PHAGOCYTE SABOTAGE: DISRUPTION OF MACROPHAGE SIGNALLING BY BACTERIAL PATHOGENS

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Macrophages function at the front line of immune defences against incoming pathogens. But the ability of macrophages to internalize bacteria, migrate, recruit other immune cells to the site of infection and influence the nature of the immune response can provide unintended benefits for bacterial pathogens that are able to subvert or co-opt these normally effective defences. This review highlights recent advances in our understanding of the many interference strategies that are used by bacterial pathogens to undermine macrophage signalling.

ADAPTIVE IMMUNE RESPONSE
In this host defence system, which evolved in vertebrates, T and B cells respond specifically to a given antigen. This type of immune response includes antibody production and the killing of pathogen-infected cells, and is regulated by cytokines such as interferon-α.

NEUTROPHIL
A phagocytic cell of the myeloid lineage that has an important role in the inflammatory response, undergoing chemotaxis towards sites of infection or wounding.

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A key strategy that is used by pathogens to survive in a hostile host environment is to interfere with normal cell signalling to disable the defences that are aimed at controlling and eliminating foreign invaders. The recent availability of microbial genome sequences shows that bacterial pathogens have acquired a multitude of genes, the products of which undermine host-cell signalling. These bacterially encoded proteins, which are known as effectors, use many mechanisms to interfere with normal host-cell signalling, by blocking or mimicking eukaryotic signalling molecules (TABLE 1). During the course of an infection, bacterial pathogens often use a distinct subset of bacterial proteins to alter the targets of host-cell signalling in cell-type-specific ways. Different bacterial pathogens use diverse strategies to achieve a common result: to undermine host-cell functions and therefore establish a permissive niche in which they can survive and replicate (for reviews, see REFS 1,2).

Macrophages are a common target for those bacterial pathogens that benefit from avoiding an encounter with the immune system, as well as those that are aiming to secure systemic spread. As the central determinants of the course of an infection, this well-situated, mobile and expandable cell population provides an early warning system for infection (for a review on macrophages, see REF. 3). As is outlined in BOX 1, macrophages have several qualities that allow them to function as both sentinels and the first line of defence against infection. Numerous signal-transduction pathways are activated when cells contact bacteria (FIG. 1). These pathways can be common to many cell types or unique to macrophages. Signalling functions to coordinate the antibacterial effectors of the macrophage, as well as antigen presentation and cytokine release to recruit other cells to the site of infection and coordinate their responses to clear the microbe (BOX 2). Macrophages form an essential barrier that pathogens must overcome to be successful, and diverse strategies are used by different bacterial pathogens to subvert macrophages.

Macrophage responses to bacteria are significant, as these cells can be considered to be the journeymen of the immune system. They are proficient generalists that are well situated to recognize rapidly, internalize and degrade bacterial pathogens to contain an infection for long enough to initiate an ADAPTIVE IMMUNE RESPONSE. Their breadth of signalling and effector mechanisms gives them certain advantages over specialized cells. For example, although they are not as adept as NEUTROPHILS in producing the reactive oxygen species and antimicrobial peptides that damage bacteria, macrophages live longer and are more potent sources of the cytokines that orchestrate other immune responses (for a review on neutrophils

DENDRITIC CELL
A 'professional' antigen-presenting cell that is found in T-cell areas of lymphoid tissues, but is also a minor cellular component in most tissues. These cells have a branched or dendritic morphology and are the most potent stimulators of T-cell responses.

INNATE IMMUNE RESPONSE
This is crucial during the early phase of host defence against infection by pathogens (such as bacteria and viruses), before the antigen-specific, adaptive immune response is induced.

TOLL-LIKE RECEPTORS
(TLRs). Receptors that are present on mammalian cells, mostly on those that are involved in innate or adaptive resistance to pathogens. They are homologous to the Toll receptor protein family in *Drosophila melanogaster*, members of which have important roles in both embryogenesis and defence against infection. TLRs have evolved to recognize molecular patterns that are conserved and shared by many microbial pathogens.

NUCLEAR FACTOR-κB
(NF-κB). A widely expressed transcription factor that is activated by cellular stress and can induce the expression of numerous proinflammatory and anti-apoptotic genes.

and bacterial infection, see REF. 4). Although they do not migrate to lymph nodes and present antigen for the activation of naive T cells as efficiently as DENDRITIC CELLS (for a review on dendritic-cell responses to bacterial infection, see REF. 5), macrophages have greater antimicrobial abilities. Their lack of specialization allows them to function as surrogate killing cells or antigen-presenting cells in the absence of, or together with, more highly specialized cells. Therefore, pathogens cannot avoid adaptive and INNATE IMMUNE RESPONSES if they do not have a strategy for dealing with macrophages. Understanding macrophage–pathogen interactions is crucial to understanding the pathogenesis of many infectious diseases.

The balance between the macrophage's ability to recognize bacterial pathogens and the pathogen's ability to modulate macrophage signalling often determines the outcome of an infection. As is shown in FIG. 2, the ability of macrophages to internalize bacteria, migrate to the spleen and lymph nodes and to recruit other immune cells to the site of infection can provide unintended benefits for successful pathogens. This review highlights select recent advances in our understanding of the interplay between normal macrophage signalling and the many interference strategies that are used by bacterial pathogens. Although conservation of signalling pathways allows other cell types that are more amenable to experimental manipulation to provide information on host–pathogen interactions, bacterial pathogens often express virulence proteins in cell-type-specific ways and cell types can express different targets for these virulence proteins[6,7]. Therefore, this review will emphasize those studies that were carried out, or confirmed, using macrophages.

Identifying the problem

As macrophages need to recognize many diverse foreign microbes rapidly, they express a repertoire of receptors that bind characteristic conserved microbial molecular patterns[8]. Signalling that is instigated by these 'pattern-recognition receptors' increases the macrophage's antimicrobial abilities. However, many successful bacterial pathogens can disguise themselves, by either modifying their surface to prevent binding by macrophage receptors or by engaging alternative receptors, which ultimately allows them to escape macrophage surveillance.

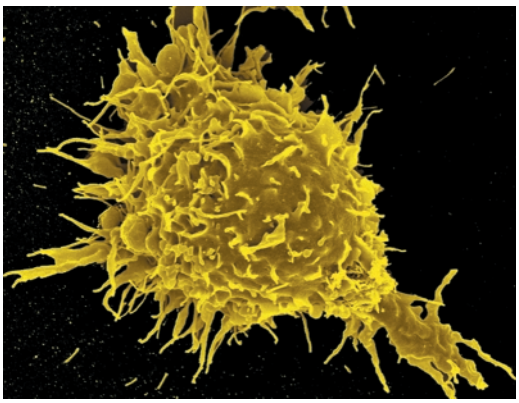
Pathogen recognition. The recent identification of the family of TOLL-LIKE RECEPTORS (TLRs) — on the basis of their similarity to the *Drosophila melanogaster* Toll receptor protein, which is responsible for antifungal responses — has significantly advanced our understanding of how macrophages recognize microbes. TLRs, of which there are known to be ten, mediate the recognition of one or several distinct microbial structures that are not expressed by eukaryotes, and they can cooperate with each other to expand the repertoire of ligands they recognize[9,10]. The signalling pathway that is activated by the recognition of these foreign structures is outlined in FIG. 3. These diverse microbial ligands trigger common downstream signal-transduction pathways, which culminates in various antimicrobial responses such as activation of the transcription factor NUCLEAR FACTOR κB (NF-κB) and transcription of proinflammatory cytokine genes (for a review, see REF. 11). Indeed, gene expression profiling has shown that most of the macrophage's transcriptional responses to infection can be mediated by TLR signalling[12–14]. Although further research is required to discover how downstream signalling can be tuned to allow unique responses to different pathogens, it has been shown that

Table 1 | **The enzymatic activities of bacterial proteins that interfere with macrophage signalling**

Strategy	Activity	Gene product	Pathogen	Target
Avoidance of phagocytosis	Tyr phosphatase	YopH	*Yersinia*	FAK, Paxillin, p130cas
	Ser/Thr kinase	YpkA (YopO)	*Yersinia*	Actin, RhoA, Rac
	Cysteine protease	YopT	*Yersinia*	RhoA, Rac1, Cdc42
	GTPase activating	YopE	*Yersinia*	Rho, Rac, Cdc42
	GTPase activating	ExoT, ExoS	*Pseudomonas*	Rho, Rac, Cdc42
	ADP-ribosyltransferase	ExoS	*Pseudomonas*	Ras family
Disruption of trafficking	GEF	RalF	*Legionella*	ARF1
Promotion of inflammation	Protease activation	IpaB	*Shigella*	Caspase-1
	Protease activation	SipB	*Salmonella*	Caspase-1
Dampening of inflammation	Protease	Lethal factor	*Bacillus*	MEK kinase
	Ubiquitin-like protease	YopJ (YopP)	*Yersinia*	MAPKK, IKKβ
	Protease	?	*Pseudomonas*	IFN-γ, TNF-α
Cytotoxicity	Adenylate cyclase	CyaA	*Bordetella*	↑ cyclic AMP
	ADP-ribosyltransferase	SpvB	*Salmonella*	?
Alteration of signalling	Adenylate cyclase	EF	*Bacillus*	↑ cAMP

The above bacterial virulence proteins are expressed during infection — their cellular targets and biological effects are given where known. The enzymatic activity of these bacterial effectors has often been determined in non-macrophage cell lines or *in vitro* and has not always been confirmed in macrophages. These bacterial components can have several effects. For example, the virulence proteins IpaB and SipB target caspase-1 to cause macrophage apoptosis as well as release of the proinflammatory cytokine interleukin-1β. ARF1, ADP-ribosylating factor 1; CyaA, adenylate cyclase; EF, oedema factor; ERK, extracellular signal-regulated kinase; FAK, focal adhesion kinase; GEF, guanine nucleotide exchange factor; IFN-γ, interferon-γ; IKKβ, inhibitor of NF-κB (IκB) kinase β; MAPK, mitogen-activated protein kinase; MEK, MAPK and ERK kinase; Ser, serine; Thr, threonine; TNF-α, tumour necrosis factor-α; Tyr, tyrosine.

Box 1 | **The role of macrophages in innate responses to bacterial infection**



The innate immune system allows a general and rapid response to an infectious agent. During this early stage of infection, macrophage roles include: ingestion of bacteria by phagocytosis; destruction of bacteria within the phagolysosome; and recruitment of inflammatory cells to the site of infection, using chemokines and acute-phase proteins.

Macrophages (see figure, which shows a 30 μm × 25 μm scanning electron micrograph of a macrophage) normally reside in tissues and beneath mucosal surfaces, but they can also infiltrate infected tissue in large numbers and migrate to central sites, such as lymph nodes, to interact with other cells. In contrast to many other cell lineages, unstimulated macrophages constitutively express unique receptor repertoires to detect bacteria rapidly and trigger cell signalling, and inflammatory stimuli such as cytokines can further enhance these responses. Macrophages also express phagocytic receptors that bind to sugars or lipids on bacterial surfaces or to microbes that are coated with serum complement proteins or antibodies. When macrophages make contact with bacteria several signal-transduction pathways are activated, including tyrosine kinase (interferon-γ signalling), serine kinase (mitogen-activated protein kinase signalling), small GTPase and lipid signalling pathways. Signal transduction facilitates the cytoskeletal rearrangements and membrane trafficking events that are responsible for the phagocytosis and trafficking of bacteria to the degradative phagolysosome.

Signalling coordinates macrophage killing mechanisms by the activation and recruitment of antibacterial effectors to the phagolysosome. Two enzymes that are crucial for the macrophage's microbicidal activity are the phagocyte NADPH oxidase and inducible nitric oxide synthase. These enzymes catalyse the OXIDATIVE BURST: synthesis of antibacterial reactive oxygen and nitrogen intermediates that include superoxide, nitric oxide and peroxynitrite. Macrophages destroy phagocytosed bacteria by the concerted effects of the oxidative burst, acidification, nutrient starvation, lysosomal enzymes and antimicrobial peptides. Bacterial antigens from degraded bacteria can be bound to major histocompatibility complex molecules and presented on the cell surface to alert other immune cells to the identity of the invader. Signalling also culminates in the activation of transcription factors such as nuclear factor κB, activator protein-1, and signal transducer and activator of transcription proteins, which leads to the expression of cytokine genes. Cytokines and chemokines that are secreted by macrophages recruit cells to the site of infection and coordinate their responses to clear the microbe.

the soluble cytokine colony-stimulating factor 1 (CSF-1) can alter signalling downstream of TLRs to enhance responses to certain bacterial products, while downregulating other responses[15], and that different TLR agonists activate distinct signalling networks[16–18].

The best characterized member of the TLR family, TLR4, traffics with its ligand, LIPOPOLYSACCHARIDE (LPS), between the cell surface and the intracellular Golgi compartment. Whereas many TLRs are expressed on the macrophage surface, TLR9, which facilitates the response to bacterial DNA, associates with endosomes[19], and TLR2, which mediates the response to various surface molecules of GRAM-POSITIVE BACTERIA, has been observed trafficking to phagosomal membranes[20]. So it has been suggested that TLRs could sample both the extracellular space and phagosomal compartments and then tailor a response that is most effective for clearing the particular type of infection that is encountered[20]. Their ability to recognize a repertoire of microbial patterns, their presence on cell-surface and phagosomal membranes, and their robust and tunable signalling makes TLRs an excellent system for pathogen recognition.

Changing the target. To avoid detection by macrophages, some bacteria modify their surface by camouflaging or directly modifying the molecules that trigger TLR signalling. Many Gram-negative bacteria can alter their LPS structure during infection, to impair recognition or to protect themselves from host-derived antibacterial peptides that can damage bacterial membranes[21].

Pseudomonas aeruginosa causes a chronic infection in the lungs of cystic fibrosis patients. It alters its LPS structure during disease, which is then recognized differently by human TLR4. During the initial stages of host–pathogen interaction, the bacteria express LPS that has a penta-acylated lipid A structure — this triggers the release by macrophages of 100-fold less tumour necrosis factor-α (TNF-α) and interleukin 8 (IL-8) when compared with hexa-acylated LPS. Inhibiting the release of these cytokines, which are essential for recruiting cells to the site of infection and activating their antimicrobial activities, could facilitate the initial colonization of the lung. Later in infection, after *P. aeruginosa* has successfully colonized the lung, the bacteria express a hexa-acylated LPS that triggers a stronger proinflammatory response when recognized by macrophage TLR4. This modification of LPS structure could explain how *P. aeruginosa* avoids macrophage responses while it is establishing infection but triggers the lung pathology that is observed in patients at later stages of infection[22].

OXIDATIVE BURST
This consists of antibacterial reactive oxygen intermediates (ROIs), such as superoxide and hydroxyl radicals, that are produced by the phagocyte NADPH oxidase.

LIPOPOLYSACCHARIDE
(LPS). A component of the outer membrane of Gram-negative bacteria that is made of a lipid, a core oligosaccharide and an *O*-linked sugar side chain.

GRAM-POSITIVE BACTERIA
The cell walls of these bacteria retain a basic blue dye during the Gram-stain procedure. These cell walls are relatively thick (15–80 nm across) and consist of a network of peptidoglycans.

Crossing the wires. Pattern-recognition receptors recognize evolutionarily conserved microbial molecules that comprise essential structures. As such, there is a restricted number of changes that can be made to these microbial molecules to avoid recognition before the changes become detrimental to bacterial survival.

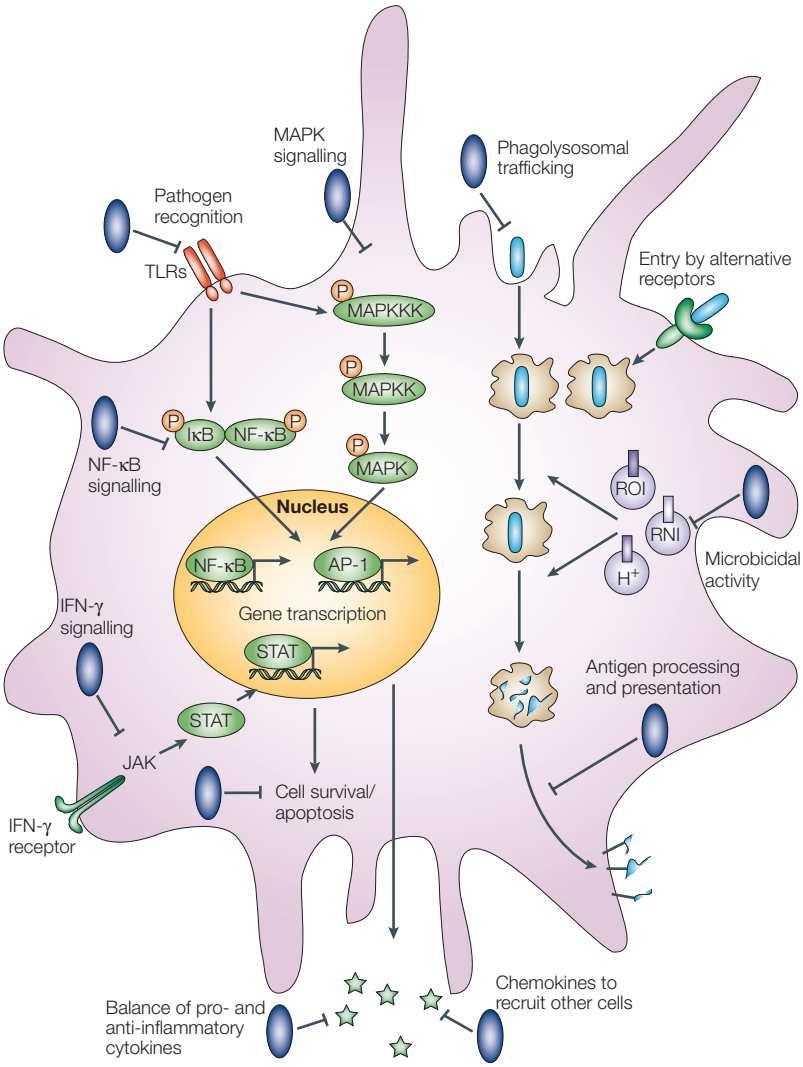


Figure 1 | **Bacterial pathogens subvert macrophage signalling and effector mechanisms.** Macrophages are both sentinels and the first line of defence against infection, and bacterial pathogens have numerous mechanisms for subverting macrophage functions, as illustrated in dark blue. Microbes can interfere with receptor-mediated recognition, phagocytosis and trafficking of bacteria to the degradative lysosome. Bacteria that enter macrophages can avoid destruction by perturbing the signalling that is required for the production of reactive oxygen intermediates (ROI), reactive nitrogen intermediates (RNI) and acidification (H^+). By interfering with antigen processing or presentation, pathogens can prevent macrophages from alerting other cells of the immune system to the identity of the infectious agent. Bacterial pathogens have several mechanisms for interfering with kinase and lipid signalling within infected macrophages. Perturbation of macrophage signalling can alter cell survival and the transcription and secretion of soluble cytokines to recruit cells to the site of infection and coordinate their responses to clear the microbe. Further details on individual signalling pathways are provided in FIGS 3–6 and the enzymatic activities of bacterial virulence proteins used to subvert macrophage functions can be found in TABLE 1. AP-1, activator protein-1; IFN, interferon; IκB, inhibitor of NF-κB; JAK, Janus kinase; MAPK, mitogen-activated protein kinase; MAPKK, MAPK kinase; MAPKKK, MAPKK kinase; NF-κB, nuclear factor κB; STAT, signal transducer and activator of transcription; TLR, Toll-like receptor.

Therefore, a common strategy of pathogens is to allow recognition, but to interfere with downstream TLR-mediated signalling or to express TLR agonists. One interesting example is a virulence protein that is known as LcrV, which is produced by *Yersinia pestis*. LcrV interacts with TLR2 to modify macrophage cytokine production by increasing the secretion of IL-10, which is a cytokine that attenuates the inflammatory response and increases bacterial survival. Ironically, bacterial interference with TLR2 signalling makes TLR-based recognition a detriment to the host response to *Yersinia*, as mice expressing TLR2 are more susceptible to *Yersinia* infection[23,24]. Pathogens can also use other receptors to interfere with TLR signalling. The lectin dendritic-cell-specific intercellular adhesion molecule 3 (ICAM3)-grabbing nonintegrin (DC-SIGN) is expressed on dendritic cells and some macrophages, and is an important receptor for the binding and internalization of *Mycobacteria* by dendritic cells. The binding of *Mycobacterium bovis* bacillus Calmette–Guerin (BCG) to DC-SIGN interferes with normal TLR-mediated signalling and prevents dendritic-cell maturation, leading to IL-10 release and immunosuppression[25].

Sneaking in the back door. Some bacteria minimize their detection by pattern-recognition receptors on the cell surface by entering the cytosol of the host cell. Bacteria such as *Shigella* sp. and *Listeria* sp. express proteins that facilitate their invasion of macrophages and escape from the membrane-bound compartment[26], thereby potentially minimizing the amount of time in which they might activate TLR-based signalling from the cell surface and vacuolar compartment. Not to be foiled, though, macrophages have recently been shown to have a cytosolic surveillance system that recognizes an unidentified *Listeria* structure — this causes p38 mitogen-activated protein kinase (MAPK) signalling and the subsequent transcription of interferon-β (IFN-β) and IL-8 (FIG. 3; REF. 27). Epithelial cells have a family of cytosolic proteins, one of which, Nod1 (nucleotide-binding oligomerization domain), has been shown to mediate the response to the bacterial component muramyl dipeptide[28,29]. The identity of the macrophage cytosolic receptors that mediate the signalling that is triggered by cytosolic bacteria remains to be determined. The ability of macrophages to discriminate between foreign molecules and to assess their cellular location theoretically allows these cells to activate the signalling pathways that are best suited to facilitating the clearance of each type of pathogen in its particular niche. Perhaps the ability of some bacterial pathogens to survive and replicate in the macrophage cytosol is determined by their ability to avoid cytosolic recognition or microbicidal mechanisms.

Perturbation of phagocytosis

The collection of receptors that is expressed on a macrophage's surface facilitates the binding and uptake of microbes, in addition to recognizing their foreign nature. As described in BOX 1, microbes might be internalized after binding these receptors by a process known

Box 2 | Macrophages and adaptive responses to bacterial infection

For bacterial pathogens that breach the pre-existing innate mechanisms that are described in BOX 1, an adaptive or acquired immune response is necessary for clearance of the infection. This later phase of the immune response is mediated by T and B cells and offers several advantages over innate immunity: remarkable specificity and immunological memory. Macrophage recognition of bacterial pathogens controls adaptive immune responses[103]. Macrophages shape these later responses through cytokine production and antigen presentation, to activate cytotoxic $CD8^+$ T cells to kill infected cells or $CD4^+$ T cells to interact with B cells to facilitate antibody production. Dendritic cells also have a fundamental role in initiating adaptive immune responses to bacterial pathogens. Both dendritic cells and macrophages can identify bacteria using pattern-recognition receptors, phagocytose bacteria and process antigen for presentation, but dendritic cells have the added ability to migrate more readily to lymph nodes and activate naive T cells. Bacterial subversion or evasion of dendritic cells can interfere with T-cell activation or skew cytokine production to undermine the adaptive immune response and prevent clearance of the microbe (for a review, see REF. 5). For example, recognition by dendritic cells of bacterial components triggers interleukin-6 cytokine release that modulates the activity of regulatory T cells, highlighting the interconnection of innate and adaptive immune responses to infection[104].

Adding to the complexity of host–pathogen interactions, macrophages are not a homogeneous population of cells. Macrophage location and activation state can markedly influence their interactions with microbes, and there is heterogeneity between and within different types of macrophages[3,105,106]. Therefore the macrophage phenotype can influence data interpretation and comparisons between experiments. Technical advances in strategies for depleting macrophages *in vivo* using LIPOSOMES[107] and live-cell imaging of cell interactions in tissues[108,109] will surely provide insights into how macrophages respond to bacterial infection in tissues.

LIPOSOME
A lipid-based delivery system that is used to deliver DNA or protein into cells.

PHAGOCYTOSIS
An actin-dependent process, by which cells engulf external particulate material by extension and fusion of pseudopods.

GTPASE-ACTIVATING PROTEINS
Proteins that inactivate small GTP-binding proteins, such as Ras family members, by increasing their rate of GTP hydrolysis.

COMPLEMENT
Nine interacting serum proteins (C1–C9), mostly enzymes, that are activated in a coordinated way and participate in bacterial lysis and macrophage chemotaxis.

FIMH TYPE 1 PILUS
An attachment organelle that extends from the bacterial surface and contains an adhesin that is encoded by the *FimH* gene.

LYSOSOME
A membrane-bounded organelle with a low internal pH (4–5) that contains hydrolytic enzymes and that is the site of degradation of proteins in both the biosynthetic and the endocytic pathways.

as PHAGOCYTOSIS — a key mechanism that is used by macrophages to control bacterial infection (for reviews, see REFS 30,31). In response to receptor binding, signalling facilitates cytoskeletal rearrangements and the phagocytic internalization of foreign objects that are destined for phagolysosomal degradation. Similar to the examples that were discussed in the previous section, some bacteria can perturb phagocytosis by altering their recognition. Other bacterial pathogens either encourage or discourage their uptake into the cell (discussed below) by interacting with different receptors or by interfering with downstream signal transduction. These various strategies are reviewed in REF. 32 and illustrated schematically in FIG. 4.

Avoiding uptake. For some bacteria, it is advantageous to remain extracellular to avoid being killed within the macrophage. Minimizing bacteria–macrophage interactions could also impair the macrophage signalling that is required to activate an adaptive immune response, which would make the extracellular niche more hostile. Several recent reviews discuss how bacterial pathogens such as *Yersinia* sp., *P. aeruginosa* and enteropathogenic *Escherichia coli* deliver proteins that interfere with signalling downstream of their recognition by phagocytic receptors and prevent bacterial uptake directly into host cells[1,33,34]. *Yersinia* sp. has the best characterized ability to interfere with phagocytosis. This interference is mediated by a set of proteins that is delivered into macrophages and interrupts the signalling required for phagocytosis. The *Yersinia* virulence proteins have an array of enzymatic activities to achieve this goal: YopH is a protein tyrosine phosphatase that targets cytoskeletal proteins such as Cas and Fyb in macrophages; YopE is a GTPASE-ACTIVATING PROTEIN that inactivates the small GTPases RhoA, Rac and Cdc42 to prevent the actin polymerization that is required for phagocytosis; and YopT is a papain-like cysteine protease that cleaves the lipid moiety of small G proteins such as RhoA to depolymerize actin filaments[35,36] (for a review, see REF. 37). Enteropathogenic *E. coli* targets a different signalling pathway by secreting an unidentified bacterial protein into macrophages to inhibit the activity of phosphatidylinositol 3-kinase (PI3K)[38]. Pathogens that subvert macrophage phagocytic signalling to remain outside the cell avoid phagolysosomal degradation, but they must have mechanisms to contend with extracellular defences, such as killing by COMPLEMENT or antimicrobial peptides[39].

Promoting uptake. Various bacteria, including *Salmonella* sp., *Mycobacterium* sp. and some *E. coli*, either actively or passively promote their uptake into macrophages. As shown in FIG. 4, pathogenic *E. coli* that adhere to host cells with a FIMH TYPE 1 PILUS avoid phagolysosomal trafficking by entering cells through lipid rafts — lipid microdomains on the cell surface that are targeted by various bacterial, parasitic and viral pathogens (for reviews, see REFS 40–42). This strain of *E. coli* uses the pilus protein FimH to bind to the macrophage receptor CD48, which is located in lipid rafts, and is internalized by the host cell after receptor binding. By entering macrophages through lipid rafts, $FimH^+$ *E. coli* persist inside membrane-bound compartments that are protected from the antimicrobial OXIDATIVE BURST and acidification[43]. *Bordetella pertussis* expresses various proteins that promote entry into macrophages using the host CR3 complement receptor as opposed to Fc receptors. CR3-mediated entry avoids triggering the oxidative burst and increases bacterial survival[44], because an essential subunit of the NADPH oxidase, Rac1, is not recruited to the membrane that surrounds bacteria in complement receptor-mediated phagosomes[45]. As discussed below, bacterial pathogens that trigger their own uptake through phagocytic receptors must have other strategies to protect themselves from the ensuing onslaught of antimicrobial components that are in the endosomal pathway.

Bacteria alter trafficking to phagolysosomes

Bacterial pathogens can survive in a remarkably diverse set of compartments within macrophages (for a review, see REF. 46; FIG. 4). Bacterial pathogens that reside intracellularly and alter macrophage signalling to divert themselves from fatal trafficking to the LYSOSOME benefit from protection against humoral and cell-mediated immune responses. As has been reviewed extensively, various intracellular bacterial pathogens reside in niches that resemble an assortment of organelles: recycling early endosomes (*Mycobacterium* sp.), late endosomes (*S. typhimurium*), lysosomes (*Coxiella burnetii*) and rough endoplasmic reticulum (*Legionella pneumophila*)[47–49].

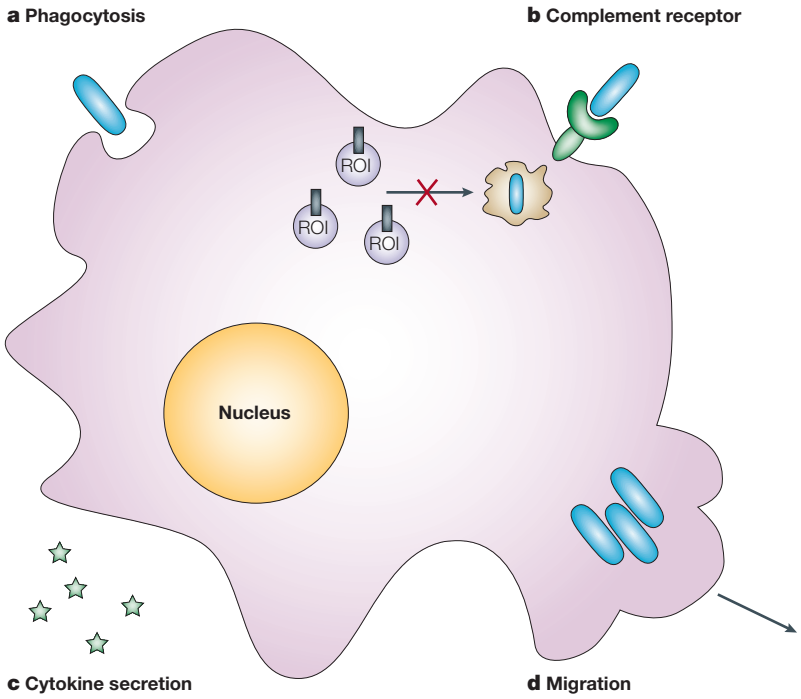


Figure 2 | **Bacterial pathogens can benefit from targeting macrophages.** Many bacterial pathogens have evolved mechanisms that capitalize on normal macrophage functions to establish or maintain infection. **a** | Securing an intracellular niche through phagocytosis protects bacteria from many other components of the immune response. **b** | Entering the cell by engaging complement receptors avoids the harmful oxidative burst of reactive oxygen intermediates (ROIs) that are produced by the NADPH oxidase. **c** | Altering the balance of cytokines that are secreted can perturb the recruitment and activation of other host cells and increase tissue damage and the spread of infection. **d** | Macrophages can function as a vehicle for systemic spread.

Several bacterial components that are responsible for altering phagosomal maturation have been identified, although the precise mechanism of action and cellular targets of most remain to be determined. Some of these strategies are outlined in FIG. 4. For example, *L. pneumophila* expresses the Dot secretion system to inject proteins into the macrophage cytosol[50] to intercept early secretory vesicles from the endoplasmic reticulum and create a replicative organelle that resembles rough endoplasmic reticulum[51]. In macrophages that have been infected by *S. typhimurium*, there seems to be a balance between the activities of the bacterial type III effector proteins SifA and SseJ in regulating the acquisition of lipids to, and the stability of, the membrane surrounding the bacteria[52].

Although bacteria can alter their location within macrophages to avoid destruction in the phagolysosome, they also alter the localization of host antimicrobial proteins. For example, *Mycobacteria* phagosomes recruit early phagosomal proteins such as coronin-1 (REF.53) but avoid acidification as the bacteria specifically exclude the vesicular PROTON ATPASE from the phagosomal membrane[54,55]. *M. tuberculosis* arrests phagosomal maturation using mannose-lipoarabinomannan (ManLAM) — a surface lipid — by disrupting early endosome autoantigen (EEA1) and Vps34 (PI3K) activities, which regulate endosomal trafficking events downstream of the small GTPase Rab5 (REF. 56). In *Salmonella*, secreted bacterial protein effectors prevent the trafficking of antimicrobial effectors such as NADPH oxidase and inducible nitric oxide synthase (iNOS) to the *Salmonella*-containing vacuole, although the mechanism of action remains undiscovered[57–59]. Not only does this protect *Salmonella* from direct damage by reactive oxygen and nitrogen species, but it also could potentially alter oxidant signalling in infected macrophages[60]. Using a different approach to achieve protection from reactive intermediates, *Helicobacter pylori* produces arginase to degrade the iNOS substrate L-arginine and thereby downregulates the macrophage-mediated production of nitric oxide[61].

Altering phagosome trafficking increases the ability of many bacteria to survive and replicate, perhaps in part by allowing pathogens to avoid triggering the signalling that is normally activated by the ingestion of bacteria. For example, modified vacuolar compartments often limit the accessibility of bacteria to antigen processing/presentation machinery, which effectively hides the bacteria from adaptive immune responses. Altered endosomal trafficking possibly limits bacterial interactions with compartments that are accessible to TLRs, and could therefore result in the evasion of recognition and subsequent TLR-derived signalling. However, it remains to be determined whether TLRs can still traffic to the modified vacuolar compartments in which the intracellular pathogens live.

Disruption of NF-κB signalling

In addition to perturbing the targeting of macrophage effectors, altering the location of signalling molecules to impair their function is a successful strategy for surviving within macrophages.

Dampening the signal. NF-κB signalling relies on the targeting of IκB (inhibitor of NF-κB) subunits to the proteasome to allow NF-κB to translocate from the cytosol to the nucleus where it activates gene transcription (FIG. 5). *Yersinia enterocolitica* binds to IκB kinase-β (IKKβ) to prevent the phosphorylation of IκB, which is essential for its degradation, thereby trapping NF-κB in the cytosol away from its gene targets[6,62,63]. *Mycobacterium ulcerans* inhibits nuclear translocation of NF-κB independently of IκB, possibly by altering the phosphorylation of NF-κB or interfering with its DNA-binding ability[64]. Inhibition of NF-κB signalling leads to the decreased release of proinflammatory cytokines, such as TNF-α, and increased apoptosis, both of which can shield bacteria from the immune response.

Amplifying the proinflammatory signal. Pathogens that are equipped to survive within macrophages often use an opposing strategy to that discussed above — they actively increase NF-κB activity in addition to allowing TLR-based NF-κB signalling to proceed, to exacerbate the proinflammatory response and recruit more potential host cells to the site of infection. This can

PROTON ATPASE
A membrane protein that mediates proton influx and acidification of the phagolysosome.

then presumably be used to spread the bacteria. Listeriolysin O and InlB, which are two virulence proteins that are produced by *Listeria monocytogenes*, activate NF-κB, the latter in a PI3K-dependent manner, and it has been suggested that this increased inflammatory response promotes pathogen spread by recruiting more monocyte vehicles to the site of infection[65,66]. Activation of NF-κB also induces anti-apoptotic signalling within macrophages, which prolongs cell survival — a potential advantage to bacteria that successfully survive and replicate inside macrophages.

Disruption of MAPK signalling

Pathogens can exert the same effects on NF-κB activation by targeting signalling components that regulate NF-κB activity, such as MAPKs. As MAPK signalling is crucial for many responses to infection (for a review, see REF. 67), it also presents a strategic target for bacterial subversion tactics (FIG. 6). The kinetics of kinase signalling can also influence a macrophage's phenotype. For example, the duration of signalling through one of the MAPK pathways, Raf–MEK–ERK/MAPK (where ERK is extracellular signal-regulated kinase, and MEK is MAPK and ERK kinase), determines whether a macrophage proliferates or activates in response to a stimulus[68]. Indeed, Raf–MEK–ERK/MAPK signalling that is sustained for several hours after infection impairs *Salmonella* replication within macrophages independently of early kinase signalling[69]. Therefore, bacteria could alter a macrophage's antibacterial phenotype through the kinase pathway that is targeted, as well as the timing of the activation or inhibition.

Cleaving the messenger. Anthrax toxin, which is produced by *Bacillus anthracis*, interrupts several MAPK signalling pathways by proteolytically degrading all MAPK kinases (MAPKKs) except MAPKK5. The tripartite anthrax toxin, which comprises three subunits (protective antigen, lethal factor and oedema factor), catalyses its entry into macrophages through lipid rafts. Binding of the protective-antigen subunit to the cell-surface anthrax-toxin receptor clusters the receptor, and this induces its association with lipid rafts, which catalyses efficient internalization of the anthrax toxin by endocytosis. Endosomal acidification triggers protective antigen to form a channel in the membrane, which delivers lethal factor, a METALLOPROTEINASE, to the cytosol where it inactivates MEK1 by cleaving between its amino terminus and catalytic domain. Chemical interference with lipid-raft integrity prevents toxin uptake and MEK1 cleavage[70]. Cleavage of the MAPKK that activates p38 MAPK, which is mediated by lethal factor, induces macrophage apoptosis, possibly by interfering with the p38-dependent expression of NF-κB target genes that are necessary for cell survival[71].

Blocking post-translational modifications. *Yersinia* sp. use an alternative mechanism to disrupt MAPK signalling and the downstream activation of NF-κB in macrophages, which impairs synthesis of the

METALLOPROTEINASE
A proteinase that has a metal ion at its active site.

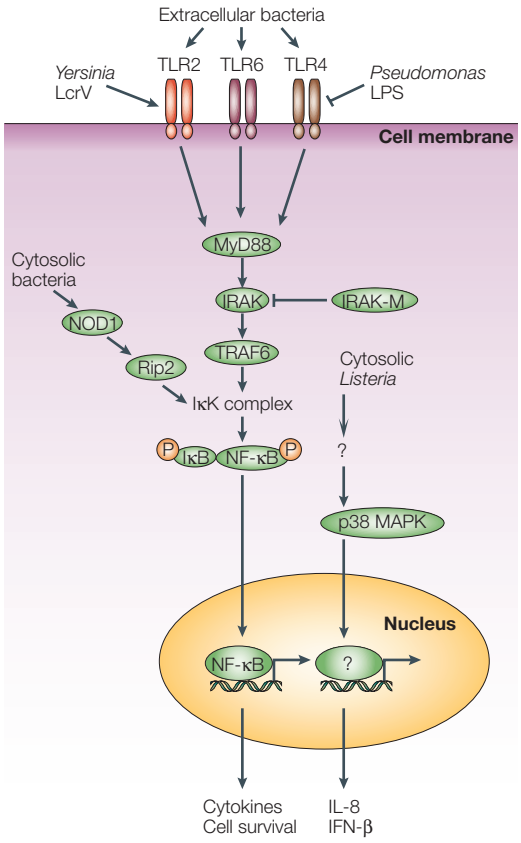


Figure 3 | **Macrophage surveillance systems to detect bacteria.** Toll-like receptors (TLRs) are pattern-recognition receptors that recognize bacterial structures such as the surface structures lipopolysaccharide (LPS), lipoteichoic acid, mycobacterial lipoproteins and lipoarabinomannan, and bacterial DNA-containing methylated CpG motifs. TLRs can also interact with each other to increase the repertoire of recognized ligands. The diverse microbial ligands trigger common downstream signal-transduction pathways, which are similar to those that are induced by interleukin (IL)-1-receptor signalling, culminating in various antimicrobial responses such as the activation of nuclear factor κB (NF-κB) and transcription of proinflammatory cytokine genes. IL-1 receptor-associated kinase-M (IRAK-M) functions as a control mechanism to downregulate TLR4-mediated macrophage responses to LPS by inducing tolerance. As described in the text, bacteria such as *Pseudomonas aeruginosa* decrease TLR4-mediated signalling whereas *Yersinia pestis* produces the virulence protein LcrV that increases TLR2-mediated signalling and secretion of the anti-inflammatory cytokine IL-10. Bacteria that escape from phagosomes can be detected by a cytosolic surveillance system. Cytosolic *Listeria* initiates p38 mitogen-activated protein kinase (MAPK) signalling and subsequent transcription of interferon-β (IFN-β) and IL-8, independently of TLRs. Although it has not been reported in macrophages, epithelial cells have a family of proteins, one of which, nucleotide-binding oligomerization domain (NOD1), mediates a response to cytosolic bacterial muramyl dipeptide. This activates the serine/threonine kinase receptor-interacting protein 2 (Rip2; also known as RICK or CARDIAK), which, through activation of the inhibitor of NF-κB (IκB) kinase complex (IκK), activates NF-κB-mediated transcription. MyD88, Myeloid differentiation primary response protein 88; TRAF6, tumour necrosis factor receptor-associated factor 6.

proinflammatory cytokine TNF-α. *Yersinia pseudotuberculosis* delivers the bacterially encoded cysteine protease YopJ protein into infected macrophages[63]. Experiments in fibroblasts showed that YopJ binds specifically to MAPKKs and inhibits kinase activity by preventing phosphorylation[72]. YopJ also interferes with the post-translational modification of proteins that are involved in MAPK signalling, by acting on the ubiquitin-like protein SUMO-1 (for small ubiquitin-related modifier), inhibiting its conjugation to target proteins and thereby interrupting normal MAPK signalling[63]. Therefore, pathogens produce proteins with varying enzymatic activities that commonly target macrophage kinases to inhibit NF-κB activation, cytokine and chemokine release, or macrophage viability.

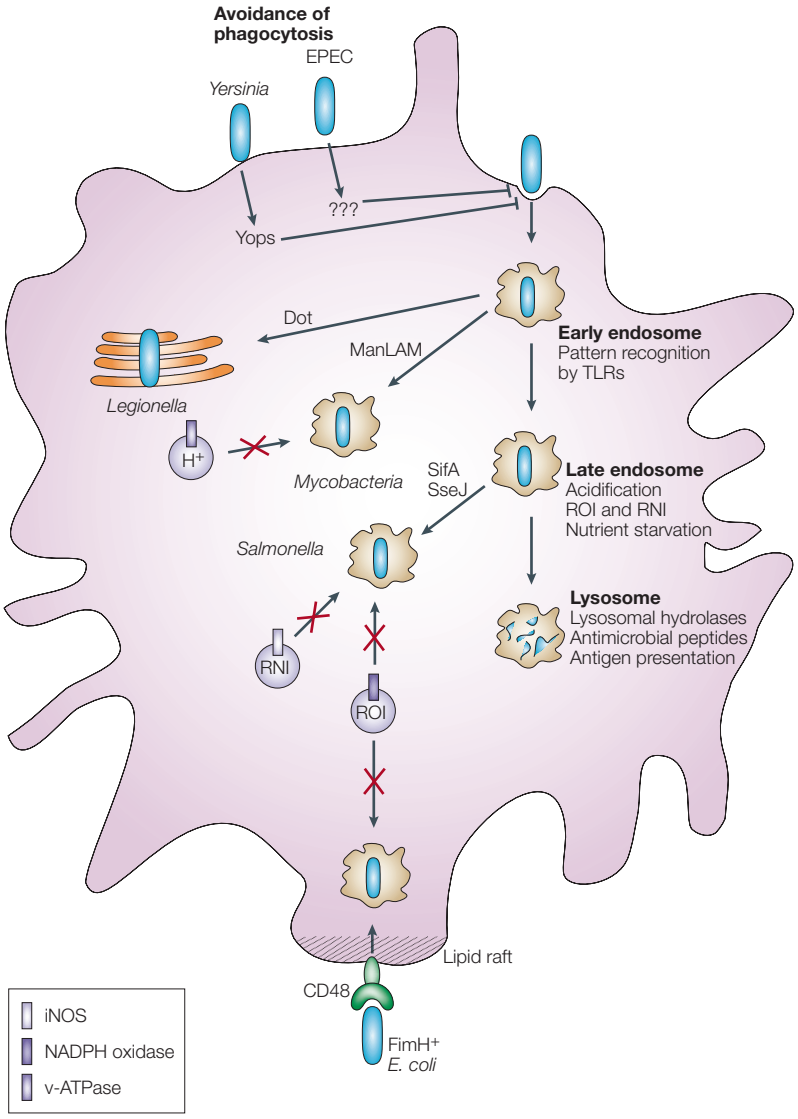


Figure 4 | **Pathogen avoidance of phagolysosomal degradation.** Signalling that is triggered by receptor binding facilitates cytoskeletal rearrangements and phagocytic internalization of foreign objects. Phagocytosed bacteria traffic through a series of increasingly hostile compartments before being fully degraded in the lysosome. Pathogens, as well as cytokines that are produced during infection, can upregulate a macrophage's antibacterial phenotype by initiating signalling to regulate phagosome trafficking through the endosomal compartment, starve the bacteria by limiting nutrients and cations within the phagosome, and enhance enzymatic degradation of the bacteria and antigen presentation. *Mycobacterium tuberculosis* blocks acidification and uses mannose-lipoarabinomannan (ManLAM) to prevent interactions with other endosomal compartments. *Salmonella* resides in an acidified compartment that resembles late endosomes but blocks the acquisition of NADPH oxidase, inducible nitric oxide synthase (iNOS) and degradative lysosomal enzymes, and uses the bacterial type III effector proteins SifA and SseJ to modify the vacuolar membrane composition. FimH$^+$ *Escherichia coli* engages alternative receptors to enter macrophages using lipid rafts and avoids the oxidative burst. *Legionella pneumophila* secretes proteins through the Dot secretion system to establish a replicative organelle resembling rough endoplasmic reticulum. *Yersinia* and enteropathogenic *E. coli* (EPEC) express virulence proteins (such as Yops) to inhibit phagocytosis altogether. CD48, human leukocyte antigen/macrophage receptor CD48; FimH, pilus adhesion subunit; TLR, Toll-like receptor.

Disruption of interferon signalling

In addition to the serine kinase signalling discussed above, macrophages have a robust tyrosine kinase signalling network. The best characterized is the Janus kinase (JAK)–signal transducer and activator of transcription (STAT) signal-transduction pathway that originates from binding of IFNs to their receptors on the cell surface and leads to transcriptional responses (for reviews, see REFS 73,74). IFN-γ exerts pleiotropic effects on macrophages to augment their ability to control bacterial pathogens[75]. First, IFN-γ signalling activates various enzymes within the macrophage that increase the production of damaging reactive oxygen and nitrogen species, starve the bacteria of tryptophan within the phagolysosome and increase lysosomal degradation of the bacteria. Second, IFN-γ enhances the ability of macrophages to coordinate antibacterial immunity, by augmenting MAJOR HISTOCOMPATIBILITY COMPLEX (MHC) class I and II antigen presentation and synthesis of cytokines such as IL-12 and TNF-α[76,77]. This IFN-γ signalling network allows macrophages to be primed to respond more rapidly and robustly to bacterial infection, which produces macrophages with an 'activated' phenotype[78]. *In vitro*, IFN-γ-activated macrophages control the intracellular replication of *S. typhimurium, Chlamydia psittaci* and *L. pneumophila*, and are essential for the clearance of various bacterial pathogens in both mice and humans[69,79,80]. As such, the ability to interfere with IFN-γ signalling within macrophages can determine the success of a pathogen.

De-activating the macrophage. Bacterial impairment of IFN-γ signalling is best characterized in macrophages that have been infected by *Mycobacteria* species. *Mycobacterium avium* infection causes decreased transcription of the IFN-γ receptor α- and β-chains and their decreased expression on the cell surface through an uncharacterized mechanism, leading to impaired downstream STAT activation[81]. *M. tuberculosis* uses an uncharacterized mycobacterial surface component to affect a later step in IFN-γ signalling. Although STAT phosphorylation, dimerization, nuclear translocation and DNA binding is intact in macrophages that are infected by *M. tuberculosis*, there is a decrease in the association of STAT1 with the transcriptional co-activators CREB and p300. This causes impaired transcription of IFN-γ-responsive genes such as the gene encoding the FcγRI antibody receptor that is involved in phagocytosis[82].

Interference modulates inflammatory responses

An important downstream response of normal macrophage signalling is the production of cytokines. These cause inflammation, recruit other cells to the site of infection and link innate and adaptive immune responses (BOXES 1,2). Macrophages need to control signalling that leads to inflammatory responses tightly, as shown, for example, by the damage that is caused by the dysregulated inflammation observed during endotoxic septic shock. One level of control is to regulate the intensity and duration of signalling, which often originates from TLRs. It has recently been discovered that the macrophage protein interleukin-1 receptor-associated kinase-M (IRAK-M) has a pivotal role in downregulating macrophage responses to LPS by inducing tolerance. Without IRAK-M, *Salmonella* infection causes increased tissue damage[83]. Another level of control is the balance between proinflammatory cytokines, such as TNF-α and IL-12, and predominantly anti-inflammatory cytokines, such as IL-10 and transforming growth factor-β (TGF-β), which are produced during infection. Bacterial pathogens target signalling that leads to the expression of cytokine genes or their post-translational modifications that perturb the balance of cytokines to their advantage (for a review, see REF. 84). Macrophages and bacteria can therefore both control the size and shape of the immune response through cytokine production.

Capitalizing on inflammation. Some pathogens capitalize on the destructive consequences of an overexuberant inflammatory response on the host. In macrophages that have been infected with *Shigella flexneri* or *S. typhimurium*, intracellular stores of IL-1β and IL-18 are proteolytically cleaved into biologically active forms by the cysteine protease caspase-1 (IL-1β-converting enzyme) and released. Studies using caspase-1-deficient mice show that the release of these cytokines is essential for initiating the inflammation that is required for bacterial clearance. *S. flexneri* and *S. typhimurium* each produce a virulence protein, IpaB and SipB, respectively, that is delivered into the macrophage and directly binds and activates caspase-1. This amplifies the release of these proinflammatory cytokines, resulting in local tissue damage and enhanced recruitment of potential host cells to the site of infection[85]. In addition, the activation of caspase-1 by these bacterial proteins triggers rapid apoptosis of the infected macrophages. Therefore, although signalling that leads to release of the proinflammatory cytokines IL-1β and IL-18 is essential to initiate inflammation to clear the bacteria, it is co-opted by bacterial virulence proteins to promote macrophage apoptosis and systemic pathogen spread[86–88]. Host-cell death would be a disadvantage for bacteria that benefit from residing in an intracellular niche, so pathogens that induce macrophage apoptosis probably have mechanisms for escaping apoptotic cells or surviving in other cell types.

Avoiding inflammation. Other pathogens maintain infection by avoiding or attenuating the inflammatory response that is orchestrated by macrophages. The mechanism of entry into a macrophage can influence the types of cytokines that are produced, as well as their trafficking within the cell. For example, pathogens that promote entry through the CR3 complement receptor directly impair IL-12 production (for a review, see REF. 89). Other pathogens skew the cytokine milieu to their advantage by activating IL-10 release to minimize inflammation. Bacteria such as *M. tuberculosis*, *L. pneumophilia*, *S. typhimurium* and *Y. pestis* induce IL-10 production, and this often correlates with virulence. IL-10 can benefit pathogens by delaying macrophage activation, decreasing the amount of reactive oxygen and nitrogen species, downregulating the production of proinflammatory cytokines such as TNF-α and IFN-γ, prolonging the survival of infected macrophages by delaying TNF-α-mediated apoptosis, and lowering the expression of MHC II that is required for antigen presentation (for a review, see REF. 90).

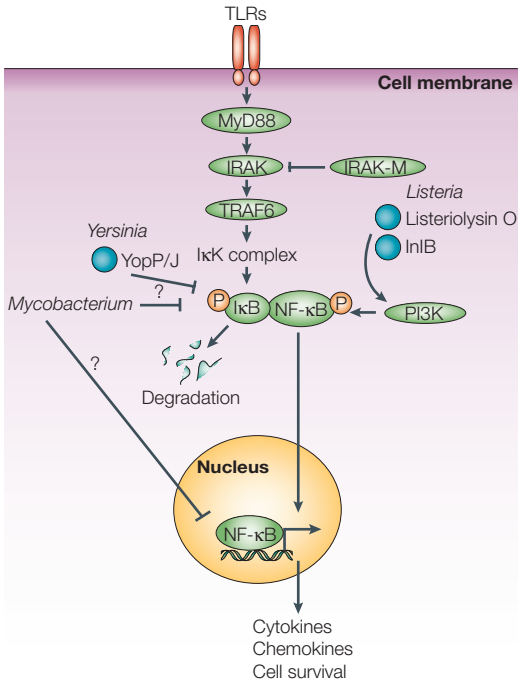


Figure 5 | **Disruption of NF-κB signalling by bacterial pathogens.** In unstimulated macrophages, the transcription factor nuclear factor κB (NF-κB) is sequestered in the cytosol bound in an inactive complex with the inhibitor of NF-κB, IκB. Signalling downstream of Toll-like receptors (TLRs) leads to phosphorylation of IκB by the IκB kinase (IκK) complex, which targets IκB for proteasomal degradation and releases phosphorylated NF-κB. This translocates to the nucleus to initiate transcription. *Yersinia enterocolitica* uses its virulence protein YopP (YopJ in *Yersinia pseudotuberculosis*) to interfere with this nuclear translocation by preventing the phosphorylation of IκB. *Mycobacterium ulcerans* inhibits NF-κB nuclear translocation by a different mechanism, possibly through altering phosphorylation of NF-κB or interfering with its DNA-binding ability. Using the converse strategy, *Listeria monocytogenes* expresses the proteins Listeriolysin O and Internalin B (InlB), which activate NF-κB signalling in a phosphatidylinositol 3-kinase (PI3K)-dependent pathway. IRAK, interleukin-1 receptor-associated kinase; MyD88, Myeloid differentiation primary response protein 88; TRAF6, tumour necrosis factor receptor-associated factor 6.

MAJOR HISTOCOMPATIBILITY COMPLEX
(MHC). The genes encoding the MHC molecules are the most polymorphic in the genome. MHC molecules are a family of surface molecules that present antigenic peptides from foreign microbes to T cells and help the immune system to recognize self from non-self — they are the ones recognized by T cells during transplant rejection.

CREB
(cyclic AMP response-element-binding protein). A transcription factor that functions in glucose homeostasis and growth-factor-dependent cell survival, and has also been implicated in learning and memory.

Undermining communication with other cells

By perturbing signal transduction that is essential for the production of, or response to, cytokines, bacterial pathogens can impair not only the individual macrophages they infect, but also the cells that are regulated by macrophages. In this way, many of the bacterial strategies that are used to interfere with macrophage signalling also function to subvert the immune response as a whole. By altering the balance of cytokines and chemokines that are produced, pathogens can alter the homing signals and receptor expression that are necessary for cells to migrate to the lymph nodes or the site of infection. By inducing apoptosis, bacteria can prevent the macrophage from communicating (using cytokine production or antigen presentation) essential information on the type of response to mount. On the other hand, though, the internalization of apoptotic macrophages by bystander dendritic cells results in the effective presentation of *Salmonella* antigens to T cells[91], so whether macrophage death is of greater advantage to the bacteria or the host is a controversial area[88,92].

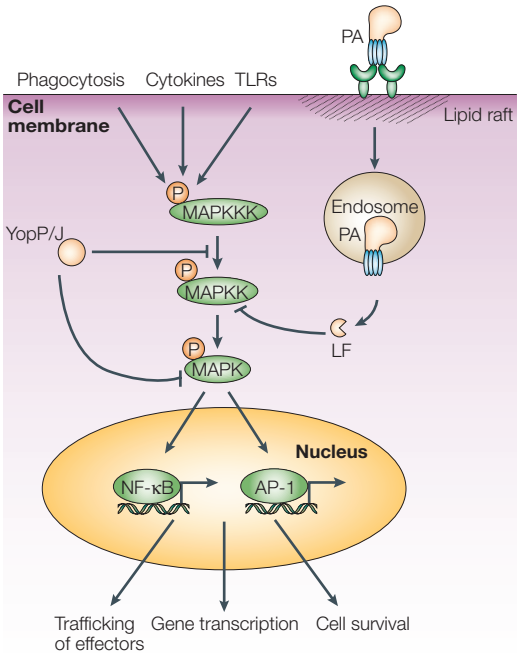


Figure 6 | **Disruption of MAPK signalling.** Several mitogen-activated protein kinase (MAPK) signalling pathways are activated by the presence of pathogens (by Toll-like receptors; TLRs), uptake of bacteria (by phagocytosis), or by infection of neighbouring cells (by cytokines). Kinase signalling can lead to activation of the transcription factors nuclear factor κB (NF-κB) and AP-1. This culminates in the expression of inflammatory cytokine and chemokine genes, as well as the regulation of endosomal trafficking and localization of antimicrobial effectors, such as NADPH oxidase and inducible nitric oxide synthase. *Bacillus anthracis* produces the anthrax toxin subunit protective antigen (PA) to catalyse entry through lipid rafts, and the toxin subunit lethal factor (LF) is released from acidified endosomes to enzymatically cleave MAPK kinases (MAPKKs). *Yersinia pestis* produces the virulence protein YopJ (YopP in *Yersinia enterocolitica*), which blocks both phosphorylation of MAPKKs and post-translational modification of MAPKs. AP-1, activator protein-1; MAPKKK, MAPKK kinase.

By modulating the response to cytokines or phagosomal trafficking, bacteria can alter the expression of, or accessibility to, molecules that are required for antigen presentation (for a review on the diversity of strategies employed, see REF. 93). Bacterial protein and lipid antigens are processed into fragments that are presented on the cell surface by MHC and related molecules, thereby alerting T and B cells to the identity of the invader. *M. tuberculosis* has an abundance of strategies for impairing antigen presentation, which perhaps mediates its persistent infection. The bacterium's disruption of IFN-γ-triggered signalling, which was discussed earlier, reduces the abundance of both the MHC that is available to present antigen as well as the co-stimulatory molecules that must also be present on the cell surface to activate T and B cells. *Mycobacteria* sp. also interfere with normal MHC II endosomal trafficking in macrophages to cause intracellular sequestration of MHC II (REF. 94), increase IL-6 production to suppress T-cell responses[95,96] and alter phagolysosomal trafficking, which potentially limits the interactions of the phagocytosed bacteria with the compartments that contain MHC II (REFS 97,98). *Brucella abortus* allows normal antigen processing and MHC II expression on the surface; however, *Brucella* LPS complexes with MHC to down-regulate the activation of $CD4^+$ helper T cells that are necessary for antibody production[99]. Macrophages infected by *Chlamydia trachomatis* not only show impaired cell-surface expression of MHC[100,101] but also use an alternative strategy by inducing apoptosis of T cells, which provides a possible mechanism of escape from T-cell surveillance for *Chlamydia*-infected cells[102]. By disrupting antigen processing or presentation, pathogens that establish a niche within macrophages can remain hidden and prevent their infected macrophage host from being killed by antigen-specific T cells. As macrophages are at the interface between innate and adaptive immunity, infection of this cell type can have profound effects on the success of the immune response that is launched.

Concluding remarks

This review has discussed how bacterial toxins, virulence proteins and conserved microbial structures can initiate macrophage signalling. Macrophages can use specific receptors and common signalling pathways to integrate this information on pathogen type and location, but this leaves them vulnerable to subversion by bacterial pathogens that can interfere with crucial kinase, trafficking or transcriptional networks. However, there are redundancies in macrophage signalling pathways and the recent discovery of a cytosolic detection system in macrophages is a good example of how avoiding one component of a macrophage's arsenal — in this case phagolysosomal degradation — makes pathogens vulnerable to another. It seems that the combination of mechanisms that a pathogen has to alter specific macrophage signalling cascades dictates their most successful niche.

For a complete understanding of host–pathogen interactions, it is important to avoid a reductionist approach that considers an individual signalling cascade

to be 'good' or 'bad' for host or pathogen survival. For example, blocking NF-κB signalling decreases the inflammatory immune response, but can also lead to host-cell death by blocking NF-κB-mediated pro-survival signalling. Likewise, a host succumbs more rapidly to many bacterial infections if it cannot initiate TLR4-mediated signalling, which leads to NF-κB activation and proinflammatory cytokine production, but also succumbs if it cannot turn this signalling off using IRAK-M. Disease can result from dysregulated macrophage signalling that is driven by the host or pathogen and does not necessarily benefit either player. Genome sequencing projects have identified an overwhelming number of host and bacterial genes that encode proteins with unknown functions. The characterization of the biological functions of these proteins will probably add to the ever-increasing number and diversity of strategies that are used by macrophages to detect and contain the invaders and by bacterial pathogens to subvert and evade host responses.

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