of infection during rapid mycobacterial growth and inflammation, and facilitation of the persistence of a small number of bacilli within APCs when adaptive immunity controls *M. tuberculosis* growth. The ability to take advantage of negative-feedback regulation in macrophages could explain, in part, one of the main paradoxes of the host–pathogen

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interaction in *M. tuberculosis* infection: that an organism that elicits strong innate immune responses, through multiple adjuvant-like molecules in its cell wall, as well as strong T cell responses is able to survive and persist in APCs, which are the ‘launch pad’ of the host's adaptive immune system.

Mechanisms of inhibiting antigen presentation

Transcriptional control of MHC class II might be an important mechanism in the regulation of APC function during infection with *M. tuberculosis*. The MHC class II transactivator (CIITA) is the master transcriptional regulator of MHC class II molecules[96–102]. CIITA also regulates transcription of both the invariant chain, which functions as a chaperone to guide MHC class II molecules to antigen-processing compartments, and human leukocyte antigen HLA-DM (histocompatibility protein H2-DM in the mouse), which catalyses the exchange of peptides bound to MHC class II molecules to facilitate binding of antigenic peptides during intracellular antigen processing[102–104]. CIITA does not bind directly to MHC class II promoters; it coordinates the assembly and action of numerous transcription factors (for example, the regulatory factor X (RFX) family, nuclear transcription factor Y (NFY) and cyclic AMP-responsive-element-binding (CReB) proteins), resulting in the induction of MHC class II transcription.

CIITA expression is controlled through three promoters (pI, pIII and pIV) that drive expression of CIITA types I, III and IV, which incorporate different first exon sequences[97]. Different APC types use different CIITA promoters and control these promoters by various mechanisms[105]. Macrophages use pI and pIV, DCs use pI and B lymphocytes use pIII[106]. In macrophages, pI and pIV are both responsive to IFNγ (that is, IFNγ induces CIITA, which induces expression of MHC class II genes); in immature DCs, pI drives expression of CIITA type I independently of IFNγ[106].

Following their synthesis in the endoplasmic reticulum, MHC class II molecules associate with the invariant chain (FIG. 3). The invariant chain serves as a surrogate ligand before the loading of MHC class II molecules with antigenic peptides, and its cytoplasmic tail directs targeting of MHC class II molecules from the Golgi complex to late endocytic compartments or phagolysosomes that contain internalized antigen and vacuolar proteases. In these compartments, vacuolar proteases process the antigen to produce antigenic peptides and also catabolize the invariant chain. HLA-DM catalyses the dissociation of a peptide fragment of the invariant chain that occupies the peptide-binding groove of the MHC class II molecule to allow association of antigenic peptides. The resulting peptide–MHC class II molecule complexes are transported to the plasma membrane for presentation to $CD4^+$ T cells.

There are multiple steps at which *M. tuberculosis* can interfere with the synthesis of MHC class II molecules and their participation in antigen processing. There is abundant evidence from *in vitro* experiments that *M. tuberculosis* inhibits antigen processing by macrophages through a mechanism involving the decreased synthesis and expression of MHC class II molecules[20,22–26,30,32,35,38]. Although some studies have suggested that this regulation is intrinsic to MHC class II gene promoters[28], several studies indicate that MHC class II molecule synthesis is decreased following *M. tuberculosis*-induced (that is, TLR-dependent) inhibition of CIITA[20,23,25,26,38]. *M. tuberculosis* inhibits antigen processing, antigen presentation and MHC class II molecule expression by macrophages after a delay of 15–24 hours[23,32,35], indicating that the inhibitory mechanism involves steps that dictate these delayed kinetics. This is consistent with a model that we have proposed (FIG. 4), in which TLR signalling initiates a series of steps that result in decreased expression of MHC class II and other molecules that contribute to antigen processing and presentation[19,21,23,32,40]. The

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signalling pathway includes a role for mitogen-activated protein kinase (MAPK) activation[26], a possible role for nitric oxide[107] and the induction of inhibitory transcription factors (for example, CCAAT/enhancer-binding proteins(C/eBPs))[25]. It remains unclear whether these effects are mediated by direct TLR2 signalling or by TLR2-induced cytokines, including IL-6 (REFS [108,109]); our studies with IL-10-knockout cells indicate that IL-10 is not required for the inhibitory effect (W.H.B., C.V.H. and E. Noss unpublished data). The result is decreased expression of *CIITA* mRNA (associated with decreased histone acetylation at both the pI and pIV promoters)[20,23,25,26,38], consequent decreased transcription and decay of MHC class II mRNA[23,35], decay of nascent MHC class II molecules transiting through the endoplasmic reticulum and Golgi and decay of the pool of nascent MHC class II molecules in antigen processing compartments (which, alone, can take several hours in macrophages).

Regulatory T cell

A $CD4^+$ T cell that naturally expresses high levels of CD25 (the IL-2 receptor subunit-α) and the transcription factor forkhead box P3 (FOXP3) and that has suppressive regulatory activity towards effector T cells and other immune cells.

Pyogenic bacterium

A pus-forming bacterium that is associated with exudative inflammation and neutrophil recruitment.

M. tuberculosis can inhibit antigen presentation by mechanisms other than the reduction in expression of MHC class II molecules. In macrophages, *M. tuberculosis* inhibits the expression of several genes involved in antigen processing and presentation[24], and *M. tuberculosis* can also inhibit antigen presentation in DCs by mechanisms other than control of MHC class II molecule expression[110]. To study the interference with antigen processing and presentation that is independent of MHC class II molecule expression requires functional assessment of antigen presentation, and this is more difficult than simply measuring the MHC class II molecules on APCs. Mechanisms that inhibit antigen processing and presentation with little effect on MHC class II molecule expression might be more important in certain human macrophage populations in which TLR2 stimulation results in notable inhibition of MHC class II antigen presentation with more subtle effects on MHC class II levels[111].

M. tuberculosis can also inhibit post-translational functions of MHC class II molecules[34] and can blunt antigen-processing mechanisms (for example, through inhibition of phagosome maturation[112], although this does not seem to substantially block the processing of *M. tuberculosis* antigens[112–114]). Although post-translational regulation can alter MHC class II function in important ways (for example, by regulating MHC class II molecule stability during DC maturation), most studies suggest that the major cause of decreased antigen presentation by *M. tuberculosis*-infected macrophages is decreased MHC class II gene transcription (consequent to the inhibition of CIITA expression).

Differences between DCs and macrophages

In vivo and *ex vivo* studies of mice following aerosol infection with *M. tuberculosis* or *M. bovis* BCG show that several types of APC in the lung are infected, including alveolar macrophages, lung macrophages and lung DCs[110,115–118]. Infected DCs migrate to draining mediastinal lymph nodes[116]. DCs are the primary APCs for naive T cells and are essential to

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'prime' T cells to become effector and memory cells. Macrophages are excellent activators of effector and memory T cells (for example, at sites of infection), although they are not important for priming T cell responses. As *M. tuberculosis* can infect both macrophages and DCs, the differences between these cells in MHC class II antigen presentation and its regulation by *M. tuberculosis* have important implications for the activation of naive and effector $CD4^+$ T cells (FIG. 5).

Macrophages and DCs differ in how MHC expression is regulated in response to TLR signalling. Prolonged TLR signalling (that is, signalling for > 24 hours) reduces MHC class II molecule levels in macrophages[23,32,35,40,69,111]. By contrast, TLR agonists drive DCs to undergo maturation, which includes their migration from peripheral sites to lymph nodes and enhances levels of MHC class II molecules and co-stimulators, resulting in increased antigen presentation to naive T cells in lymph nodes[119–122]. Importantly, the increased levels of MHC class II molecules on mature DCs is due to the increased stability of the proteins[121,123], which results from the decreased ubiquitylation of the MHC class II molecules on maturation of DCs, leading to decreased ubiquitin-dependent endocytosis and degradation of the proteins[124–126].

Although the regulation of MHC class II molecule expression differs in macrophages and DCs, owing to different post-translational mechanisms for the control of MHC class II molecule stability, the effects of TLR signalling on the transcriptional regulation of MHC class II genes are similar and include decreases in the expression of *CIITA* and the MHC class II genes. Thus, DCs reduce the levels of *CIITA* and MHC class II mRNAs upon maturation[106,127], which is similar to the reduction in *CIITA* and MHC class II mRNA levels that is seen in macrophages after TLR signalling[19,21,23,32,35].

In vitro exposure to *M. tuberculosis* or its lipoproteins drives DC maturation and the increased expression of co-stimulators and MHC class II molecules[41,128–131], despite decreased CIITA expression and MHC class II molecule synthesis. Antigen processing and the generation of peptide–MHC class II complexes before the loss of MHC class II molecule synthesis allows infected DCs to present mycobacterial antigens to T cells[32,132].

Despite the proposal that TLR stimulation increases DC antigen presentation, mycobacterial infection might interfere with MHC class II antigen processing and the presentation of *M. tuberculosis* antigens by DCs. The ManLAM of *M. tuberculosis* is a DC-SIGN ligand on DCs[133] that can inhibit DC maturation[11]. Some *in vitro* studies indicate that infection of DCs with *M. bovis* BCG ultimately leads to loss of MHC class II molecules on DCs[134], as observed in macrophages, making DCs another possible niche for immune evasion by mycobacteria. In addition, induction of DC maturation and subsequent loss of the antigen-processing function can occur before sufficient MHC class II processing of *M. tuberculosis* antigens has occured, although CD1-based antigen processing persists[128]. It was also observed that lung DCs from mice that were infected with *M. tuberculosis* by aerosol had low antigen presentation capacities, despite continued expression of MHC class II molecules[110]. Furthermore, *in vivo* infection of lung DCs after aerosol administration of GFP-expressing *M. bovis* BCG led to decreased expression of MHC class II molecules relative to expression in uninfected DCs from the same lung[117].

In vivo studies present a more complex situation in which to assess the effects of mycobacterial infection of APCs on MHC class II molecule expression. Several studies[115,135,136] have suggested that some macrophage subsets have decreased expression of MHC class II molecules at certain stages during mycobacterial infection of the lung. As noted above, simply measuring MHC class II molecule levels might underestimate the inhibition of antigen presentation, as some effects on antigen processing can only be

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assessed functionally. In addition, *in vivo* studies have not distinguished the small proportion of APCs that are directly infected by mycobacteria from the large proportion of uninfected APCs that are present in infected tissue (for example, only ~1% of macro phages are infected at the peak of pulmonary infection after aerosol infection of mice with *M. bovis* BCG[117]). T cell responses generate IFNγ, which induces MHC class II molecule expression in macrophages. Thus, the inflammatory response to mycobacterial infection increases expression of MHC class II molecules in most of the uninfected macrophages (as observed in mycobacteria-induced exudates[137]), but this does not reveal the level of MHC class II molecule expression in the small proportion of these cells that are actually infected with mycobacteria.

To determine whether *M. tuberculosis* inhibits the expression of MHC class II molecules in APCs *in vivo*, it is necessary to distinguish the small proportion of infected APCs from uninfected APCs, by infection with GFP-expressing mycobacteria, for example. when this approach was taken for the aerosol infection of mice with *M. bovis* BCG, lung macrophages and DCs that harboured mycobacteria were found to have lower levels of MHC class II molecule expression than cells from the same lungs that did not harbour mycobacteria[117]. However, when a similar approach was used to study lung cells from mice infected with *M. tuberculosis* H37Rv, no significant changes were observed in the expression levels of MHC class II molecules in macrophages[21]. Thus, *in vivo* studies have produced varying results with different models, but several reports using different approaches suggest that small subsets of lung APCs that harbour mycobacteria have suppressed levels of MHC class II molecule expression, even though expression is increased in other APCs. Importantly, inhibition of MHC class II molecule expression in even a small proportion of APCs would provide sufficient niches for the small number of *M. tuberculosis* bacilli that persist during chronic infection.

Explaining the differing MHC class II regulation

Are there teleological models to explain why APCs decrease synthesis of MHC class II molecules after TLR signalling, and why macrophages and DCs differ in their post-translational control of MHC class II molecule expression after TLR signalling? For DCs, TLR stimulation results in a final burst of MHC class II molecule synthesis at the time of PAMP exposure, which should correspond to the time of exposure to, and processing of, other pathogen-derived antigens. Subsequently, MHC class II molecule synthesis declines, and expression is prolonged for the cohort of peptide–MHC class II complexes that is enriched in pathogen-derived peptides. This focuses antigen presentation by DCs on the presentation of pathogen-derived antigens, and the prolongation of this presentation allows for efficient priming of naive T cells in lymph nodes. This might explain why $CD4^+$ T cell responses are readily induced in animals and humans after *M. tuberculosis* infection.

Macrophages differ from DCs in their roles in both antigen presentation and its regulation. Although macrophages are not major contributors to the priming of T cell responses, they have an important role in presenting antigen to effector T cells at sites of infection. This is crucial for T cell effector functions that are important for host defence (such as cytokine release), but these effector functions can also seriously damage host tissues if they are induced to excessive levels. Macrophages, like DCs, decrease MHC class II molecule synthesis after prolonged TLR signalling, but the stability of MHC class II molecules on macrophages is not enhanced by this signalling. Therefore, expression of MHC class II molecules decays after prolonged TLR signalling.

We propose that this decay of APC function could be a homeostatic downregulatory mechanism to limit effector T cell activation and prevent excessive tissue damage. As a

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consequence of this mechanism, MHC class II antigen presentation might be downregulated in macrophages that are directly infected with intra-vacuolar pathogens that provide chronic TLR stimulation. Other uninfected macrophages could remain uninhibited and competent for antigen presentation, so the downregulatory mechanism limits, but does not eliminate, antigen presentation in infected tissue. In the context of chronic bacterial infections, such as *M. tuberculosis* infection, delayed downregulation might allow sufficient T cell activation to induce adequate host defences. The ability of *M. tuberculosis* to survive in phagosomal compartments and inactivate microbicidal molecules allows its persistence in macrophages until MHC class II antigen presentation is inhibited, reducing the ability of $CD4^+$ T cells to recognize infected cells and, thus, providing a niche for survival and evasion of $CD4^+$ T cell-mediated immunity.

The infected APC as a niche for immune evasion

One of the paradoxes of the host–pathogen interaction in *M. tuberculosis* infection is that the organism is rich in cell wall and secreted molecules that are TLR agonists and in antigens that readily prime $CD4^+$ T cell responses, but it can survive and persist in APCs, avoiding elimination. $CD4^+$ T cells are essential for the control of primary infection and necessary for ongoing immune surveillance to control the persistent infection that forms the reservoir for reactivation TB, which is the most common form of the disease worldwide. There are many mechanisms involved in the survival of *M. tuberculosis*, including its ability to prevent phagosome fusion with lysosomes and resist the microbicidal molecules (such as superoxide, nitric oxide and defensins) that are produced by phagocytes. The host's tendency to form granulomas might interfere with cell trafficking and with the access of T cells to *M. tuberculosis*-infected macrophages.

The ability of *M. tuberculosis* to inhibit MHC class II antigen presentation through prolonged stimulation of TLR2, thereby directly inhibiting its recognition by $CD4^+$ T cells, is an additional means of immune evasion. This mechanism takes advantage of a natural regulatory mechanism of macrophages to decrease MHC class II molecule expression and antigen presentation in order to down-modulate the excessive inflammation that would be induced by prolonged stimulation of the innate immune system. However, the pathogen must survive its initial interaction with macrophages and deliver ligand to TLR2 on the cell surface or phagosomal membrane. This mechanism could be especially relevant during the chronic phase of infection, when few organisms are present and immune surveillance is focused on macrophages. In this way, *M. tuberculosis* is able to turn a liability (namely, a cell wall that is rich in TLR agonists) into a survival mechanism that relies on inhibition of macrophage-mediated presentation of *M. tuberculosis* antigens and induction of effector T cell responses.

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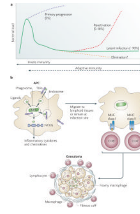

Figure 1. The host response to *Mycobacterium tuberculosis* infection

a | The natural history of *Mycobacterium tuberculosis* infection. The innate and adaptive immune systems allow most healthy people (> 90%) to control the growth of *M. tuberculosis*, although they harbour latent infection (it is not known whether host immune responses can eliminate infection). Some individuals, especially those with impaired T cell function, develop active tuberculosis (TB), either as primary progression or as a reactivation. **b** | The interaction of *M. tuberculosis* (Mtb) with the immune system. *M. tuberculosis* is phagocytosed by antigen-presenting cells (APCs), including macrophages, monocytes and dendritic cells, and survives in phagosomes. *M. tuberculosis* ligands, including lipoproteins and glycolipids, are recognized in APCs by Toll-like receptors (TLRs) and nucleotide-binding oligomerization domain (NOD) proteins[10,138–140], resulting in the secretion of inflammatory cytokines and chemokines. Infected APCs migrate to regional lymphoid tissues, where adaptive immunity develops through antigen presentation to naive T cells. APCs process *M. tuberculosis* antigens by intravacuolar proteolysis to produce peptides that bind to major histocompatibility complex (MHC) class II molecules, which then translocate to the cell surface to mediate presentation of *M. tuberculosis* peptides to $CD4^+$ T cells. *M. tuberculosis* peptides are also presented by MHC class I molecules to $CD8^+$ T cells. Effector and memory T cells migrate back to sites of infection to control *M. tuberculosis* growth. Granulomas develop through the secretion of tumour necrosis factor and other effector cytokines.

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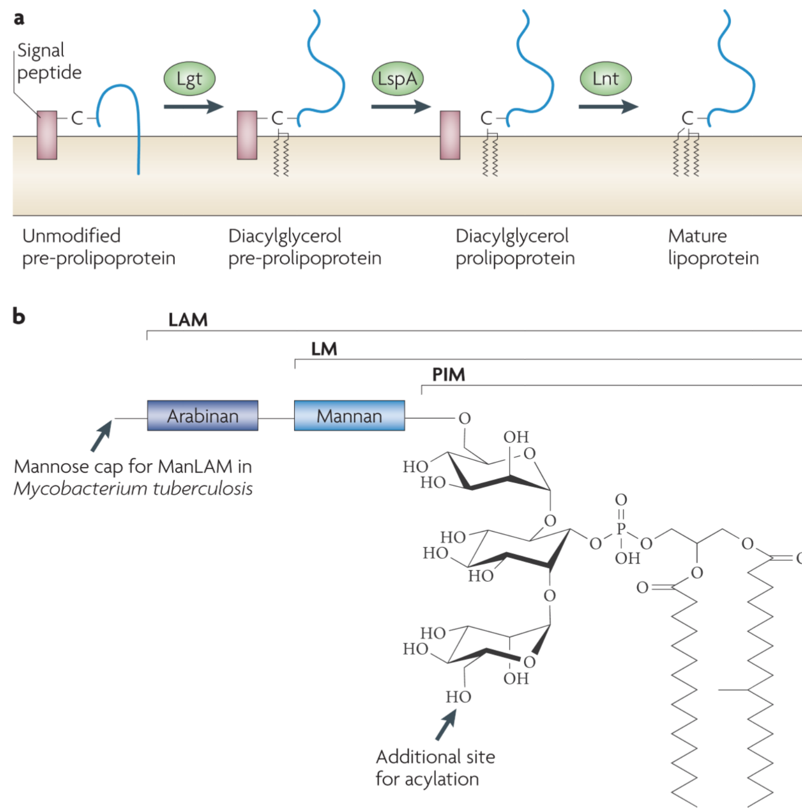

Figure 2. Toll-like receptor 2 ligands expressed by *Mycobacterium tuberculosis*

a | The synthesis of mycobacterial lipoproteins involves the production of an unmodified pre-prolipoprotein, the addition of a diacylglycerol to a cysteine residue (C) adjacent to the signal peptide by a pre-prolipoprotein diacylglyceryl transferase (Lgt), the removal of the signal peptide by lipoprotein signal peptidase (LspA) to expose the cysteine at the amino terminus, and the addition of a third acyl moiety on the free amino group of the cysteine residue by lipoprotein *N*-acyltransferase (Lnt). The acyl chains are thought to be inserted into the *Mycobacterium tuberculosis* plasma membrane. **b |** The structure of the *M. tuberculosis* glycolipids lipoarabinomannan (LAM), lipomannan (LM) and phosphatidylinositol mannoside (PIM). The PIM core shown is a diacylated molecule formed by the addition of two mannose residues to phosphatidylinositol. Additional mannose residues can be added to form higher-order PIM molecules and additional acylation can also occur. Longer mannan chains are added to form LM, and arabinan chains are added to form LAM. Variations in these structures occur between different mycobacterial species and even between different strains. *M. tuberculosis* usually adds mannose caps to the LAM structure, forming ManLAM. Figure part **a** is modified, with permission, from REF. [61] © (2000) American Society for Biochemistry and Molecular Biology.

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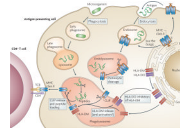

Figure 3. Major histocompatibility complex class II synthesis and function

Major histocompatibility complex (MHC) class II molecules are assembled in the endoplasmic reticulum (ER) as α-chain–β-chain heterodimers in a nonameric complex containing three heterodimers, each associated with a single invariant chain (Ii) molecule. The Ii molecule prevents premature loading of the MHC class II molecules with peptides before their delivery to vacuolar antigen-processing compartments. The Ii–MHC class II complex is transported from the ER through the Golgi to reach late endocytic or lysosomal compartments (some may transit to the plasma membrane and reach endocytic compartments by endocytosis). In the case of phagocytosed bacteria, MHC class II molecules target to phagolysosomes, where Ii is degraded by proteases, leaving an invariant chain-derived peptide, known as class II-associated Ii peptide (CLIP), that remains in the peptide- binding groove, inaccessible to proteases. Human leukocyte antigen (HLA)-DM interacts with MHC class II molecules to catalyse the dissociation of CLIP as well as the exchange of other peptides, resulting in the formation of complexes of MHC class II molecules with high-affinity peptides (including both self and non-self peptides, which MHC class II molecules cannot discriminate). HLA-DO can bind HLA-DM and inhibit its function, negatively regulating peptide loading of MHC class II molecules in some subcellular compartments in certain types of antigen-presenting cell. Exogenous antigens are internalized by endocytosis or phagocytosis. Proteolysis by lysosomal proteases produces peptides (usually 10–16 amino acids long) that bind MHC class II molecules. Peptide–MHC class II complexes are transported from vacuolar processing compartments to the plasma membrane, where they are presented to $CD4^+$ T cells. As antigen-processing mechanisms and MHC class II molecules do not discriminate self and non-self peptides, this discrimination is the function of T cell receptors (TCRs). Figure is modified, with permission, from *Nature Reviews Immunology* REF. [141] © (2009) Macmillan Publishers Ltd. All rights reserved.

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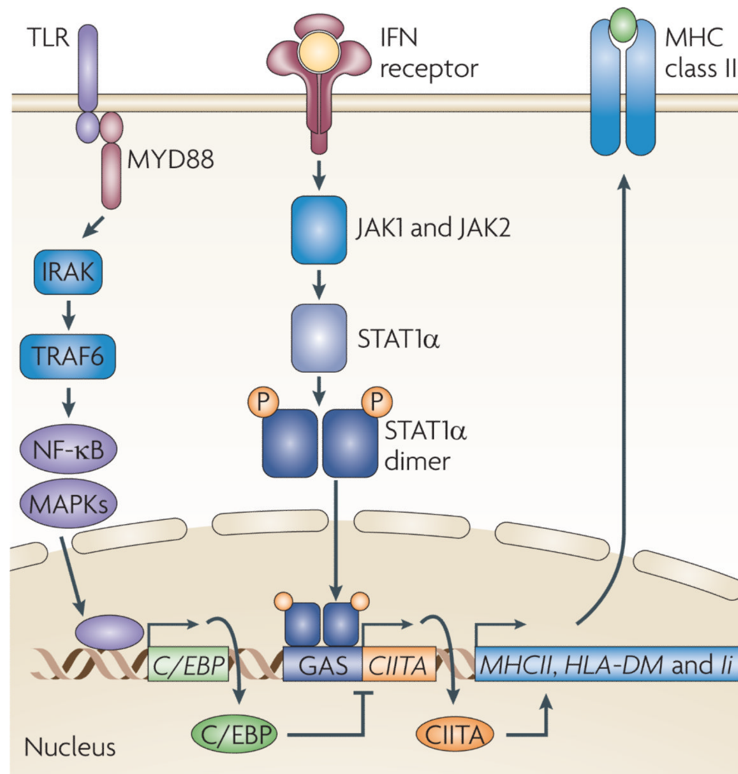

Figure 4. inhibition of major histocompatibility complex class II by *Mycobacterium tuberculosis* through Toll-like receptor signalling

In macrophages, major histocompatibility complex (MHC) class II expression is induced by interferon-γ (IFNγ), which signals through its receptor to activate Janus kinase–signal transducer and activator of transcription (JAK–STAT) signalling, resulting in STAT1α phosphorylation and dimerization. STAT1α dimers translocate into the nucleus and bind to interferon-γ-activated sequence (GAS) sites in promoters, resulting in the induction of genes, including the gene encoding MHC class II transactivator (CIITA). CIITA assembles with other transcription factors to bind to the promoters of MHC class II genes and other genes related to antigen processing (for example, the human leukocyte antigen gene *HLA-DM*), driving their expression. The resulting MHC class II molecules participate in antigen processing and presentation (see FIG. 3). Most studies suggest that *Mycobacterium tuberculosis* infection and Toll-like receptor (TLR) signalling do not interfere with the proximal steps of IFNγ signalling that lead to activation of STAT1α. TLR signalling occurs through the adaptor molecule myeloid differentiation primary-response protein 88 (MYD88) and downstream signalling pathways that result in the activation of nuclear factor-κB (NF-κB) and mitogen-activated protein kinases (MAPKs) and the induction of expression of many genes. CCAATT/enhancer-binding protein-β (C/EBPβ) and C/EBPδ are induced by TLR2 signalling and bind to CIITA promoters, contributing to the inhibition of CIITA, which results in decreased MHC class II molecule expression and inhibition of antigen presentation[25]. Ii, invariant chain; IRAK, interleukin-1 receptor-associated kinase; TRAF6, tumour necrosis factor receptor-associated factor 6.

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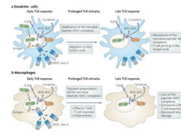

Figure 5. The different responses of macrophages and dendritic cells to Toll-like receptor signalling

Although macrophages and dendritic cells (DCs) both secrete cytokines and produce antimicrobials (such as reactive oxygen species (ROS) and reactive oxygen intermediates (ROI)) in response to Toll-like receptor (TLR) signalling, these cell types differ in how their major histocompatibility complex (MHC) class II antigen presentation function is regulated by TLR signalling. **a** | In DCs, TLR signalling induces maturation, which involves increased expression of peptide–MHC class II molecule complexes and co-stimulatory molecules. DC maturation also involves migration to lymph nodes, where DCs present antigen to naive T cells, priming the T cell response to pathogens. **b** | By contrast, macrophages initially show little decrease in MHC class II antigen presentation and then, after prolonged stimulation (approximately 24 hours or more), show inhibition of antigen presentation with decreased expression of MHC class II molecules. This contrast in MHC class II levels results from regulation of the post-translational stability of MHC class II molecules, which is greatly enhanced during DC maturation but is not increased in macrophages upon TLR stimulation. Accordingly, TLR stimulation of DCs by microorganisms results in a final burst of antigen processing and the accumulation of a kinetic cohort of peptide–MHC class II molecule complexes that include microbial peptides; this cohort is expressed for a prolonged period and provides effective stimulation of naive T cells. DCs present antigen in lymph nodes to activate naive T cells, whereas macrophages present antigens to effector T cells at sites of infection, which produces inflammatory responses that can damage host tissues if they are not controlled. This model proposes that macrophages decrease antigen presentation after prolonged TLR stimulation as a means of homeostatic negative-feedback regulation to limit tissue damage from excessive activation of effector T cells. CIITA, MHC class II transactivator; Mtb, *Mycobacterium tuberculosis*.

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Table 1

Mycobacterial agonists of Toll-like receptor 2

Agonist	Bacterial location	Acylation status	TLR2 co-receptor	Accessory receptor
Lipoproteins				
LpqH	Cell wall	Triacylated	TLR1*	CD14*
LprA	Cell wall	Triacylated	TLR1 or TLR6?*	CD14*
LprG	Cell wall	Triacylated	TLR1*	CD14*
PhoS1	Cell wall	Triacylated	TLR1*	None?*
Glycolipids				
LAM	Cell wall	2–4 acyl chains	TLR1?	CD14
LM	Cell wall	2–4 acyl chains	TLR1?	CD14
PIM	Cell wall	2–4 acyl chains	TLR1?	CD14?
TDM	Cell wall	Dimycolate	TLR2?‡	CD14 and MARCO?‡

LAM, lipoarabinomannan; LM, lipomannan; PIM, phosphatidylinositol mannoside; TDM, trehalose dimycolate; TLR, Toll-like receptor.

*See REF. [142].

‡See REF. [67].