Microbial manipulation of receptor crosstalk in innate immunity

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Abstract | In the arms race of host–microbe co-evolution, successful microbial pathogens have evolved ingenious ways to evade host immune responses. In this Review, we focus on 'crosstalk manipulation' — the microbial strategies that instigate, subvert or disrupt the molecular signalling crosstalk between receptors of the innate immune system. This proactive interference undermines host defences and contributes to microbial adaptive fitness and persistent infections. Understanding how pathogens exploit host receptor crosstalk mechanisms and infiltrate the host signalling network is essential for developing interventions to redirect the host response and achieve protective immunity.

Pattern recognition receptor (PRR). A host receptor that can sense pathogen-associated molecular patterns and initiate signalling cascades that lead to an innate immune response. PRRs can be membrane bound (such as Toll-like receptors) or soluble cytoplasmic receptors (such as NOD-like receptors).

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Front-line defence cells, such as neutrophils, macrophages and dendritic cells (DCs), detect invading pathogens through germline-encoded pattern recognition receptors (PRRs). Soluble and membrane-bound PRRs alert the mammalian immune system through both extracellular and intracellular activation cascades, such as the complement and Toll-like receptor (TLR) pathways, respectively. The aim is to elicit innate antimicrobial and inflammatory responses and initiate adaptive immunity for the control or elimination of infection[1,2]. PRRs recognize relatively invariant microbial structures, often referred to as pathogen-associated molecular patterns (PAMPs), which are shared by related groups of microorganisms[3]. Different PRRs generally recognize distinct PAMPs, a concept that is best illustrated by the diverse ligand specificities of TLRs, which are the prototypical and best characterized PRR family[1,4]. The broad but distinct specificities of the PRRs and their ability to form functional multi-receptor complexes in lipid rafts[5,6] allow for the generation of large combinatorial repertoires. This further diversifies the recognition and signalling capacities of cooperating PRRs and, at least in principle, enables the host to detect almost any type of infection, discriminate between different pathogens and mount a context-relevant immune response.

Sentinel cells receive a variety of 'input messages' from their environment, including those communicated by pathogen-sensing PRRs. The cell needs to appropriately process and integrate this information, which is relayed intracellularly through nonlinear signalling cascades. A systematic analysis of intracellular signalling crosstalk has shown that a large number of pathways converge on a relatively limited set of interaction mechanisms, which include both synergistic and antagonistic interactions[7]. Synergistic pathways greatly increase the sensitivity of detection, in that several individually weak stimuli can combine to elicit a vigorous cellular response. Antagonistic pathways increase the specificity of the host response by restraining it and preventing collateral tissue damage. Signalling crosstalk is therefore important for the normal function of the immune system; it can synergistically activate host defences to clear infections or it can antagonistically dampen unwarranted host responses[2,8–11]. Two characteristic examples are the cooperation of TLR2 with the C-type lectin dectin 1 (also known as CLEC7A) to stimulate antifungal immunity[12] and the homeostatic suppression of TLR-induced pro-inflammatory responses by the glucocorticoid and adenosine receptors[13,14]. Briefly stated, coordinated signalling crosstalk can help to maintain a fine balance between protective immunity and inflammatory pathology.

However, chronic infections and disease can ensue when bacterial, viral or eukaryotic parasitic pathogens successfully evade, neutralize or subvert immune detection, signal transduction or effector killing functions[15–17]. Microbial pathogens that disable host defences preferentially target the innate immune system[16]. In part, this is because the innate defences of the host are the first to be encountered by pathogens. In addition, by subverting innate immunity, pathogens can undermine the overall host defence system, given the instructive role of innate immune mechanisms in the development of the adaptive immune response[3].

One way in which pathogens could undermine host immunity to promote their adaptive fitness is through the manipulation of crosstalk interactions between innate immune receptors. Indeed, despite the physiological

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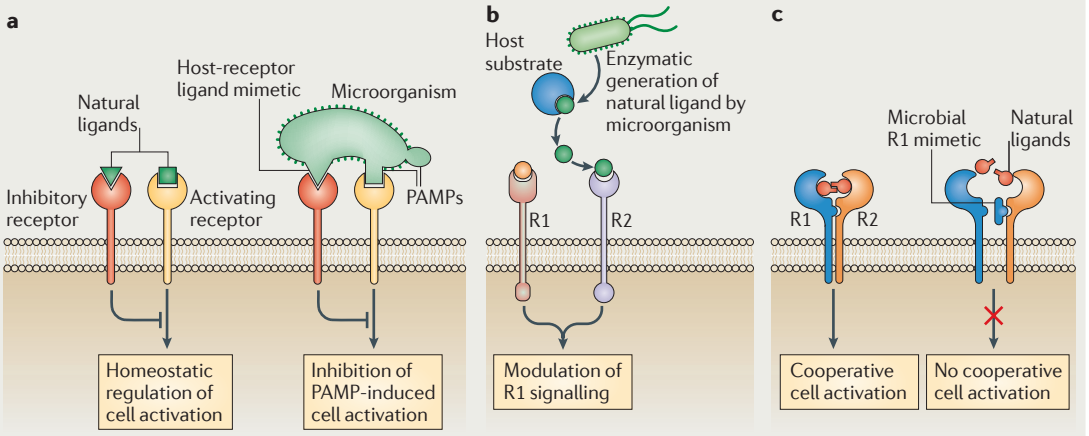
Box 1 | **PRRs and microbial virulence proteins**

To exploit or subvert the functions of pattern recognition receptors (PRRs), pathogens use virulence factors rather than pathogen-associated molecular patterns (PAMPs), which are evolutionarily selected targets of pattern recognition. Unlike PAMPs, virulence proteins are characterized by mutability and lack of conservation, and they can readily respond to environmental changes. Therefore, virulence proteins can contribute to the adaptive fitness of pathogens and are unlikely to have been targeted for pattern recognition in the course of host–microorganism co-evolution[3]. However, the converse notion, that virulence proteins might have evolved to bind and possibly exploit PRRs, is suggested by documented examples of microbial protein interactions with Toll-like receptors (TLRs) and other innate immune receptors[72,116,122,128,129]. A characteristic example involves LcrV, a virulence protein of pathogenic *Yersinia* spp. that induces TLR2-mediated immunosuppression[122]. The TLR2-interacting epitope of LcrV maps to a 19-amino-acid amino-terminal sequence that is divergent from that of *Pseudomonas aeruginosa* PcrV, an LcrV homologue that does not bind TLR2. Moreover, a point mutation in the TLR2-binding epitope of LcrV abrogates its capacity to interact with TLR2 and decreases the virulence of the organism[122]. The plasticity of virulence proteins enables pathogens to manipulate the crosstalk interactions of host receptor signalling through various mechanisms. Molecular mimicry of host ligands (or counter-receptors) by microbial structures can activate inhibitory receptors[24,50] (see the figure, part **a**). Virulence enzymes can convert host molecules into active agonists or ligands to manipulate modulatory receptors[31,74] (see the figure, part **b**). Microbial proteins can function as host-receptor mimetics to block functional interactions of cooperative host receptors[43] (see the figure, part **c**).



Pathogen-associated molecular pattern
(PAMP). A conserved molecular pattern that is found in pathogens but not mammalian cells. Examples include terminally mannosylated and polymannosylated compounds, which bind the mannose receptor, and various microbial products, such as bacterial lipopolysaccharides, hypomethylated DNA, flagellin and double-stranded RNA, which bind Toll-like receptors.

Lipid raft
A membrane microdomain rich in cholesterol, sphingolipids and glycosylphosphatidyl-inositol-anchored proteins. These domains partition receptors for various cellular signalling and trafficking processes.

Inside-out signalling
The process by which integrins (such as complement receptor 3) become activated (assume a high-affinity binding state) through intracellular signalling initiated by other receptors, such as anaphylatoxin receptors or Toll-like receptors. By contrast, outside-in signalling refers to intracellular signalling initiated by the activated and ligated integrins.

Immunoreceptor tyrosine-based inhibitory motif
(ITIM). A structural motif containing a tyrosine residue that is found in the cytoplasmic tails of several inhibitory receptors, such as Fcγ receptor IIB and paired immunoglobulin-like receptor B (PIRB). The consensus six-amino-acid ITIM sequence is (I/V/L/S)XYXX(L/V), in which X denotes any amino acid. Ligand-induced clustering of these inhibitory receptors results in tyrosine phosphorylation, often by SRC-family tyrosine kinases, which provides a docking site for the recruitment of cytoplasmic phosphatases.

significance of innate receptor crosstalk for immunity and homeostasis, undesirable outcomes can arise when these same mechanisms come under the control of pathogens. At least in principle, pathogens could induce antagonistic pathways, leading to immune suppression. Furthermore, pathogens might induce synergistic interactions to skew the host immune response away from protective immunity — for example, by promoting a T helper 2 (T_H2) cell response when protective immunity is mediated by T_H1 cells. A growing body of literature indicates that diverse pathogens use specific virulence factors (BOX 1) to exploit mechanisms of PRR cooperation, either at the receptor level or during downstream signalling (see Supplementary information S1 (table)).

In this Review, we focus on the microbial strategies that instigate, subvert or disrupt innate immune receptor crosstalk, thereby contributing to microbial adaptive fitness and persisting infections. These mechanisms are referred to as 'crosstalk manipulation'. We do not cover general immune evasion strategies of pathogens[16,18–20] or the other ingenious tactics used by pathogens to interfere with intracellular signalling pathways through the direct targeting of signalling intermediates (such as the inactivation of signalling molecules through cleavage or dephosphorylation by virulence proteins)[21–23]; these topics have been extensively covered in excellent recent reviews. The objective of this Review is to summarize and discuss the relevant literature identifying virulence factors and hijacked receptors that mediate 'crosstalk manipulation', and to organize distinct microbial tactics into common themes. We discuss the ways in which pathogens can: co-opt host inhibitory receptors (which are normally involved in homeostatic crosstalk), sometimes by expressing host-ligand mimetics[24–28] (FIG. 1); instigate crosstalk pathways for the synergistic induction of immunosuppressive mediators, such as interleukin-10 (IL-10)[29,30] (FIG. 2) or cyclic AMP[31,32] (FIG. 3); induce inside-out signalling to transactivate safe uptake pathways (which are intended for apoptotic cells)[33–36] (FIG. 4); selectively inhibit T_H1 cell-mediated immunity by capitalizing on complement–TLR regulatory crosstalk[37–39] (FIG. 5); exploit TLR–TLR cross-inhibition[40,41]; and disrupt the functional receptor interactions that are required for cooperative protective signalling[42–44] (TABLE 1). Through these diverse mechanisms, pathogens hack into the host receptor crosstalk network to dysregulate the innate immune system for their own benefit.

Co-option of host inhibitory receptors

A distinct set of host inhibitory immune receptors signal through immunoreceptor tyrosine-based inhibitory motifs (ITIMs), which recruit phosphatases such as SH2 domain-containing protein tyrosine phosphatase 1 (SHP1; also known as PTPN6), SHP2 (also known

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as PTPN11) or SH2 domain-containing inositol-5-phosphatase (SHIP). These phosphatases attenuate signalling induced by juxtaposed receptors by dephosphorylating signalling intermediates[8]. ITIM-bearing receptors often, but not exclusively, interact with and inhibit immunoreceptor tyrosine-based activation motif (ITAM)-coupled receptors, such as the Fcγ receptors (FcγRs) and triggering receptor expressed on myeloid cells 1 (TREM1)[45]. ITAMs are found in the cytoplasmic domains of certain transmembrane adaptors (such as the FcR common γ-chain (FcRγ) and DAP12 (also known as TYROBP)) and generally mediate activating signals through the activation of spleen tyrosine kinase (SYK). However, ITAM-mediated cell activation requires high-avidity ligation of the ITAM-coupled receptors, whereas low-avidity (or tonic) ligation of these receptors generates inhibitory signals mediated by SHP1 (REFS 46,47). An ITAM that is functioning in an inhibitory mode is referred to as an ITAMi[47].

Several microorganisms exploit ITIM-bearing or ITAMi-coupled receptors, which they can co-ligate with targeted receptors[24–27]. The resulting juxtaposition of ITIM-bearing or ITAMi-coupled receptors with the targeted receptors (such as TLRs or phagocytic receptors) allows the induction of inhibitory crosstalk that suppresses cellular activation and/or phagocytosis. For example, *Moraxella catarrhalis* and *Neisseria meningitidis* use virulence proteins (UspA1 and Opa, respectively) to bind and activate carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1) on respiratory epithelia, resulting in inhibition of TLR2 signalling. The potential significance of this inhibitory effect is underscored by the heavy involvement of TLR2 in the inflammatory and antimicrobial responses of pulmonary epithelial cells. At the molecular level, TLR2 associates with CEACAM1 following their co-activation by these respiratory pathogens, although the microbial TLR2 ligands involved have not been identified[26]. Subsequently, phosphorylation of the cytoplasmic ITIM of activated CEACAM1 recruits SHP1, which limits phosphorylation of the p85α regulatory subunit of phosphoinositide 3-kinase (PI3K) that is recruited to the TLR2 cytoplasmic domain[26] (FIG. 1a). This in turn inhibits the activation of the PI3K–AKT pathway and decreases the production of IL-8 and granulocyte–macrophage colony-stimulating factor, which regulate the development, mobilization and activation of granulocytes in response to respiratory infections. These data indicate a mechanism of immune evasion through CEACAM1 exploitation, which might be shared by other CEACAM1-interacting pathogens such as *Neisseria gonorrhoeae* and enteropathogenic *Escherichia coli* and *Salmonella* spp. strains. However, the lack of appropriate animal models (*M. catarrhalis* and *N. meningitidis* infections are specific to humans) prevents *in vivo* confirmation of the model.

Group B *Streptococcus* (GBS) can bind to sialic acid-binding immunoglobulin-like lectins (Siglecs) on leukocytes in a sialic acid-dependent or -independent manner, depending on the GBS serotype. For example, the sialylated capsular polysaccharide of serotype III GBS mimics host sialylated glycans and binds Siglec-9 (REF. 24), and the cell wall-anchored β-protein (also known as Bac) of serotype Ia and III GBS strains binds Siglec-5 (REF. 25). GBS engagement of ITIM-bearing Siglec-5 or Siglec-9 activates inhibitory SHP2-dependent signals that interfere with cellular activation (FIG. 1b). This leads to the inhibition of several leukocyte antimicrobial functions, including phagocytosis, induction of the oxidative burst and formation of extracellular DNA traps, and this allows GBS to escape killing by monocytes and/or neutrophils[24,25]. The receptor(s) that crosstalk with Siglecs in this immune evasion mechanism have not been identified, but they could be TLRs, as Siglec-E (the mouse orthologue of Siglec-9) downregulates TLR4 signalling through SHP2 (REF. 48).

An evasion strategy analogous to that of GBS might be used by *Staphylococcus aureus* in mouse macrophages, in which the TLR2-induced inflammatory response is counteracted by co-activation of another ITIM-containing receptor, the murine paired immunoglobulin-like receptor B (PIRB)[49] (FIG. 1c). Under physiological conditions, PIRB regulates the activation of the ITAM-coupled PIRA. The identity of the PIRB-interacting ligand(s) of *S. aureus* is uncertain, although polyanionic molecules, such as dextran sulphate and polyinosinic acid, competitively inhibit the binding of this bacterium to PIRB[49]. PIRB has human orthologues — Ig-like transcript 2 (ILT2; also known as LIR1) and ILT5 (also known as LIR3) — that are also bound by *S. aureus* and other bacteria[49]. Whether the ILT receptors (also known as leukocyte immunoglobulin-like receptors (LIRs)) in humans are exploited by bacterial pathogens remains to be investigated. However, ILT2 is used by human cytomegalovirus (HCMV) to suppress natural killer (NK) cell-mediated cytolysis, through SHP1-dependent crosstalk signals that interfere with activating NK cell receptors[28,50] (FIG. 1d). Intriguingly, activation of ILT2 and phosphorylation of its ITIM are initiated by interaction with HCMV UL18, a viral glycoprotein that not only mimics but also outcompetes MHC class I molecules, as it binds ILT2 with >1,000-fold higher affinity[50]. Another virus, HIV-1, binds DC immunoreceptor (DCIR; also known as CLEC4A), an ITIM-containing C-type lectin, and this interaction promotes HIV-1 infection of DCs[51]. Through signalling crosstalk, endocytosed DCIR inhibits the production of TLR8-induced IL-12 and TLR9-induced interferon-α (IFNα) in conventional and plasmacytoid DCs, respectively[52,53] (FIG. 1e). Therefore, this pathway might contribute to immune evasion by HIV-1 and other DCIR-binding viruses.

E. coli evades phagocytic killing by inducing ITAMi signals through FcγRIII (also known as CD16) and its ITAM-bearing signalling adaptor, FcRγ, which crosstalk with the class A scavenger receptor macrophage receptor with collagenous structure (MARCO)[27] (FIG. 1f). Specifically, *E. coli* binds FcγRIII directly in a non-opsonic manner (that is, without antibody), and this low-avidity interaction induces FcRγ phosphorylation, followed by SHP1 recruitment. SHP1 dephosphorylates PI3K, which is thereby unable to support MARCO-mediated phagocytosis of *E. coli*[27]. Mice deficient in FcγRIII or FcRγ have increased survival rates in models of sepsis induced by caecal ligation and puncture, and this is attributed, in part, to their enhanced ability to clear *E. coli*[27].

Immunoreceptor tyrosine-based activation motif
(ITAM). A structural motif containing two tyrosine residues that is found in the cytoplasmic tails of several signalling adaptor molecules. The motif has the form YXX(L/I) X_{6-12}YXX(L/I), in which X denotes any amino acid. The tyrosine residues are targets for phosphorylation by SRC-family protein tyrosine kinases and subsequent binding of proteins that contain SRC homology 2 (SH2) domains, such as spleen tyrosine kinase (SYK).

Oxidative burst
The process in phagocytic cells by which molecular oxygen is reduced by the NADPH oxidase system to produce reactive oxygen species, such as hydrogen peroxide and hydroxyl radicals. These are toxic oxidants that can destroy targeted microorganisms (for example, in the phagosome lumen).

Extracellular DNA trap
Often referred to by the acronym NET (neutrophil extracellular trap). Upon activation (for example, through Toll-like or Fcγ receptors), neutrophils release nuclear content such as chromatin (DNA, histones and other proteins). This forms a web-like scaffold for the exposure of released antimicrobial molecules at high local concentrations, resulting in the trapping and extracellular killing of bacteria.

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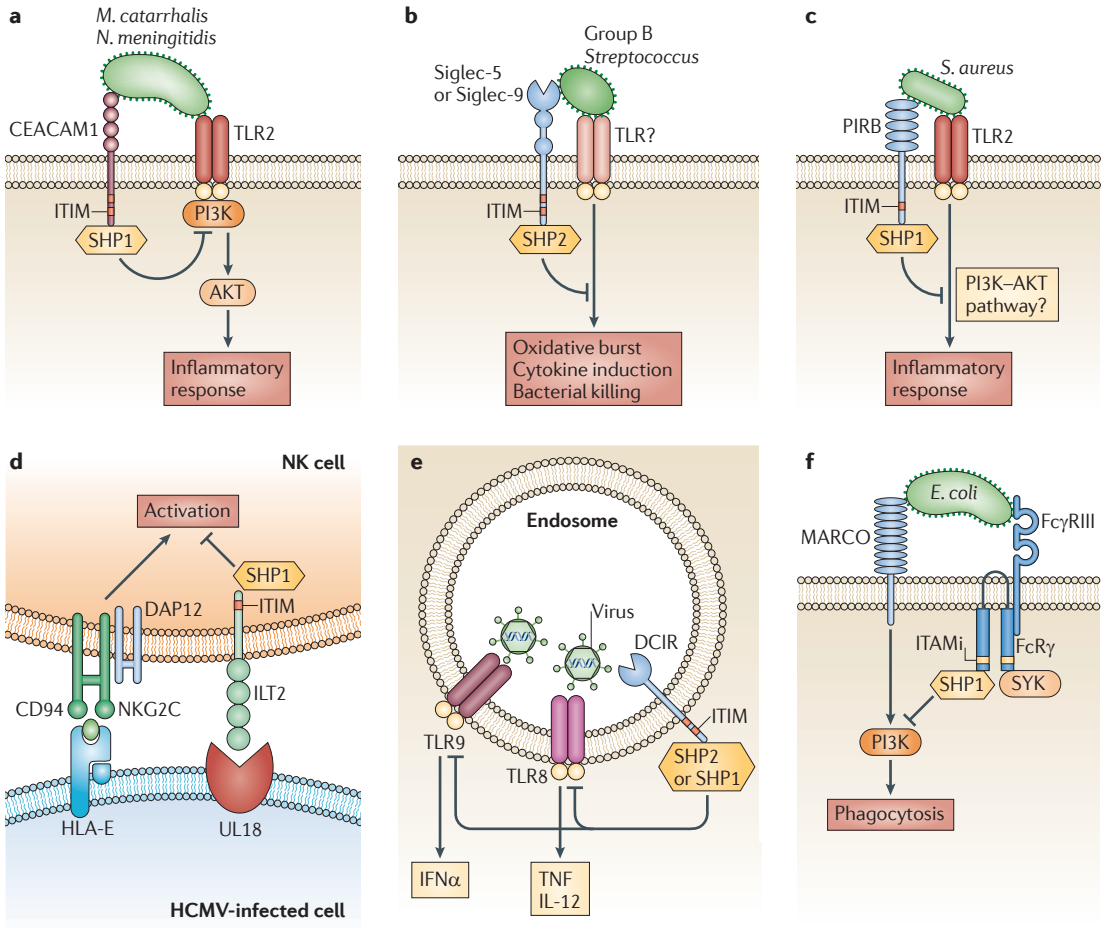

Figure 1 | **Inhibition of cell activation by pathogen-ligated ITIM-bearing or ITAMi-coupled receptors. a** | *Moraxella catarrhalis* and *Neisseria meningitidis* use specific virulence proteins to activate carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1), which co-associates with and inhibits Toll-like receptor 2 (TLR2) signalling. The underlying crosstalk involves phosphorylation of the CEACAM1 immunoreceptor tyrosine-based inhibitory motif (ITIM), which recruits SH2 domain-containing protein tyrosine phosphatase 1 (SHP1; also known as PTPN6); this suppresses the phosphorylation of phosphoinositide 3-kinase (PI3K) and downstream activation of the AKT-mediated pro-inflammatory pathway. **b** | Serotypes of Group B *Streptococcus* (GBS) bind sialic acid-binding immunoglobulin-like lectins (Siglecs), either through molecular mimicry of host sialylated glycans or through a cell wall-anchored protein. The activation of ITIM-bearing Siglec-5 or Siglec-9 by GBS activates inhibitory SHP2 (also known as PTPN11)-dependent signals that interfere with TLR-mediated cellular activation and antimicrobial functions. **c** | *Staphylococcus aureus* uses the ITIM-containing paired immunoglobulin-like receptor B (PIRB) to crosstalk with and inhibit the TLR2-induced inflammatory response, possibly by inhibiting the PI3K–AKT pathway. **d** | Human cytomegalovirus (HCMV) expresses an MHC class I homologue, UL18, which interacts with immunoglobulin-like transcript 2 (ILT2; also known as LIR1) and activates ITIM-dependent and SHP1-mediated signalling. This inhibits natural killer (NK) cell activating receptors, such as the NK group 2, member C (NKG2C)–CD94 complex, and interferes with NK cell-mediated cytolysis of the HCMV-infected cell. **e** | Upon activation by viruses, the ITIM-bearing DC immunoreceptor (DCIR; also known as CLEC4A) becomes internalized into endosomes and inhibits endosomal TLR signalling — specifically, it inhibits production of TLR8-induced interleukin-12 (IL-12) and TLR9-induced interferon-α (IFNα) in conventional and plasmacytoid dendritic cells, respectively. **f** | *Escherichia coli* evades macrophage receptor with collagenous structure (MARCO)-dependent phagocytic killing through inhibitory crosstalk with Fcγ receptor III (FcγRIII; also known as CD16). Specifically, non-opsonized *E. coli* binds with low affinity to FcγRIII and induces partial phosphorylation of the FcR common γ-chain (FcRγ) ITAM (ITAMi), leading to weak mobilization of spleen tyrosine kinase (SYK) but strong recruitment of SHP1. SHP1 dephosphorylates PI3K and impairs MARCO-dependent phagocytosis. TNF, tumour necrosis factor.

It is conceivable that at least some of these inhibitory receptors could be used by the immune system to limit and control unnecessary inflammatory responses, as is the case for commensal Gram-negative bacteria in the gut[54]. In this context, the interaction of a receptor such as PIRB with a commensal organism might avoid a vigorous host immune response, an outcome that is beneficial for both the host and the microorganism. Although it might sound counterintuitive to suggest that commensal and pathogenic microorganisms share immune evasion mechanisms, the latter express additional factors, such as invasins and/or toxins, that enable them to breach epithelial barriers and invade host cells.

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Induction of immunosuppressive mediators

Many pathogens capitalize on the immunosuppressive properties of IL-10 and cAMP to undermine aspects of innate immune defence[55–58]. Although IL-10- and cAMP-dependent signalling pathways have important regulatory functions in maintaining homeostasis of the immune system, excessive and sustained production of these mediators impairs the killing capacity of phagocytes. The functions that are suppressed by IL-10 and cAMP include the production of reactive oxygen or nitrogen intermediates and pro-inflammatory cytokines, and phagolysosomal fusion and acidification. However, differences between the effects of IL-10 and cAMP do exist; for example, IL-10 additionally inhibits the induction of co-stimulatory molecule expression by antigen-presenting cells, whereas cAMP inhibits degranulation of neutrophils more consistently than IL-10 (REFS 55, 58–60). Moreover, cAMP upregulates rather than inhibits IL-6 production[32,58]. Because cAMP is generated through enzymatic action (by adenylyl cyclase), its levels can increase markedly within minutes of stimulation and, in fact, cAMP subsequently enhances transcription of the *IL10* gene[61].

Some microorganisms have genetically encoded mechanisms for regulating cAMP or IL-10 production. Specifically, bacteria such as *Bordetella pertussis* and *Bacillus anthracis* express their own adenylyl cyclase enzyme for unregulated production of cAMP[56,57], and certain viruses (such as Epstein–Barr virus, orf virus and equine herpesvirus 2) encode their own viral homologue of IL-10 (REF. 55). Alternatively, other pathogens can elicit IL-10 or cAMP production through the induction of synergistic crosstalk pathways[29–32,62,63] (see Supplementary information S1 (table)).

Induction of IL-10. Several clinically important human pathogens (including *Mycobacterium tuberculosis*, *Mycobacterium leprae*, *Helicobacter pylori, Candida albicans*, measles virus and HIV-1) induce crosstalk between TLRs and the C-type lectin DC-SIGN (DC-specific ICAM3-grabbing non-integrin; also known as CD209), and this leads to high levels of IL-10 production by DCs[29,62,63] (FIG. 2a). For example, mycobacterial mannosylated lipoarabinomannan (ManLAM) binds DC-SIGN and induces a complex signalling pathway that activates the serine/threonine kinase RAF1 (FIG. 2a). RAF1, in turn, induces phosphorylation of the p65 subunit of nuclear factor-κB (NF-κB) on Ser276 and subsequent acetylation on several lysines. The acetylation of p65 requires the activation of both DC-SIGN and TLR signalling, and is mediated by the related acetyltransferases CREB-binding protein and p300, which are recruited to p65 by binding to its phosphorylated Ser276 residue. This acetylation allows NF-κB to mediate prolonged and enhanced transcription of *IL10*; the underlying mechanism involves the enhanced DNA-binding affinity and transcriptional activity of acetylated p65 and its prolonged presence in the nucleus[29]. The same pathway was later shown to induce enhanced transcription of the *IL12A* and *IL12B* genes[64]. The upregulation of both T_H1 cell-promoting and T_H1 cell-repressing cytokines (IL-12 and IL-10, respectively) is consistent with earlier observations that mycobacteria induce IL-10-producing T_H cells without a T_H1 or T_H2 bias[64,65]. The interaction of mycobacteria with DC-SIGN has also been associated with impaired or intermediate-stage maturation of DCs[62,66], although it is not clear whether this represents immune evasion or a host mechanism to decrease inflammatory pathology.

By contrast, the interaction of *H. pylori* with DC-SIGN through fucose-containing lipopolysaccharide (LPS) Lewis antigens leads to increased IL-10 and decreased IL-12 production and, eventually, to the inhibition of T_H1 cell development[63,64] (FIG. 2b). The two pathways for IL-10 production through DC-SIGN (FIG. 2a,b) are therefore divergent. Indeed, in contrast to mannose-containing DC-SIGN ligands (such as mycobacterial ManLAM, fungal mannan and HIV-1 gp120), the binding of fucose-containing DC-SIGN ligands (such as Lewis X of *H. pylori*) proactively excludes RAF1 and other select components from the DC-SIGN signalling complex and thereby modifies downstream signal transduction[64].

The spirochaete *Borrelia burgdorferi* (the causative agent of Lyme disease) induces DC-SIGN crosstalk with TLR2, although the DC-SIGN ligand is contributed by its tick vector (*Ixodes scapularis*)[67] (FIG. 2c). Specifically, TLR2 and DC-SIGN are activated, respectively, by *B. burgdorferi* lipoproteins and *I. scapularis* salivary protein Salp15, which is captured by outer surface protein C of the bacterium. Although the composition of the DC-SIGN signalling complex for *B. burgdorferi* was not reported in the same detail as that for *H. pylori* (described in the study discussed above[64]), Salp15 contains mannose structures and induces RAF1 signalling[67]. However, in this case, RAF1 activation does not lead to acetylation of the NF-κB p65 subunit, as described for ManLAM. It is thought that because Salp15 can also bind CD4, this receptor might participate in the DC-SIGN–TLR2 signalling crosstalk and alter the RAF1-dependent signalling pathway (FIG. 2c). In this pathway, Salp15-induced RAF1 activation stimulates MAPK/ERK kinase (MEK) signalling, which promotes *IL6* and *TNF* mRNA decay and impairs nucleosome remodelling and, hence, transcriptional activation at the *IL12A* promoter. The same pathway does not destabilize *IL10* mRNA; in fact, co-activation of DCs with *B. burgdorferi* and Salp15 synergistically enhances IL-10 production[67]. As IL-12 inhibits IL-10 production, it is possible that the observed down-regulation of IL-12 expression could contribute to the increased IL-10 levels. In terms of biological relevance, the consequences of Salp15 utilization by *B. burgdorferi* include inhibition of TLR-dependent maturation of DCs and their capacity to activate T cells, which is advantageous for both the arthropod vector and the bacterial pathogen[67].

In neutrophils, mycobacteria use another C-type lectin (potentially CLEC5A), which is coupled to the ITAM-containing adaptor protein DAP12, to induce SYK-dependent crosstalk with the TLR2–MYD88 (myeloid differentiation primary response protein 88) pathway. This crosstalk synergistically upregulates

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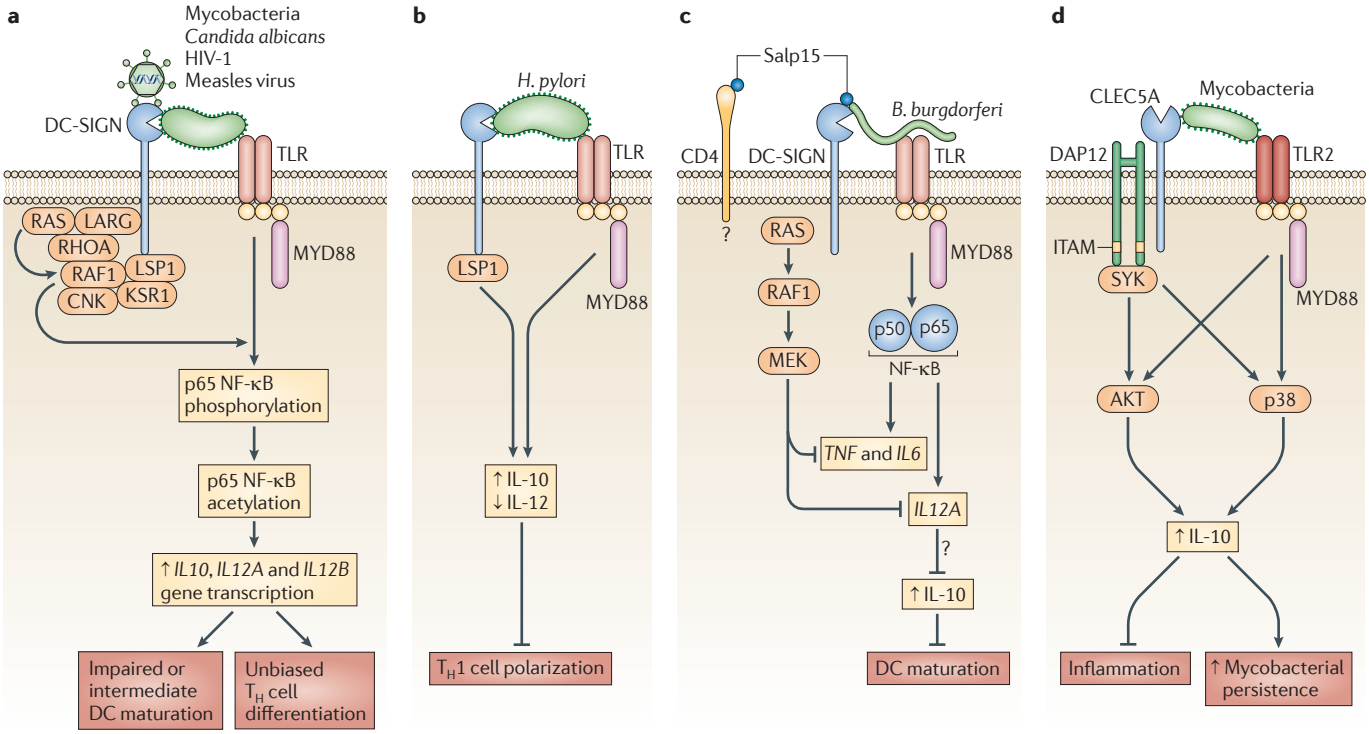

Figure 2 | **Pathogen-induced host receptor crosstalk to stimulate IL-10 production. a** | The indicated pathogens express mannose-containing ligands that bind DC-specific ICAM3-grabbing non-integrin (DC-SIGN; also known as CD209) and induce crosstalk with Toll-like receptors (TLRs) through RAF1. Induction of RAF1 signalling involves the participation of the LSP1–KSR1–CNK scaffolding complex and upstream activators (LARG, RAS and RHOA), and this pathway then mediates the phosphorylation and acetylation of TLR-activated nuclear factor-κB (NF-κB) p65 subunit. This results in increased transcription of the interleukin-10 (*IL10*), *IL12A* and *IL12B* genes owing to the enhanced DNA-binding affinity and transcriptional activity of acetylated p65. **b** | *Helicobacter pylori* binds DC-SIGN through fucose-containing lipopolysaccharide Lewis antigens and activates leukocyte-specific protein 1 (LSP1)-dependent (but RAF1-independent) signalling, leading to increased IL-10 production, decreased IL-12 production and the inhibition of T helper 1 (T_H1) cell development. **c** | *Borrelia burgdorferi* uses the salivary protein Salp15 of its tick vector to induce DC-SIGN–TLR crosstalk. Here, DC-SIGN-induced RAF1 signalling does not lead to p65 acetylation but stimulates MAPK/ERK kinase (MEK) signalling, which promotes *IL6* and tumour necrosis factor (*TNF*) mRNA decay and impairs nucleosome remodeling at the *IL12A* promoter. Conversely, *IL10* mRNA is not destabilized but, rather, IL-10 production is synergistically increased, and this leads to inhibition of dendritic cell (DC) maturation. This divergent RAF1 pathway might be attributed to Salp15 binding to CD4, which may participate in the crosstalk. **d** | In neutrophils, mycobacteria interact with a C-type lectin (possibly CLEC5A) linked to immunoreceptor tyrosine-based activation motif (ITAM)-bearing DAP12. This interaction induces spleen tyrosine kinase (SYK)-dependent crosstalk with the TLR2–MYD88 (myeloid differentiation primary response protein 88) pathway, and this synergistically upregulates IL-10 production through sustained phosphorylation of AKT and p38 mitogen-activated protein kinase. This decreases lung inflammation but increases the persistence of a high mycobacterial burden in a mouse lung infection model. CNK, connector enhancer of KSR; KSR1, kinase suppressor of RAS1; LARG, leukaemia-associated RHO guanine nucleotide exchange factor (also known as RhoGEF12).

IL-10 production through rapid and sustained phosphorylation of two kinases: AKT and p38 mitogen-activated protein kinase (p38 MAPK)[30] (FIG. 2d). In a chronic infection model in mice, neutrophil-derived IL-10 decreased lung inflammation but contributed to the persistence of a high mycobacterial burden[30].

Induction of cAMP. *Porphyromonas gingivalis* is a periodontal pathogen that is also implicated in systemic conditions such as atherosclerosis and rheumatoid arthritis[68,69]. This Gram-negative bacterium uses an array of virulence factors to evade immune elimination and chronically persist in human hosts[70]. Recent evidence indicates that *P. gingivalis* achieves this partly by subverting immune receptor signalling crosstalk[31,32] (FIG. 3). Specifically, *P. gingivalis* induces the recruitment and co-association in macrophage lipid rafts of TLR2 and two G protein-coupled receptors (GPCRs) — CXC-chemokine receptor 4 (CXCR4) and the complement C5a receptor (C5aR) — leading to the induction of high and sustained levels of cAMP[31,32]. *P. gingivalis* activates TLR2 through its surface fimbriae and lipoproteins. Notably, it does not rely on immunological means for C5aR activation. Indeed, the bacterium can generate C5a through its own C5 convertase-like enzymatic activity, mediated by Arg-specific cysteine proteinases (the RgpA and RgpB gingipains)[31]. In addition, *P. gingivalis* can directly activate CXCR4 through its

G protein-coupled receptors (GPCRs). Also known as seven-transmembrane-domain receptors, this large group of receptors can bind a diverse set of molecules (such as chemokines, complement anaphylatoxins, hormones and neurotransmitters) and can induce intracellular signalling by coupling to heterotrimeric GTP-regulated signalling proteins.

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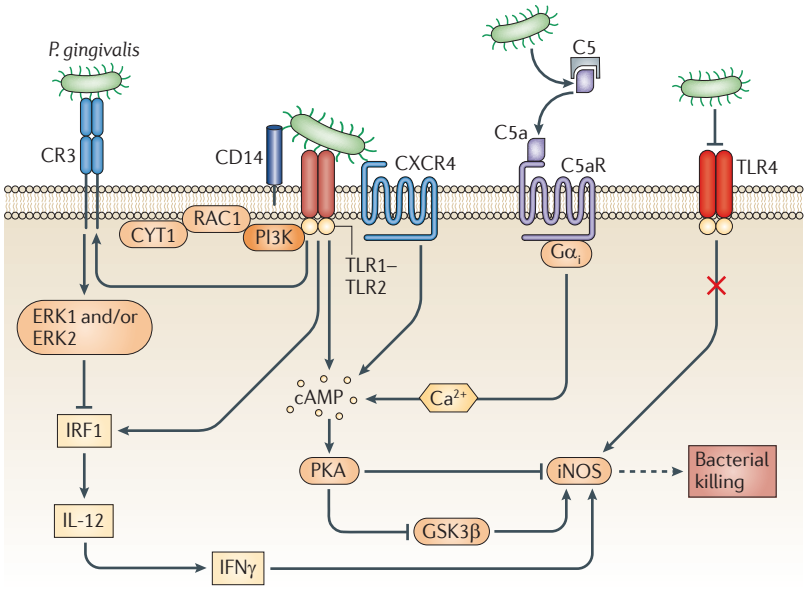

Figure 3 | **Integration of subversive crosstalk pathways, leading to inhibition of pathogen killing.** *Porphyromonas gingivalis* interacts with Toll-like receptor 2 (TLR2) (specifically with the CD14–TLR2–TLR1 complex) and TLR4. The latter receptor is blocked by the bacterium's atypical lipopolysaccharide (which functions as a TLR4 antagonist) and so cannot induce protective responses. The TLR2 response is proactively modified through crosstalk with other receptors that are regulated by *P. gingivalis*. For example, *P. gingivalis* controls the C5a receptor (C5aR) using bacterial Arg-specific cysteine proteinases, which cleave C5 to release biologically active C5a. This C5a stimulates intracellular Ca^{2+} signalling, which synergistically enhances the otherwise weak cyclic AMP (cAMP) response induced by TLR2 activation alone. Maximal cAMP induction requires the participation of CXC-chemokine receptor 4 (CXCR4), which is activated directly by the pathogen's fimbriae and associates with both TLR2 and C5aR in lipid rafts. The ensuing activation of cAMP-dependent protein kinase (PKA) inactivates glycogen synthase kinase-3β (GSK3β) and impairs the inducible nitric oxide synthase (iNOS)-dependent killing of the pathogen in macrophages. An additional pathway induced downstream of TLR2 is an inside-out signalling pathway, mediated by RAC1, phosphoinositide 3-kinase (PI3K) and cytohesin 1 (CYT1), that transactivates complement receptor 3 (CR3; also known as αMβ2 integrin or CD11b–CD18). Activated CR3 binds *P. gingivalis* and induces extracellular signal-regulated kinase 1 (ERK1) and/or ERK2 signalling, which in turn selectively downregulates the expression of the interleukin-12 (IL-12) p35 and p40 subunits through the suppression of interferon regulatory factor 1 (IRF1). This inhibitory ERK pathway is also activated downstream of C5aR (not shown here for clarity; see FIG. 5). Decreased production of bioactive IL-12 and, secondarily, of interferon-γ (IFNγ), leads to impaired immune clearance of *P. gingivalis*.

surface fimbriae (albeit using different epitopes from those mediating TLR2 binding[71,72]), without requiring CXCL12 as a ligand[32]. Recognition of *P. gingivalis* by TLR2 alone induces a weak cAMP response, whereas activation of CXCR4 or C5aR alone fails to induce cAMP. Strikingly, however, *P. gingivalis*-stimulated TLR2 cooperates with activated C5aR and CXCR4 to synergistically increase cAMP production. This, in turn, greatly increases cAMP-dependent protein kinase (PKA) signalling, which inactivates glycogen synthase kinase-3β and, hence, impairs inducible nitric oxide synthase (iNOS)-dependent killing of bacteria *in vitro* and *in vivo*[31,32] (FIG. 3). The C5aR–TLR2 crosstalk depends on $G\alpha_i$-coupled C5aR signalling and the mobilization of intracellular calcium[31], which potentiates concurrent cAMP signalling and, hence, PKA activation[7]. Although the C5aR–TLR2 and CXCR4–TLR2 crosstalk pathways can proceed independently of each other, maximal cAMP induction requires the cooperation of all three receptors[31]. *P. gingivalis* interacts with at least one other TLR, TLR4, although the ligands involved are atypical LPS molecules that only weakly activate TLR4 (in the case of LPS with a 5-acyl monophosphate lipid A structure) or even antagonize TLR4 (4-acyl monophosphate lipid A)[73] (FIG. 3).

The G_s protein-coupled A2A and A2B adenosine receptors (A2AR and A2BR) respond to extracellular adenosine and increase intracellular cAMP levels. Activated A2AR (which has a higher affinity than A2BR for adenosine) crosstalks with and inhibits TLR-induced inflammatory pathways[14]. Intriguingly, *S. aureus* expresses cell wall-associated adenosine synthase A (AdsA), which converts adenosine monophosphate to adenosine[74]. The pathogen exploits the immunosuppressive properties of the adenosine it generates to 'disable' phagocytes in the blood and escape immune clearance. AdsA-deficient mutants of *S. aureus* have a survival disadvantage in the blood that can be reversed by the addition of exogenous adenosine. The authors of this paper also identified another ten species of Gram-positive bacteria (such as *B. anthracis*, *Clostridium perfringens* and *Listeria monocytogenes*) that express homologues of the adenosine synthase domain of AdsA. A study of *B. anthracis* showed that it also uses AdsA to escape phagocytic clearance, which suggests that additional AdsA-expressing bacteria share this evasion mechanism[74]. Given that A2AR signalling inhibits T_H1 and T_H17 cell development, while promoting the generation of adaptive regulatory T cells[75], AdsA-expressing pathogens might also be able to manipulate T cell-mediated immunity.

Inside-out signalling

Complement receptor 3 (CR3; also known as αMβ2 integrin or CD11b–CD18) is a versatile β2 integrin that binds multiple ligands or counter-receptors (such as the complement component iC3b and intercellular adhesion molecule 1 (ICAM1)) and contributes to the phagocytosis of apoptotic cells, leukocyte trafficking and the regulation of cytokine production[2]. Its adhesive activity is tightly regulated; whereas CR3 has a low-affinity conformation in resting cells, a rapid and transient shift to a high-affinity state can be triggered through inside-out signalling by chemokine or anaphylatoxin receptors[76]. TLRs can also induce inside-out signalling for CR3 activation, as originally shown for TLR2 (REFS 77,78) and recently confirmed for TLR4 (REF. 79). The TLR2 inside-out signalling pathway proceeds through RAC1, PI3K and cytohesin 1 (REFS 77,78,80) (FIG. 4). In contrast to TLR4, however, the TLR2 inside-out pathway does not depend on MYD88 (REF. 81). This is because PI3K can be recruited directly to the TLR2 cytoplasmic tail, which contains PI3K-binding motifs that are absent from this region of TLR4 (REF. 82). In terms of physiological significance, the ability of pathogen-sensing receptors, such as the TLRs, to transactivate CR3 might contribute to leukocyte recruitment to sites of infection[80].

Anaphylatoxins
The pro-inflammatory fragments C3a and C5a that are generated during the activation of the complement system. They mediate various inflammatory responses through their corresponding G protein-coupled receptors, such as chemotaxis, oxidative burst and histamine release (from mast cells), but they (in particular, C5a) can also regulate other innate immune components (such as TLRs) through crosstalk signalling pathways.

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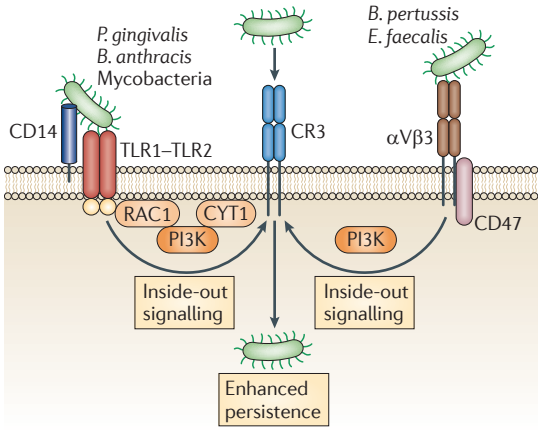

Figure 4 | **Pathogen-induced transactivation of CR3-mediated internalization.** Certain bacteria (such as *Porphyromonas gingivalis*, *Mycobacterium tuberculosis* and *Bacillus anthracis*) bind CD14 and induce Toll-like receptor 2 (TLR2)–TLR1 inside-out signalling for activating and binding complement receptor 3 (CR3; also known as αMβ2 integrin or CD11b–CD18), which leads to a relatively 'safe' uptake of these organisms by macrophages. The signalling pathway that activates the high-affinity state of CR3 is mediated by RAC1, phosphoinositide 3-kinase (PI3K) and cytohesin 1 (CYT1). *Enterococcus faecalis* and *Bordetella pertussis* stimulate their uptake by CR3 through an alternative inside-out signalling pathway. This mechanism is activated by the interaction of these bacteria with a receptor complex comprising αVβ3 integrin and CD47, and is dependent on PI3K signalling. Similarly, CR3-mediated uptake of these bacteria prevents their intracellular killing and promotes their persistence in the mammalian host.

Although CR3 is a phagocytic receptor, it is not linked to vigorous microbicidal mechanisms such as those activated by FcγR-mediated phagocytosis[83,84] and, under certain conditions, CR3-derived phagosomes do not fuse with lysosomes[85]. This is possibly related to the physiological role of CR3 in the uptake of apoptotic cells, which are not normally recognized as a danger that warrants a strong host immune response[2]. Not surprisingly, therefore, the TLR2–CR3 crosstalk pathway is a target of immune subversion by several pathogens. *P. gingivalis*, *M. tuberculosis* and *B. anthracis* use, respectively, their fimbriae, lipoarabinomannan and the BclA glycoprotein to interact with the CD14–TLR2 receptor complex and induce TLR2-mediated transactivation of CR3 (REFS 33,77,78) (FIG. 4). This mechanism allows these bacteria to hijack the phagocytic functions of CR3 for a relatively safe 'outside-in' entry into macrophages. The subversive effect of *P. gingivalis* is evident from the finding that CR3-deficient macrophages are superior to wild-type controls at intracellular killing of this pathogen[34,86]. Moreover, compared with CR3-deficient mice, wild-type mice have increased susceptibility to infection with *B. anthracis* spores; this is attributed to the 'safe' storage of the spores in macrophages after uptake by CR3, and their carriage to sites of spore germination and bacterial growth[33]. Similarly, the ability of *M. tuberculosis* to survive in macrophages might depend, at least in part, on the stimulation of TLR2-induced CR3-mediated phagocytosis[78], although an additional step involves the recruitment of coronin 1A to CR3-derived phagosomes, preventing their fusion with lysosomes[85].

Two other organisms, *Enterococcus faecalis* and *B. pertussis*, activate CR3-mediated phagocytosis through alternative inside-out signalling pathways. Specifically, the 'aggregation substance' glycoprotein of *E. faecalis* and the filamentous haemagglutinin of *B. pertussis* interact with a signalling complex comprising the αVβ3 integrin and the integrin-associated protein CD47, leading to CR3 transactivation in macrophages and neutrophils[36,87] (FIG. 4). CR3-mediated uptake of *E. faecalis* by phagocytes does not induce the oxidative burst and thus promotes the survival of the bacterium[35,88]. *B. pertussis* takes advantage of CR3-mediated phagocytosis to escape immune clearance *in vivo*[89]; the pathogen is readily cleared if it is phagocytosed through FcγRIII[89], which, unlike CR3, is coupled to potent microbicidal mechanisms[83,84].

Interestingly, at least in two cases, TLR2-mediated inside-out signalling is activated by the same virulence proteins (namely, *P. gingivalis* fimbriae[34] and *B. pertussis* filamentous haemagglutinin[36]) that bind transactivated CR3 for non-opsonic phagocytosis. Given the role of CR3 in the phagocytosis of iC3b-coated apoptotic cells, the pathogens that target CR3 as a 'preferred' portal of entry (FIG. 4) might have evolved to co-opt a homeostatic, anti-inflammatory mechanism to evade the innate immune system.

Subversive complement–TLR crosstalk

Complement and TLRs are rapidly activated in response to infection, and common PAMPs (such as LPS and CpG DNA) function as both complement activators and TLR ligands[2]. In fact, the early innate immune response is shaped, to a large extent, by bidirectional crosstalk between the two systems[11]. For example, activation of the complement anaphylatoxin receptors (the GPCRs C3aR and C5aR) synergistically enhances TLR-induced production of pro-inflammatory and antimicrobial mediators[2,90,91]. The signalling pathways involved in complement–TLR4 crosstalk converge at the level of MAPKs, specifically extracellular signal-regulated kinase 1 (ERK1), ERK2 and JUN N-terminal kinase (JNK)[90]. This synergy could potentially enhance innate resistance to infection, and similar crosstalk effects explain, at least in part, why pharmacological inhibition of C5aR protects against sepsis that is induced by high doses of LPS. In a reciprocal reinforcing manner, TLR activation induces the expression of complement components and/or receptors[2]. Moreover, TLR signalling decreases the desensitization of GPCRs by downregulating the expression of GPCR kinases that induce receptor phosphorylation and internalization[92]. This TLR activity would be expected to prolong the activation of C3aR and C5aR in response to infection.

Regulation of IL-12 production. The crosstalk between anaphylatoxin receptors (particularly C5aR) and TLRs also has specific antagonistic effects, at least in macrophages, that selectively alter the induction of

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IL-12-family cytokines. The underlying mechanism of C5aR–TLR crosstalk, which depends on ERK1 and/or ERK2 and PI3K signalling, suppresses the activation of interferon regulatory factor 1 (IRF1) and IRF8. These factors regulate the expression of IL-12 and related cytokines such as IL-23 (REFS 37,93). The C5aR–ERK–IRF1 pathway preferentially inhibits IL-12p70 production, whereas the C5aR–PI3K–IRF8 pathway mainly downregulates the production of IL-23 (REF. 37). Similar inhibitory effects on IL-12 induction are seen when other complement receptors (including gC1qR, CD46 and CR3) are co-activated with TLR4 or TLR2 in mouse macrophages or human monocytes[39,94,95]. The activation of these complement receptors by their natural ligands (such as C3b and C5a), which are produced during the complement cascade or by non-complement host enzymes (such as thrombin and kallikreins)[2], might be a regulatory mechanism to attenuate T cell-mediated inflammation, given the important role of IL-12 in T_H1 cell differentiation and activation[96]. For example, inhibition of IL-12p70 production by C5aR–TLR4 crosstalk can suppress T_H1 cell-mediated pathology. Moreover, decreased IL-12p70 production following the interaction of CR3 on macrophages with iC3b-coated apoptotic cells might prevent unwarranted inflammation and T_H1 cell activation during the phagocytosis of apoptotic cells[2]. Conversely, C1q deficiency in humans and mice causes inflammatory autoimmune pathology[97], although it is uncertain whether, and to what extent, this results from a lack of C1q–gC1qR-mediated homeostatic regulation of T cells through crosstalk with TLR signalling pathways in antigen-presenting cells.

The significance of these antagonistic crosstalk interactions becomes more evident in the context of microbial pathogenesis, in which complement receptors seem to modify TLR signalling and skew the T_H cell response in a manner that interferes with protective immunity[37–39]. Certain non-complement innate immune receptors are also implicated in the selective inhibition of TLR-induced IL-12 production[98], and examples of pathogens that exploit complement and non-complement receptors are given below (FIG. 5).

Leishmania major, an intracellular parasite of macrophages, seems to benefit from complement activation and C5aR-induced inhibition of T_H1 cell-mediated immunity[37]. This conclusion is based on the finding that BALB/c mice, which are normally susceptible to cutaneous leishmaniasis, acquire T_H1 cell-dependent resistance to *L. major* infection when C5aR is genetically ablated[37]. Microbial C5 convertase-like enzymes (such as the gingipains of *P. gingivalis*), which generate C5a, have been implicated in the selective downregulation of IL-12 production and the inhibition of IL-12-dependent immune clearance *in vivo*[99]. Similar evasive strategies, involving alternative complement receptors, are used by other pathogens. These include measles virus, human herpesvirus 6 and adenovirus (groups B and D), which all interact with CD46 through specific virulence proteins or ligands[39,100–103] (see Supplementary information S1 (table)). Measles virus also inhibits TLR4-induced IL-12 production in DCs, although in this case the inhibitory signal is delivered by CD150 (also known as SLAM)[104]. In both cases, however, the virus uses its haemagglutinin to bind CD150 or CD46. Some other pathogens (such as *N. gonorrhoeae*, *N. meningitidis* and Group A *Streptococcus*)[105] also use CD46 as a receptor, thus raising the possibility that these organisms also inhibit IL-12 production.

Hepatitis C virus uses its core protein to bind gC1qR on macrophages or DCs and thereby inhibit IL-12 production and T_H1 cell-mediated immunity; this is thought to be an important mechanism whereby the virus can establish persistent infections[38]. This evasion mechanism might be shared by other pathogens, such as *L. monocytogenes* and *S. aureus*, which can also interact with gC1qR using specific virulence proteins[106,107]. Mycobacteria can downregulate TLR4-induced IL-12 production through the mannose receptor, although this mechanism has broader anti-inflammatory effects[108,109].

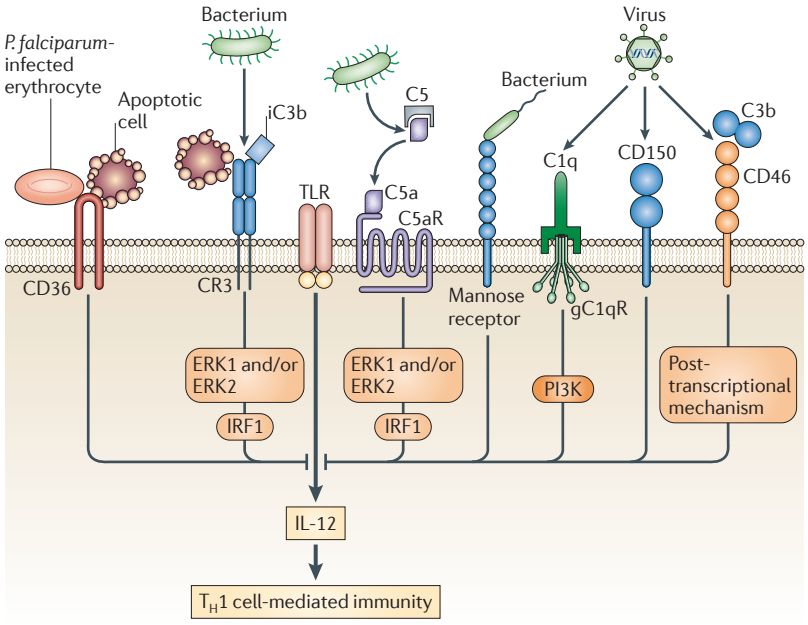

Figure 5 | **Selective inhibition of TLR-induced IL-12 production by pathogen-instigated PRR crosstalk.** The crosstalk between Toll-like receptors (TLRs) and anaphylatoxin receptors (particularly C5a receptor (C5aR)) or other complement receptors (such as complement receptor 3 (CR3; also known as αMβ2 integrin or CD11b–CD18), gC1q receptor (gC1qR) and CD46) selectively inhibits the induction of interleukin-12 (IL-12) production. Relatively little is known regarding the pathways that mediate this selective inhibition; signalling molecules that have been implicated, such as extracellular signal-regulated kinase 1 (ERK1), ERK2 and phosphoinositide 3-kinase (PI3K), are shown downstream of the corresponding receptors. At least for ERK1 and ERK2, the selectivity of IL-12 inhibition is attributed to the suppression of a crucial transcription factor, interferon regulatory factor 1 (IRF1). Post-transcriptional mechanisms might also contribute to IL-12 inhibition. Activation of the complement receptors by their natural ligands might have a homeostatic function, and this is also a possibility for other innate immune receptors (such as CD36, mannose receptor and CD150 (also known as SLAM)) that share the ability to downregulate IL-12 production. However, these same receptors can be activated by bacterial, viral or parasitic pathogens, which can thereby downregulate TLR-induced IL-12 production to interfere with host defences (such as the inhibition of T helper 1 (T_H1) cell-mediated immunity). Although microbial molecules that function as ligands for C5aR have been described, this receptor can also come under pathogen control through the enzymatic generation of high levels of C5a by microbial C5 convertase-like enzymes. *P. falciparum*, *Plasmodium falciparum*; PRR, pattern recognition receptor.

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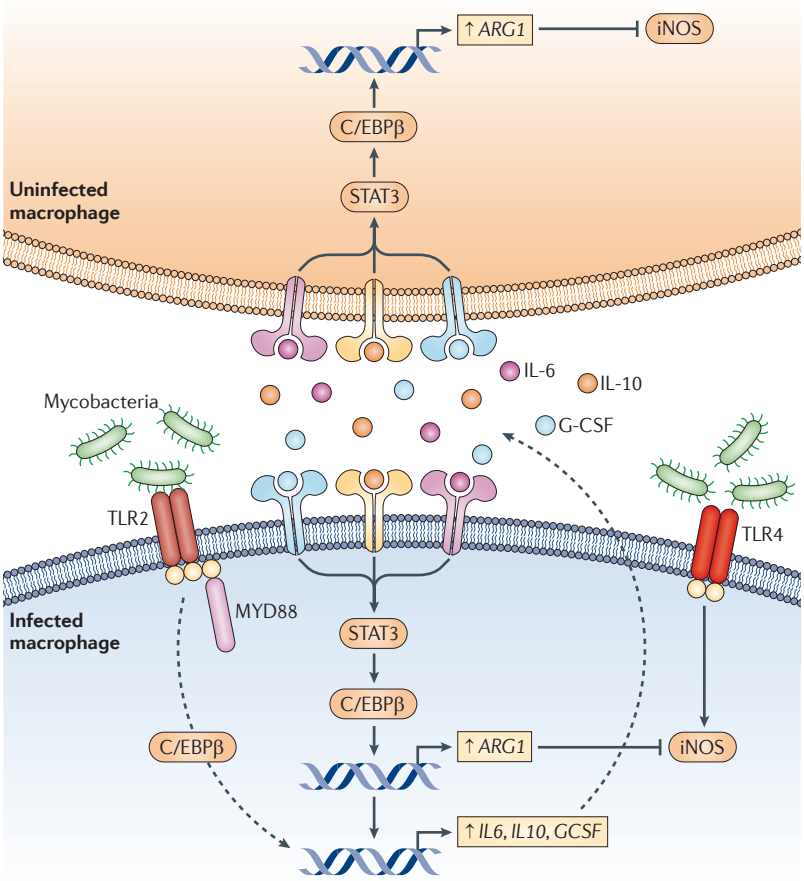

Figure 6 | **MYD88-dependent arginase induction prevents nitric oxide production in both infected and uninfected macrophages.** The activation of myeloid differentiation primary response protein 88 (MYD88) signalling by mycobacteria (at least in part through Toll-like receptor 2 (TLR2)) induces CCAAT/enhancer-binding protein-β (C/EBPβ)-mediated induction of interleukin-6 (IL-6), IL-10 and granulocyte colony-stimulating factor (G-CSF) production. These signal transducer and activator of transcription 3 (STAT3)-activating cytokines function in both autocrine and paracrine manners to induce arginase 1 (*ARG1*) expression, which is partially dependent on C/EBPβ. The ARG1 that is produced can inhibit inducible nitric oxide synthase (iNOS) activity through competition for their common substrate, arginine. The MYD88-dependent pathway for arginase production was shown to confer a survival benefit for mycobacteria *in vivo* and is thought to counteract pathways that activate nitric oxide production, such as TLR4 signalling.

Plasmodium falciparum selectively inhibits IL-12 production and suppresses DC maturation and T cell activation through interactions with the scavenger receptor CD36. Such interactions with CD36 are mediated by *P. falciparum* erythrocyte membrane protein 1 (PfEMP1), which is expressed on infected erythrocytes[110,111] and also interacts with TLR2 (REF. 112). Although PfEMP1-modulated DCs secrete high levels of IL-10, their inability to produce IL-12 is an IL-10-independent effect[110,111]. More recently, PfEMP1 was also implicated in specific suppression of the early induction of IFNγ production, although this involves a CD36-independent mechanism[113]. Moreover, the role of CD36 in malarial pathogenesis is complex and multifactorial, as suggested by a report that CD36-deficient mice have defective clearance of the parasite[112].

Several microorganisms express virulence proteins that interact directly (in a non-opsonic manner) with CR3, although specific CR3-mediated inhibition of IL-12 production has been documented for only a few cases. These examples include *Histoplasma capsulatum*, *B. pertussis* and *P. gingivalis*[95,114–116], which use specific virulence proteins to inhibit IL-12 production (see Supplementary information S1 (table)). CR3-dependent immune subversion was confirmed *in vivo* for *P. gingivalis*, which uses its fimbriae to bind CR3, activate ERK1 and ERK2 signalling, and thereby inhibit TLR2-induced IL-12 production[116] (FIG. 3). This allows *P. gingivalis* to survive in wild-type but not CR3-deficient mice (or normal mice in which CR3 is pharmacologically inhibited), as mice lacking CR3 activity produce higher levels of IL-12 and, secondarily, IFNγ. However, the host-protective effect of CR3 inhibition can be reversed by antibody-mediated neutralization of IL-12 (REF. 116).

TLR–TLR interplay

The capacity of TLR signalling pathways for cross-regulation[9] could potentially be exploited by certain pathogens. This could be achieved through the induction of conflicting signals by distinct pathogen-expressed TLR ligands, and recent papers lend support to this concept. For example, *M. tuberculosis* expresses lipoproteins and glycolipids that function in an inhibitory mode through TLR2 to downregulate TLR9 signalling pathways. This decreases the production of IFNα and IFNβ in response to bacterial CpG DNA and impairs antigen cross-presentation on MHC class I molecules[40]. This crosstalk mechanism might explain why IFNα and IFNβ are not important factors in host immunity to mycobacteria[40]. Hepatitis C virus uses its core protein to activate TLR2-mediated production of inhibitory cytokines (such as IL-10) by human monocytes, and this suppresses TLR9-induced IFNα production by plasmacytoid DCs[41]. This mechanism involves transcellular crosstalk, as human plasmacytoid DCs express TLR9 but not TLR2. By contrast, mouse myeloid DCs, which were used in the study of *M. tuberculosis*[40], express both TLR2 and TLR9; in this system, direct intracellular TLR2–TLR9 signalling crosstalk is mainly responsible for TLR2-mediated inhibition of TLR9-induced antibacterial immunity.

M. tuberculosis and *Toxoplasma gondii* were shown to promote their survival and ability to cause disease in mouse models through MYD88-dependent induction of macrophage arginase 1 (ARG1), which inhibits nitric oxide production by macrophages by competing with iNOS for the common substrate, arginine[117]. MYD88-mediated ARG1 expression in mycobacteria-infected macrophages depends, in part, on TLR2 activation, whereas strong induction of nitric oxide production is mediated by TLR4 activation[117]. In a follow-up paper, the same group showed that the TLR–MYD88-mediated expression of ARG1 is not a direct result of MYD88 signalling but is controlled by the MYD88-dependent production of IL-6, IL-10 and granulocyte colony-stimulating factor, which mediate their effects through signal transducer and activator of transcription 3 (STAT3)[118] (FIG. 6). The implication of these findings is that ARG1 can be induced in a paracrine

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Table 1 | **Microbial disruption of cooperative interactions between innate immune receptors**

Pathogen	Virulence molecules and their targets	Crosstalking receptors		Cell type	Cellular response without interference	Mechanism and outcome of disruption	Ref
		R1	R2				
Coxiella burnetii	Uncertain; possible involvement of smooth-type LPS, which is thought to target CD47	αVβ3–CD47	CR3	Monocytes	αVβ3–CD47-induced inside-out signalling activates CR3 (lectin site)-mediated phagocytosis, leading to intracellular killing*	The pathogen is taken up by αVβ3, leading to intracellular survival; the mechanism is unclear, but perhaps smooth-type LPS interferes with the co-signalling function of CD47	42
Group A *Streptococcus*	Mac, a CD11b mimetic, binds FcγRIII	FcγRIII	CR3	Neutrophils	Opsonophagocytosis, oxidative burst and killing	Mac blocks FcγRIII–CR3 interactions for outside-in signalling, thereby inhibiting the neutrophil antimicrobial response	43
Filarial nematodes	Secreted glycoprotein ES-62 forms a complex with TLR4	TLR4	FcεRI	Mast cells	FcεRI-mediated mast cell degranulation	The sequestration and degradation of PKCα, which is required for the coupling of FcεRI to PLD, results in the inhibition of mast cell activation	44

CR3, complement receptor 3 (also known as αMβ2 integrin or CD11b–CD18); FcγRIII, Fcγ receptor III (also known as CD16); FcεRI, Fcε receptor I; LPS, lipopolysaccharide; PKCα, protein kinase Cα; PLD, phospholipase D; R, receptor; TLR4, Toll-like receptor 4. *Refers to avirulent *C. burnetii*, which expresses rough-type, rather than smooth-type, LPS.

manner; therefore, mycobacteria can 'instruct' both infected and uninfected macrophages to decrease nitric oxide production, thereby rendering these cells permissive to the mycobacterial intracellular lifestyle. Interestingly, ARG1 can also be expressed by 'alternatively activated' macrophages through a STAT6-dependent pathway in the context of T_H2 cell-mediated immunity[118]. This might affect the iNOS-dependent killing of pathogens such as *Francisella tularensis*, which induces the production of IL-4 and IL-13 and thus activates macrophages through the alternative pathway[119].

C. albicans expresses ligands for both TLR2 and TLR4. Whereas TLR4 signalling confers protection against infection[120], TLR2 signalling suppresses the capacity of macrophages to kill *C. albicans* and promotes host susceptibility to invasive candidiasis[121]. The immunosuppressive effect of TLR2 is mediated through the induction of high levels of IL-10 (REF. 121). Although a direct, cell-intrinsic TLR2–TLR4 inhibitory crosstalk pathway has not yet been identified, *C. albicans* seems to use TLR2 signalling to counteract potential TLR4-dependent immunity. Pathogenic *Yersinia* spp. also induce TLR2-dependent IL-10 production and cause immunosuppression by means of the secreted virulence protein LcrV[122]. An intriguing question is how pathogen-stimulated TLR2 signalling can induce immunosuppressive levels of IL-10, given that several TLR2 ligands (including synthetic lipopeptides) induce an overall pro-inflammatory response. It is plausible that complex microbial structures activate TLR2 in tandem with functionally associated co-receptor(s), such as certain C-type lectins[29,30,64], and that IL-10 production is actually induced by the resulting receptor crosstalk between TLR2 and a particular co-receptor. This notion is consistent with the inability of mycobacteria to induce high levels of IL-10 in neutrophils through the TLR–MYD88 pathway unless they activate a C-type lectin–SYK-dependent pathway in parallel[30].

Disruption of cooperative receptor interactions

Pathogens have evolved tactics to disrupt productive cooperation between certain innate immune receptors (TABLE 1). Integrins are an important target in this regard, owing to their capacity to engage in dynamic physical and/or functional interactions with several other receptors in lipid rafts[123]. Indeed, *Coxiella burnetii* impairs the crosstalk between the αVβ3 integrin–CD47 signalling complex and CR3 that is required for activation of CR3 (REF. 42). This prevents CR3-mediated uptake and post-phagocytic killing of this bacterium by monocytes. The exact mechanism is unclear, although it is thought that the smooth-type LPS of virulent *C. burnetii* interferes with CD47 signalling functions, which are not disrupted when monocytes are exposed to avirulent *C. burnetii* expressing rough-type LPS[42]. The avoidance of CR3-mediated phagocytosis by *C. burnetii* contrasts with other bacteria that voluntarily activate this uptake pathway (FIG. 4). However, the phagocytosis of *C. burnetii* is mediated by the carboxy-terminal lectin sites of CR3 which, unlike the amino-terminal I domain used by *P. gingivalis*, *E. faecalis* and other CR3-exploitative bacteria, are linked to induction of the oxidative burst[124,125]. Group A *Streptococcus* secretes a CD11b mimetic, Mac, that binds FcγRIII and blocks its productive interaction with CR3. The disruption of this functional association inhibits cooperative outside-in signalling and impairs opsonophagocytosis, the oxidative burst and bacterial killing[43].

Pathogens may also have developed ways to disrupt productive interactions between non-integrin receptors. TLRs are functionally linked to FcRs, and this has important implications for immunity and inflammatory pathology[8,126]. Filarial nematodes express a secreted glycoprotein, aminopeptidase ES-62, that forms a complex with TLR4, leading to the sequestration and degradation of protein kinase Cα. This disrupts the coupling of FcεRI to phospholipase D and thus prevents mast cell

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degranulation[44]. It has not been specifically addressed whether this mechanism is used by the parasite to evade mast cell-mediated immunity, although ES-62 could be exploited as a potential therapeutic in allergy.

Several viruses and parasites encode soluble molecules that mimic host receptors. Such molecules include the myxoma virus M-T7 glycoprotein, which scavenges IFNγ and sequesters C-, CC- and CXC-chemokines, and the *Schistosoma mansoni* chemokine-binding protein, which binds CC-, CXC- and CX_3C-chemokines[127]. These and other decoy receptors contribute to immune evasion by preventing the interaction of cytokines or chemokines with their signalling receptors[127]. It is conceivable — although not yet specifically addressed — that such decoys disrupt crosstalk interactions between the affected chemokine receptors and PRRs.

Concluding remarks and future perspectives

Receptor crosstalk in the innate immune system is crucial to coordinate microorganism-sensing signals and allow the host to tailor an appropriate immune response. However, many pathogens subvert these functions, often by taking control of host regulatory receptors. This can be achieved by mimicking host ligands or counter-receptors[24,28,43], or the host enzymes that generate such ligands[31,74]. Moreover, pathogens can 'voluntarily' interact with TLRs (or other innate immune receptors) through virulence molecules that have evolved to recognize such receptors[25,32,122,128,129]. It is clear that these interactions do not represent pattern recognition but rather involve subversive action that promotes the adaptive fitness of the pathogens. Future research may identify additional pathogen virulence factors and hijacked host receptors, particularly among those receptors with regulatory roles. For example, it would provide a significant survival advantage if a pathogen has evolved to interact with regulatory Toll/IL-1 receptor domain-containing transmembrane proteins (such as single immunoglobulin IL-1-related receptor (SIGIRR) and IL-1 receptor-like 1 (IL1RL1; also known as ST2)), which are involved in negative crosstalk with the IL-1 receptor and/or TLRs[9]. Some of the evasion mechanisms discussed in this Review need to be substantiated in appropriate animal models, which are not always available. Identification of the mechanisms of 'crosstalk manipulation', through continued research and improved animal models, is essential in order to develop approaches that counteract immune subversion by pathogens. Indeed, antagonistic blockade of hijacked host receptors or neutralization of the virulence factors involved might offer promising options for controlling infection and associated immunopathology.

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Competing interests statement
The authors declare competing financial interests: see Web version for details.

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