Bacterial strategies for overcoming host innate and adaptive immune responses

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In higher organisms a variety of host defense mechanisms control the resident microflora and, in most cases, effectively prevent invasive microbial disease. However, it appears that microbial organisms have coevolved with their hosts to overcome protective host barriers and, in selected cases, actually take advantage of innate host responses. Many microbial pathogens avoid host recognition or dampen the subsequent immune activation through sophisticated interactions with host responses, but some pathogens benefit from the stimulation of inflammatory reactions. This review will describe the spectrum of strategies used by microbes to avoid or provoke activation of the host's immune response as well as our current understanding of the role this immunomodulatory interference plays during microbial pathogenesis.

Like all other higher organisms, humans have evolved in the continuous presence of various microbes. In fact, many body surfaces are densely populated by what we call the "normal microflora", which is mainly constituted of a variety of commensal bacteria, such as *Bacteroides thetaiotamicron* and *Lactobacillus* species. These bacteria are harmless and even beneficial under normal circumstances, but may cause local or systemic inflammatory disease if the integrity of the hosts' surface is disturbed. On the other hand, pathogenic bacteria are able to invade sterile body sites, proliferate and cause substantial tissue damage or systemic inflammation, such as is seen after infection with *Shigella dysenteriae* or *Mycobacterium tuberculosis*. The success of many pathogens relies on their ability to circumvent, resist or counteract host defense mechanisms; yet some bacteria also provoke activation of the immune system, which ultimately leads to disruption of the epithelial barrier and bacterial invasion. Consequently, pathogens have provided many examples of how to avoid and manipulate host responses.

In this review, we will discuss how pathogens mechanistically avoid recognition by the immune system, and how they interfere—or possibly could interfere—with innate or adaptive immune responses, should recognition occur. We will examine the various steps that take place during the course of infection, starting with microbial attachment and colonization of host surfaces, which can eventually lead to invasion of the epithelial cell layer and penetration to the subepithelial tissue, and finally facilitate systemic dissemination of the microbes. We will discuss the strategies these microbes use to modify or circumvent the host defense mechanisms that come into play during this process, including recognition by surface immune receptors, secretion of antimicrobial effector molecules, internalization and degradation by phagocytes and activation of the humoral as well as the cellular immune systems.

Attachment to and colonization of body surfaces

Bacteria are excluded from the host tissue by anatomical barriers that consist of the skin and mucous membranes. The integrity of the mucosal surfaces is protected by active removal of bacteria, for example by the acid environment of the stomach, the ciliary movement in the upper respiratory tract and the continuous flushing with urine of the lower urinary tract. Thus, motility and attachment factors (so-called adhesins) found in most pathogenic bacteria are essential for approaching cellular surfaces and withstanding mechanical removal. For example, lack of expression of the major attachment factor toxin-coregulated pili (TCP) in *Vibrio cholerae*, significantly reduces the severity of bacteria-induced diarrhea in humans[1]. Alternatively, the secretion of bacterial toxins impairs protective functions and facilitates colonization. For example, *Bordetella pertussis*, the agent that causes whooping cough, paralyses the ciliary clearance function of the respiratory tract *via* the release of cell wall constituents that induce nitric oxide–mediated ciliostasis[2]. Biofilm formation by the opportunistic pathogens *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* or the production of a protective bacterial extracellular matrix ("curli" surface fibers) by *Escherichia coli* shield bacteria from the hostile environment and might facilitate resistance against the host surface protective mechanisms[3]. Finally, the presence of an extensive resident microflora represents yet another means of effectively protecting the host's mucosal surfaces; this is illustrated by infection with the opportunistic pathogen *Clostridium difficile*—the agent that causes pseudomembranous colitis—in patients undergoing antibiotic treatment, which results in disturbance of the enteric microflora. Thus, colonization by pathogens in the presence of a resident flora requires successful strategies that enable invading microbes to successfully compete for nutritional and spatial resources and displace commensal organisms from the microbial niche.

Evasion of immune recognition by mucosal surfaces

Besides acting as mechanical barriers, vulnerable mucosal membranes are covered with an array of soluble opsonizing factors, such as antibodies, that immobilize and remove approaching bacteria. Bacteria counter this with the proteolytic degradation of secretory immunoglobulin. This method of evasion is used particularly by bacteria that colonize the upper respiratory tract; *Haemophilus influenzae*—an important causative agent of respiratory tract infections—is one example of a microbe that uses such mechanisms to prevent opsonization and Fc receptor–mediated phagocytosis. Many types of epithelial cells have the intrinsic ability to sense the presence of microbial organisms and

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respond specifically through the identification of conserved components of these microbes. These microbial structures are termed pathogen-associated molecular patterns (PAMPs) and include parts of the bacterial cell envelope, such as lipopolysaccharide (LPS), peptidoglycan and bacterial DNA. Recognition of microbial structures by host cells relies on diverse families of genome-encoded receptors that allow detection of infectious nonself particles and provide signals that activate the defense mechanisms[4]. One group of membrane receptors, the toll-like receptors (TLRs), has attracted substantial attention due to their role in cellular signaling and their importance during initiation of the adaptive immune response[5]. The most effective strategy for avoiding innate recognition could involve steric shielding or modification of exposed PAMPs. In fact, host-like bacterial capsular structures have long been recognized as important virulence factors. Also, various LPS species from different commensal as well as pathogenic bacteria show some variance in the capacity to induce cytokine synthesis. Multiple alterations in the structure of *Salmonella* LPS decrease the microbe's potential to provoke innate immune responses[6]. However, due to the pivotal role played by most PAMPs in essential bacterial cell functions as well as structure, major modifications might well decrease the viability and fitness of the bacterial intruders. Bacterial flagellin, which is recognized by TLR5, might represent an exception; flagellin shows in many Gram-negative bacteria as a result of phase and antigenic variation[7]. Also, although it is an important virulence factor for many bacteria (for example *V. cholerae*), it appears that flagellar expression is not an essential contributor to the pathogenicity of the prominent enteric pathogen *Salmonella enterica* serovar Typhimurium[8].

The cellular process of pattern molecule recognition is only beginning to be understood. In addition to the need for soluble as well as membrane-bound accessory proteins such as LPS-binding protein (LBP), CD14 and MD-2, the cellular localization of a given TLR seems to be highly specific. For example, TLR2 is situated on the plasma membrane of macrophages and stays bound to its ligands—such as yeast zymosan—even after internalization in the phagosome[9]. In order for hypomethylated CpG motifs—a characteristic feature of bacterial DNA—to be recognized, endocytosis must occur so that the cell can signal through the intracellularly located receptor TLR9[10]. TLR4 is found on the surface of macrophages but in the Golgi apparatus of intestinal epithelial cells, colocalized with its internalized ligand LPS[11]. These examples demonstrate the complexity of TLR-mediated recognition processes, which involve ligand internalization, cell traffic and fusion of subcellular compartments.

Although the exact relationship between ligand localization and TLR-mediated signaling has not been determined, the possibility exists that microbes inhibit or delay recognition by interference with membrane and vesicular trafficking. Alternatively, because expression of recognition receptors seems to be organ-specific, recognition might be avoided through the selection of certain favorable anatomical sites for colonization and invasion[12].

In contrast to the avoidance of immune recognition, some microbial pathogens, under certain conditions, enhance immune-activation and pro-inflammatory responses by producing maximally stimulatory pattern molecules. For example, *S. dysenteriae*, which causes bacillary dysentery in humans, contains two copies of the *msbB* gene; one of these genes is located on the virulence plasmid. The *msbB* gene product is involved in the biosynthetic pathway of lipid A, the immunostimulatory part of the LPS molecule. Deletion of the *msbB* gene in *E. coli* leads to the production of hypoacylated lipid A with strongly decreased pro-inflammatory activity. The second *msbB* gene encoded by *Shigella* might be used to ensure complete acylation of lipid A and generate maximal stimulatory LPS. Cell activation is required to induce intestinal leukocyte infiltration followed by disruption of the enteric mucosal layer, which facilitates bacterial invasion[13]. As this example demonstrates, inflammation during the early course of infection might, under certain conditions, be advantageous. In contrast, long-term microbial colonization requires that cellular stimulation and activation of host defenses are avoided. This point is illustrated by *Helicobacter pylori*, which colonizes the human gastric mucosa and causes chronic infections in a large percentage of the human population. *H. pylori* activates stomach epithelial cells in a process that is mainly dependent on proteins encoded by the CagA pathogenicity island[14]. After prolonged colonization, part of the bacterial population in the stomach tends to delete *cag* genes[15]. This may reflect a need to reduce the inflammatory response as soon as microbial colonization is established. In addition, a global modulation of virulence gene expression is associated with the transition from acute to chronic infection of mice with *S. enterica* Typhimurium[16]. In contrast, isolates of *P. aeruginosa* that chronically colonize the lungs of patients with the inherited disease cystic fibrosis continue to produce highly stimulatory LPS[17]. Biofilm formation and low susceptibility to host defense molecules (such as antimicrobial peptides and complement) might provide sufficient protection to allow *P. aeruginosa* to persist in the face of ongoing inflammation, which enhances the supply of nutrients.

Recognition *via* host receptor molecules eventually leads to the activation of signal transduction cascades—including recruitment of adaptor molecules, tyrosine phosphorylation and activation of transcription factors—and subsequent activation of defense responses such as chemokine release and antimicrobial peptide production. An alternative immune-evasion strategy might interfere with cellular signaling during the stages that follow actual recognition. However, regarding microbial interference with TLR-induced signaling, only one example—that used by the vaccinia virus—has been described[18]. Possibly, disruption of immediate TLR-mediated signaling in host cells requires a pace that simply is not easily achieved. The alternative, then, would be to interfere with downstream signaling events. Active suppression, at the molecular level, of an induced pro-inflammatory immune response is demonstrated by *S. enterica* serovar Pullorum, the agent that causes fowl typhoid. In contrast to the well studied serovar Typhimurium—which causes inflammatory gastroenteritis in humans, and high secretion of pro-inflammatory cytokines in polarized human intestinal epithelial cells—*S. enterica* Pullorum produces only a minimal cellular response. More strikingly *S. enterica* Pullorum can suppress the pro-inflammatory activation of a subsequent exposure to *S. enterica* Typhimurium through active inhibition of IκB ubiquitination[19]. Inhibition of cellular activation by commensal or pathogenic microbes may therefore represent a strategy with which gastrointestinal mucosal tolerance to pro-inflammatory stimuli can be maintained and host defenses avoided. Microbial strategies for the manipulation or avoidance of surface defense mechanisms of the host epithelial barrier are illustrated (**Fig. 1**).

Resistance to antibacterial effectors on epithelial surfaces

In addition to their ability to attract professional immune cells, the epithelial body surfaces themselves provide effective innate antimicrobial defense. A large variety of antimicrobial peptides protect the inner and outer surfaces of most multicellular organisms against environmental microbial pathogens. These locally secreted short peptides are highly resistant to enzymatic degradation and show a net positive charge, which facilitates their binding to prokaryotic cell surfaces. Antimicrobial peptide–induced bacterial killing involves attachment and integration of the

peptide into the surface of the invading prokaryote and subsequent disturbance of membrane integrity[20]. A whole spectrum of adaptive mechanisms used by bacteria lowers susceptibility to antimicrobial peptides expressed by the host. Although they are considered relatively resistant to enzymatic digestion, degradation of at least some linear antimicrobial peptides by bacterial proteases has been reported[21,22], and active transport of peptides out of the bacterial cytoplasm also occurs[23]. Some bacteria degrade extracellular matrix, and the resulting fragments bind to antimicrobial peptides and abolish their efficacy[24]. Bacterial membranes are much less susceptible to antimicrobial peptides than artificial membranes[25]. This might be explained by the fact that the negatively charged membranes of many bacteria are modified by the addition of positively charged residues. *Staphylococcus aureus*, the dominant causative agent of purulent wound infections, modifies its principal membrane lipid, phosphatidylglycerol, with lysine[26] and adds D-alanine to teichoic acid[27]. Both changes reduce the net negative charge of the membrane. Similarly, under certain circumstances Gram-negative bacteria modify the structure of their LPS so they become less susceptible to antimicrobial killing. For example, *S. enterica* Typhimurium can form hepta-acylated lipid A (*via* the addition of palmitate by the bacterial protein PagP), add phosphate and phosphoethanolamine to the core polysaccharide and modify lipid A phosphate groups with ethanolamine and aminoarabinose. These alterations decrease the susceptibility of microbes to α-helical antimicrobial peptides or the cyclic polypeptide polymyxin[6,28].

Adaptation to antimicrobial peptides seems to play a critical role in microbial virulence, as 11 out of 12 *S. enterica* Typhimurium mutants with decreased susceptibility to host peptides, showed reduced virulence *in vivo*[29]. Also, the mechanism used by the virulence factor encoded by the *S. enterica* Typhimurium *mig14* gene was recently identified: mig-14 mutants showed enhanced susceptibility to antimicrobial peptides[30]. Finally, *S. aureus* that was unable to modify phosphatidylglycerol with L-lysin and thereby reduce its negatively charged surface membrane showed attenuated virulence in mice[26]. Thus, bacteria have evolved a number of mechanisms in order to adapt to surrounding antimicrobial peptides, and these mechanisms appear to be important in the expression of full virulence. However, high concentrations of animicrobial peptides at vulnerable body sites *in vivo* do nevertheless impede microbial colonization and growth.

Why do bacteria not attempt full resistance to antimicrobial peptides? One explanation may lie in the high costs to microbial organisms that the development and expression of resistance engender. The diverse and highly specific biological functions of the microbial membrane might preclude modifications that allow resistance to the membrane-disturbing activity of antimicrobial peptides in the interest of preserving the functional and structural integrity of the microbial cell[20]. For example, a strain of *Streptococcus pyogenes* that is resistant to the murine antimicrobial peptide Cramp shows growth inhibition in enriched culture medium[31]. Therefore, the importance of decreased susceptibility to antimicrobial peptides may, in most cases, lie in the competition with resident microbial organisms for nutrients and space rather than resistance to the hosts' immune defense. Resistance-enhancing changes to LPS structure in *Salmonella* are tightly regulated by the PhoP-PhoQ and PmrA-PmrB two-component signal transduction systems, which are central regulators of bacterial virulence[6,32]. The ability to monitor the environment and accordingly modify the cell wall structure might allow the organism to adapt to specific requirements during infection and therefore minimize the accompanying high metabolic costs.

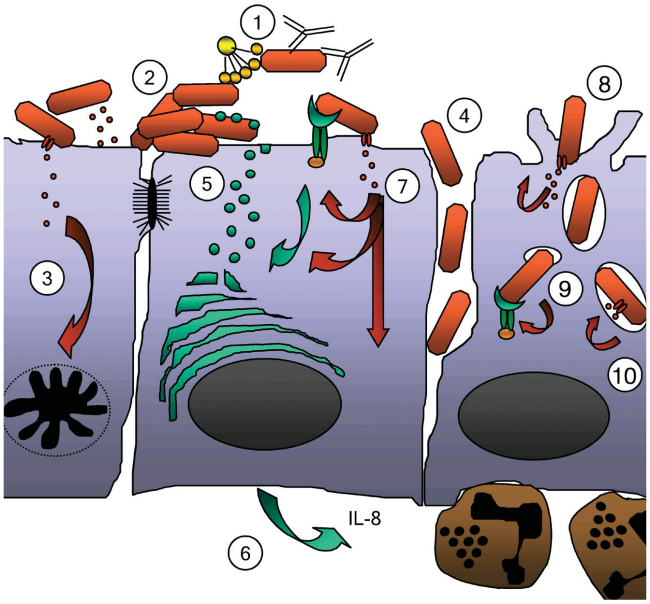

Figure 1. Strategies for bacterial escape from epithelial defense mechanisms. Prevention of opsonization (1) is required to facilitate colonization of host surfaces (2). Toxin secretion can paralyze the host's defenses (3) and disrupt its mucosal integrity (4). Microbial recognition and host responses—such as the secretion of antimicrobial peptides (5) or chemokine production (6)—can be impaired by modification of pattern molecule presentation or interference with intracellular signaling or cell trafficking (8). Microbe-induced self-uptake (7) and escape from the phagosome along with inhibition of intracellular recognition (9) or persistence in modified endosomes (10) can then impede removal by host defense mechanisms. Green, host responses; orange, bacterial components and interference with host defense strategies.

Another explanation for the lack of emergence of resistance to antimicrobial peptides is the simultaneous production of a variety of different peptides at most body sites. The simultaneous use of different antimicrobial substances significantly impairs the development of microbial resistance, as is illustrated by the modern multiple drug regimens prescribed to treat tuberculosis or HIV infection. A recent genetic analysis has identified a large number of potential antimicrobial peptides in vertebrates, which further increases the quantity and diversity of molecules identified to date[33]. Accordingly, gene-deletion strategies to prevent the expression of a single antimicrobial peptide have so far failed to reveal a clear phenotype of enhanced susceptibility to infection. (The only exception is a report on mice deficient in Cramp, the only murine member of the cathelicidin gene family, which showed enhanced susceptibility to skin infection with *S. pyogenes*[31].) In contrast, mice lacking the whole group of enteric α-defensins—as a result of deletion of the proteolytic enzyme matrilysin—showed an increased susceptibility to orally administered *S. enterica* Typhimurium; this demonstrates the importance of enteric antimicrobial peptides in host defense[34].

A third reason for the lack of fully resistant bacteria may be the fact that the activity of antimicrobial peptides seems to be highly regulated both at the transcriptional level and through enzymatic processing and secretion[35,36]. Consequently, interference with antimicrobial peptide regulation seems to represent another microbial strategy for avoiding killing[37,38]. In addition, the continuous presence of high peptide concentrations is restricted to defined and particularly vulnerable body sites such as the mouth, airways and intestinal crypts (where the intestinal

epithelium regenerates) as a means of controlling the distribution of the normal flora[39,40]. The situation in the intestinal crypts illustrates this scenario well. These small, gland-like appendices (with a volume of ~4–6 μl) contain high concentrations of antimicrobial peptides (estimated to be of the order of grams per liter) that are produced by the Paneth cells at the lower end of the crypts and which effectively inhibit bacterial entry into the crypts and protect the site of epithelial regeneration[20,40]. Diffusion into the comparatively large intestinal lumen, absorption by the mucus overlying the epithelium and consumption through the abundant intestinal microflora results in a peptide concentration below that required to inhibit bacterial growth. Therefore, restricted secretion of antimicrobial peptides might help to avoid the development of microbial resistance by minimizing the selective pressure on the surrounding resident flora.

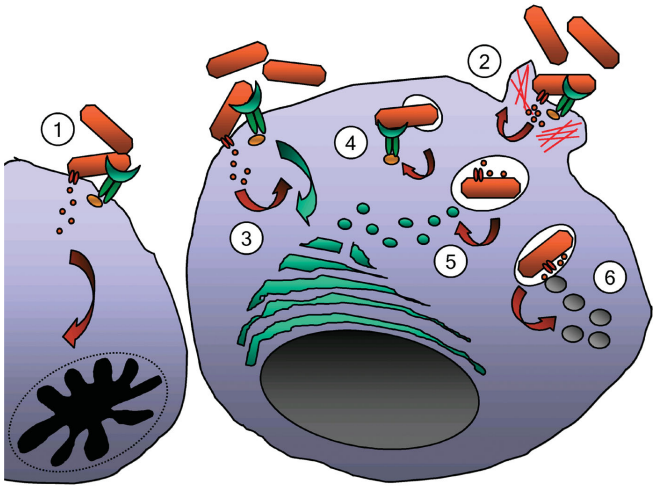

Figure 2. Bacterial defense against phagocytes. Bacterial defense strategies against phagocyte engulfment include the induction of programmed cell death (1) as well as inhibition of uptake (2) by translocated effector proteins. Effector proteins can also be used to down-regulate other host cell nuclear responses (3). Should phagocytosis occur, bacteria can escape from the endosome into the host cell cytosol (4) or interfere with endosomal trafficking as well as maturation of the phagosome (5) and the subcellular localization of defense factors (6).

Strategies for invading and crossing the epithelium

Invasion of the epithelial layer provides protection from surface defense molecules. For example, *S. enterica* Typhimurium invades epithelial cells using a mechanism by which it induces its own uptake. The microbe uses a syringe-like transfer apparatus—termed a type III secretion system—to transfer two bacterial products, SopE and SopE2, directly from the bacterial cytoplasm into the eukaryotic host cell. Both proteins act as nucleotide exchange factors that activate central regulators of the actin cytoskeleton, the small GTP-binding proteins CDC42 and Rac, and induce subsequent engulfment of the bacterium[41,42]. However, activation of these proteins also stimulates nuclear responses through the transcription factors NF-κB and AP-1, ultimately leading to the secretion of pro-inflammatory cytokines and attraction of professional phagocytes. This immune stimulation is counteracted by yet another translocated bacterial protein, SptP, which quenches the activated GTP-binding proteins involved and thereby limits cell activation[42]. Similarly, uropathogenic *E. coli* invade the bladder epithelium and thereby avoid clearance by surface host defense mechanisms[43]. Internalized *Shigella flexneri*—which has a similar clinical profile to *S. dysenteriae*—produces and secretes IpaB, which mediates lysis of the phagosome and allows the bacterium to escape into the cytoplasmic space[44]. Actin nucleation and polymerization initiated by the bacterial protein IcsA—which is located at the rear pole of the bacterium—enables *S. flexneri* to move through the cytoplasm and enter neighboring cells, facilitating microbe evasion of activated immune reponses[45]. One might assume that the cytosolic location provides optimal protection from immune recognition and response. However, even the cytosol seems to be equipped to detect the presence of bacterial pattern molecules, such as LPS, mediated by members of the nonobese diabetic (NOD) protein family leading to a pro-inflammatory cellular response[46]. Mutations in the human *NOD2* gene, which are involved in cytosolic recognition of LPA, are associated with Crohns' disease, an inflammatory bowel disease of unknown etiology[47,48].

Direct penetration of the skin is found with vector-born microbial diseases. In the case of Lyme disease, the protective skin barrier is transversed by the bite of a tick. The tick translocates *Borrelia burgdorferi* directly to the subepithelial space, where the bacteria initiate systemic infection. Infection with the spirochete *Leptospira* in an example of active transcutaneous migration, as this bacterium has the exceptional ability to actively penetrate the skin without the aid of any vector. Other bacteria such as *S. pyogenes* or *Clostridium perfringens*, both prominent causative agents of soft tissue infections, bypass the epithelial barrier *via* pre-existing injuries and use enzymatic degradation of the host's extracellular matrix, toxin-mediated cell destruction or induction of programmed cell death to spread themselves through intact tissue[49]. Yet another important mode of entry, bypassing the intestinal epithelial barrier—used, for example, by *Salmonella* and *Shigella*—occurs through a specialized cell type: the M cell. M cells overlay the Peyer´s patches in the small intestine and can translocate luminal antigens (and even intact bacteria) to the basolateral side of the epithelia for uptake and recognition by the underlying cells of the immune system[50]. However, once they are beyond this entry point, bacteria must defend themselves against resident professional immune cells.

Escape from phagocyte responses

Upon arrival at the subepithelial space, bacteria encounter locally resident as well as newly infiltrated professional phagocytic cells that are attracted by the chemokine response of the overlying epithelial cells. The strategies used by bacteria to overcome this additional defense barrier are shown (**Fig. 2**) Phagocytes are equipped with a number of receptors that detect the presence of invading microbes and bind opsonized microbial surfaces. Membrane-bound scavenger receptors, lectins, Fc receptors and complement receptors as well as signaling through TLRs may cooperate to determine the ultimate cellular response. This may lead to phagocyte maturation, activation of antimicrobial substances and secretion of pro-inflammatory cytokines, as well as phagocytosis and microbial degradation.

Consequently, bacteria use a variety of strategies to avoid engulfment and degradation by phagocytes and facilitate proliferation and spread among host tissues[51]. Examples are the inhibition of phagocytosis by capsule formation or toxin-mediated cellular destruction and necrosis. In contrast, induction of apoptosis avoids the release of pro-inflammatory signals[49]. Host-induced apoptosis of lung epithelial cells during infection with *P. aeruginosa* plays an important role in reducing leukocyte infiltration and maintaining the essential function of the lung:

the oxygenation of blood[52]. In contrast, *Salmonella* and *Shigella* both actively stimulate pro-apoptotic pathways in order to paralyze phagocytic defense: SipB from *S. enterica* Typhimurium and the similar IpaB from *S. flexneri* are translocated *via* a type III secretion apparatus into the host cytosol. These proteins bind to caspase-1, which activates downstream caspases and induces apoptosis[53,54]. The observation that caspase-1–deficient mice are resistant to infection with wild-type *Salmonella* suggests that this mechanism may contribute to the pathogenesis of this bacterium[55]. *Yersinia enterocolitica* YopP (like its homolog *Yersinia pseudotuberculosis* YopJ) can also inhibit anti-apoptotic signals *via* the repression of NF-κB activation as well as stimulation of pro-apoptotic signals through LPS-mediated activation of the TLR4 pathway[56].

Y. enterocolitica and Y. *pseudotuberculosis*—which both cause enterocolitis and abdominal lymphadenitis—can inhibit phagocytosis by the translocation of bacterial mediators that specifically disorganize the host cell cytoskeleton preventing bacterial uptake by macrophages and polymorphonuclear leukocytes. Bacterial YopE RhoGAP activity promotes the disruption of actin filaments by interaction with the Rho GTPases Rac, Rho and CDC42. YopH destabilizes focal adhesion *via* dephosphorylation of the adapter protein p130Cas and inhibits phagocytosis that is mediated by Fc receptors and complement receptors[57,58]. Once internalization has occurred, some bacteria—such as the food-born pathogen *Listeria monocytogenes*, which is responsible for serious infections in immunocompromised individuals—manage to survive, persist and even proliferate in host phagocytes. To avoid degradation in the phagolysosome, *L. monocytogenes* is able to escape into the host cell cytosol by means of a bacterial toxin, listeriolysin, which disrupts the endosomal membrane[59]. Other pathogens such as *Salmonella* are able to manipulate endosomal trafficking and recruit defense factors to the maturing vacuole[60]. *S. enterica* Typhimurium, for example, is able to reduce the recruitment of NADPH oxidase and inducible nitric oxide synthase (iNOS) to the vacuole through interference with vacuolar trafficking, thereby preventing oxygen radical production and bacterial killing in macrophages[61–63]. The fact that many different *Salmonella* mutants that are able to down-regulate host iNOS activity could be isolated in a screen of macrophage-adapted bacteria suggests that *Salmonella* use several strategies for interfering with the host NO response[60]. And like many other bacteria, *Salmonella* is able to detoxify oxygen radicals enzymatically[64]. *M. tuberculosis* inhibits phagosomal maturation by depleting H^+ ATPase molecules from the vacuolar membrane[65]. This leads to reduced acidification and allows intracellular survival and growth.

Resistance to humoral defense mechanisms

Successful escape by microbes from internalization by phagocytes opens the way for systemic spread in the host *via* the blood or lymph vessels. However, the limited supply of essential nutrients such as iron requires a high degree of adaptation to this environment. This is illustrated by the example of *Yersinia*, which carries genes that encode high-affinity uptake systems for ferric iron. In addition, bacteria will encounter humoral defenses. Soluble factors such as the C-reactive protein (CRP), mannan-binding lectin (MBL) and serum amyloid protein (SAP) are produced by the liver and function as opsonins. CRP and MBL also act as alternative recognition molecules for the antibody-independent activation of complement by binding to C1q, the activator of the classical complement activation pathway. Both *S. pyogenes* and *Streptococcus pneumoniae* possess surface structures that bind the complement regulatory component factor H[66,67]. Factor H binding consequently promotes complement factor I–mediated degradation of C3b deposited on the bacterial surface and inhibits the release of chemotactic molecules, such as C5a and C3a, as well as formation of the membrane attack complex. Additionally, certain bacteria express proteases that degrade C1q, C3, C4 and C5-C9[68]. As we mentioned before, intracellular persistence and proliferation, such as that seen with *S. enterica* Typhimurium, represents an opposite yet similarly effective strategy for avoiding the limited growth factors as well as soluble humoral defense molecules.

Bacterial interference with cytokine secretion

The innate immune system is clearly critical in the early control of bacterial replication and successful eradication of an infection. It is also linked to the adaptive immune response, which helps clear the infection and builds specific immunity with a memory component. Activation of the adaptive response occurs through cytokine secretion, antigenic processing and presentation as well as proliferation and differentiation of effector cells. Secretion of cytokines—particularly by effector T cells—killing of cells harboring intracellular pathogens by cells with cytolytic activity—such as $CD8^+$ T cells—and antibody production by B cells all then contribute to controlling bacterial infections. Examples of the strategies bacteria use to deal with this complex defense network are shown (**Fig. 3**).

The production of pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin 1 (IL-1), IL-8 and IL-12 by host cells upon sensing bacterial products is crucial in the innate and adaptive immune responses to infection. These cytokines play a role in enhancing the bactericidal capacity of phagocytes, recruiting additional innate cell populations to sites of infection, inducing dendritic cell maturation and directing the ensuing specific immune response to the invading microbes. Some bacterial pathogens have evolved mechanisms for modulating cytokine production by host cells, which modifies the host's subsequent immune response.

Mycobacteria provide a good example of bacterial manipulation of the cytokine response. These bacteria can induce the production of anti-inflammatory cytokines, which dampen the immune response. Mycobacteria-infected macrophages produce IL-6, which inhibits T cell activation[69], as well as the potent immunosuppressive cytokines IL-10[70] and transforming growth factor-β (TGF-β)[71]. IL-10 is immunosuppressive in several ways[72], including the inhibition of macrophage activation and production of reactive oxygen and nitrogen intermediates, suppression of inflammatory cytokine production as well as down-regulation of the production of molecules important in triggering specific immunity (for example the major histocompatibility complex (MHC) class II antigen presentation complex and the costimulatory molecule CD86). *Mycobacterium*-induced production of immunosuppressive cytokines may also contribute to the generation of regulatory T cells, also called T suppressor cells, that down-regulate immune activation. For example, aerosol treatment of mice with killed *Mycobacterium vaccae* induces regulatory T cells that prevent airway inflammation in an IL-10 and TGF-β–dependent manner[73]. Similarly, *B. pertussis* exploits IL-10 in order to down-regulate the host immune response. *Bordetella* filamentous hemagglutinin (FHA) induces IL-10 production by dendritic cells. This induces naïve T cells to develop into regulatory cells that suppress interferon-γ (IFN-γ) production by antigen-specific T cells[74]. Also the LcrV protein produced by *Y. enterocolitica* induces macrophages to secrete IL-10, which, in turn, suppresses TNF-α production[75]. Thus, bacterial exploitation of host cell capacity to produce immunosuppressive cytokines, particularly IL-10, provides an effective means for invading microbes to modulate host defense mechanisms and evade immune recognition.

Certain bacteria have evolved mechanisms for interfering with the signal transduction pathways important in regulating expression of cytokines and other proteins involved in inflammation. For example, YopP from *Y. enterocolitica* and YopJ from *Y. pseudotuberculosis* inhibit NF-κB and MAPK (mitogen-activated protein kinase) signal transduction pathways[76–78]. Thus, *Yersinia* avoids the detrimental effects of pro-inflammatory cytokines secretion by suppressing TNF, IL-1 and IL-8 production. In contrast to *Yersinia* inhibition of NF-κB activation, the intracellular pathogen *L. monocytogenes* activates this transcription factor as a potential means of increasing its pathogenicity[79,80]. *Listeria*-mediated NF-κB activation of endothelial cells results in increased expression of the adhesion molecules intercellular adhesion molecule 1 (ICAM-1) and E-selectin, and secretion of IL-8 and macrophage chemoattractant protein 1 (MCP-1)[80]. This attracts circulating phagocytes and promotes diapedesis and tissue infiltration. This "Trojan horse" mechanism directs *Listeria*-infected phagocytes to the subendothelial space, facilitating tissue spread and bacterial dissemination.

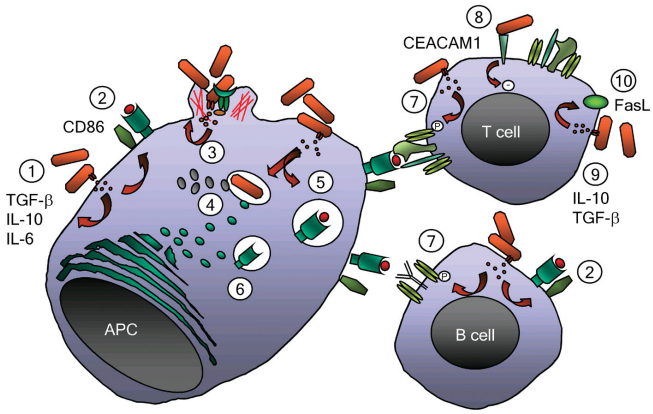

Figure 3. Bacterial defense strategies against the adaptive immune response. Strategies include the induction of immunosuppressive cytokines, such as IL-10, IL-6 and TGF-β (1); inhibition of pro-inflammatory cytokine production; and surface expression of costimulatory molecules such as CD86 (2) by antigen presenting cells (APC). Interference with bacterial uptake (3), phagosome maturation (4) and antigen processing (5) as well as MHC class I and II expression (6) also lead to diminished antigen presentation. Inhibiting tyrosine phosphorylation of the T and B cell receptors (7) and activating the inhibitory CEACAM1 receptor on T cells (8) further decreases effector cell function. Certain bacteria can also induce regulatory T cells (formerly called suppressor T cells) that dampen the immune response (9) or induce T cell apoptosis by enhancing FasL expression on T cells (10).

Bacterial interference with antigen presentation

Interfering with antigen processing and presentation is another strategy used by bacterial pathogens to prevent stimulation of an adaptive immune response. For example, *S. enterica* Typhimurium mutants that constitutively express the *phoP-phoQ* regulatory locus, which is important for survival in macrophages and bacterial virulence, are inefficiently processed by macrophages for MHC class II presentation[81,82]. Similarly, the vacuolating toxin VacA produced by *H. pylori* diminishes the capacity of antigen-presenting cells to degrade internalized antigens[83]. Also, *M. tuberculosis* shows several strategies for suppressing antigen presentation and T cell activation, including inhibition of phagosomal maturation (see above) and sequestration of mycobacterial antigens from molecules required for T cell stimulation, such as MHC class II presentation[84–86]. Mycobacteria also down-regulate surface expression of MHC class II and CD1 and interfere with the presentation of antigens by MHC class II molecules[87–90]. Mycobacteria inhibit the transcription of IFN-γ–responsive genes, including the master regulator for MHC class II expression, the class II transactivator (CIITA)[89,91]. As MHC class II expression by resting macrophages is very low and IFN-γ is a potent inducer of MHC class II on these cells, the capacity of Mycobacteria to inhibit MHC class II expression by interfering with IFN-γ–mediated signaling pathways provides a potent means for dampening critical $CD4^+$ T cell responses to this bacterium.

Chlamydia trachomatis, a sexually transmitted pathogen that causes urogenital tract and ocular infections, also inhibits surface expression of MHC molecules on infected cells[92,93]. Like Mycobacteria, *C. trachomatis* inhibits IFN-γ–inducible MHC class II expression by interfering with CIITA activation. The mechanism used by *Chlamydia* to inhibit activation of CIITA involves degrading the upstream stimulatory factor 1 (USF-1) which is required for IFN-γ–mediated CIITA induction and, thus, IFN-γ–inducible MHC class II expression[92]. In addition, *C. trachomatis* suppresses both constitutive and IFN-γ–inducible MHC class I expression on infected cells by degrading the transcription factor regulatory factor X 5 (RFX5) in addition to degrading USF-1[93]. As regulatory factor RFX5, a key component of the RFX transcription complex, is required for both constitutive and IFN-γ–inducible MHC class I expression and the RFX complex is required for MHC class II transcription[94], the ability of *C. trachomatis* to degrade these transcription factors provides an effective means of blocking adaptive immunity.

Inhibiting T and B cell effector functions

Some bacteria interfere with the capacity of T and B cells—the effector cells of the adaptive immune system—to carry out their functions. For example, *H. pylori* Cag pathogenicity island-encoded genes induce Fas ligand (FasL) expression on T cells and mediate apoptosis[95]. YopH from *Y. pseudotuberculosis* also suppresses antigen-specific T cell activation and IL-2 production by inhibiting tyrosine phosphorylation of components of the T cell receptor[96]. YopH also inhibits tyrosine phosphorylation of components of the B cell receptor and suppresses up-regulation of the costimulatory molecule CD86 after B cell receptor engagement with antigen[96]. The YopH-dependent inhibition of signaling cascades associated with antigen receptor engagement is an additional immune evasion strategy to the above-mentioned capacity of YopH to impair bacterial internalization[97].

Another bacterium that exploits the host receptor signal transduction machinery in order to modulate immunity is *Neisseria gonorrhoeae*, a sexually transmitted pathogen that causes urogenital infection. One class of the multiallelic, phase-variable *Neisseria* OPA proteins—which bind various ligands and mediate uptake by host cells—binds members of the CD66 receptor family, also known as carcinoembryonic antigen–related cellular adhesion molecule (CEACAM) family. CECAM1 is the only CEACAM molecule expressed on human lymphocytes, and the presence of an immunoreceptor tyrosine-based inhibitory motif (ITIM) in its cytoplasmic tail highlights its role as a coinhibitory receptor on $CEACAM1^+$ cells. *N. gonorroheae* expressing a CEACAM1-binding OPA protein inhibits the activation and proliferation of $CD4^+$ T cells stimulated by ligation of the T cell receptor[98]. This inhibitory effect was associated with increased recruitment of two tyrosine phosphatases, SHP-1 and SHP-2, that are critical to the inhibitory function of ITIM-containing receptors[98]. Thus, the capacity of

N. gonorrohea to inhibit T cell activation in addition to antigenic variation of surface proteins including OPAs[99] likely contributes to the poor specific immune response observed in *Neisseria*-infected individuals.

Our current knowledge of the strategies used by bacteria to interfere with innate and adaptive immunity and escape host defenses is largely incomplete. Nevertheless, the diverse disciplines of immunology, microbiology, infectious diseases and cell biology have contributed much to the exciting progress we have made in our knowledge over recent years. These studies have also revealed the complex interplay between microbial pathogens and higher organisms. However, when the role played by the host's normal microbial flora is included in the analysis, the complexity of bacteria-host interactions is even greater. How does the host differentiate between its responses to pathogens and commensals? One explanation is that the mucosal linings are tolerant to microbes at locations colonized by the normal flora, and that innate responses are induced only after bacterial intrusion beyond these barriers. However, even in the absence of pathogenic microorganisms, host defense mechanisms are required to maintain the integrity of the anatomical barrier against the resident microbial flora.

The need for continuous vigilance is illustrated by mice that are deficient in the production of bactericidal oxygen and nitrogen intermediates ($gp91^{phox-/-}NOS2^{-/-}$). Such mice spontaneously develop massive abscesses that are caused by the normal flora of the intestine, respiratory tract and skin[100]. On the other hand, down-regulation of pro-inflammatory responses as well as enhancement of the intestinal barrier function appear to represent important functions of the normal microbial flora. The intestinal commensal *Bacteroides thetaiotamicron* protects host cells from complement-mediated cytotoxicity *via* the up-regulation of DAF (decay-accelerating factor), a central regulator of complement deposition on nucleated cells, and simultaneous enhancement of cutaneous repair and barrier functions[101]. Therefore, one might assume that commensals, like pathogens, express factors that directly or indirectly interfere with immune defense. However, the extent to which commensal microbes apply similar strategies remains an important question that needs to be addressed. Another important factor that should be taken into account is the heterogeneity of the host population: the genetic polymorphisms of receptor or effector molecules, and also the diversity of environmental conditions including the constitution of the resident microflora. Upcoming studies will undoubtedly reveal further surprising details of the intriguing relationship between bacteria and host immunity, and will hopefully provide us with the knowledge to improve the prevention and treatment of infectious diseases in the near future.

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1. Tacket C. O. *et al.* Investigation of the roles of toxin-coregulated pili and mannose-sensitive hemagglutinin pili in the pathogenesis of *Vibrio cholera* O139 infection. *Infect. Immun.* **66**, 692–695 (1998).
2. Flak, T. A. & Goldman, W. E. Signalling and cellular specificity of airway nitric oxide production in pertussis. *Cell. Microbiol.* **1**, 51–60 (1999).
3. Chapman, M. R. *et al.* Role of *Escherichia coli* curli operons in directing amyloid fiber formation. *Science* **295**, 851–855 (2002).
4. Janeaway, C. A. Jr. & Medzhitov, R. Innate immune recognition. *Annu. Rev. Immunol.* **20**, 197–216 (2002).
5. Medzhitov, R., Preston-Hurlburt, P. & Janeway, C. A. Jr. A human homologue of the Drosophila Toll protein signals activation of adaptive immunity. *Nature* **388**, 394–397 (1997).
6. Guo, L. *et al.* Regulation of lipid A modifications by *Salmonella typhimurium* virulence genes phoP-phoQ. *Science* **276**, 250–253 (1997).
7. Hayashi, F. *et al.* The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. *Nature* **410**, 1099–1103 (2001).
8. Schmitt, C. K. *et al.* Absence of all components of the flagellar export and synthesis machinery differentially alters virulence of *Salmonella enterica* serovar Typhimurium in models of typhoid fever, survival in macrophages, tissue culture invasiveness, and calf enterocolitis. *Infect Immun.* **69**, 5619–5625 (2001).
9. Underhill, D. M. *et al.* The Toll-like receptor 2 is recruited to macrophage phagosomes and discriminates between pathogens. *Nature* **401**, 811–815 (1999).
10. Hemmi, H. *et al.* A Toll-like receptor recognizes bacterial DNA. *Nature* **408**, 740–745 (2000).
11. Hornef, M. W., Frisan, T., Vandewalle, A., Normark, S. & Richter-Dahlfors, A. Toll-like receptor 4 resides in the Golgi apparatus and colocalizes with internalized lipopolysaccharide in intestinal epithelial cells. *J. Exp. Med.* **195**, 559–570 (2002).
12. Backhed, F., Soderhall, M., Ekman, P., Normark, S. & Richter-Dahlfors, A. Induction of innate immune responses by *Escherichia coli* and purified lipopolysaccharide correlate with organ- and cell-specific expression of Toll-like receptors within the human urinary tract. *Cell. Microbiol.* **3**, 153–158 (2001).
13. D'Hauteville, H. *et al.* Two *msbB* genes encoding maximal acylation of lipid A are required for invasive *Shigella flexneri* to mediate inflammatory rupture and destruction of the intestinal epithelium. *J. Immunol.* **168**, 5240–5251 (2002).
14. Fischer, W. *et al.* Systematic mutagenesis of the *Helicobacter pylori* cag pathogenicity island: essential genes for CagA translocation in host cells and induction of interleukin-8. *Mol. Microbiol.* **42**, 1337–1348 (2001).
15. Akopyants, N. S. *et al.* Analyses of the cag pathogenicity island of *Helicobacter pylori*. *Mol. Microbiol.* **28**, 37–53 (1998).
16. Clements, M. O. *et al.* Polynucleotide phosphorylase is a global regulator of virulence and persistency in *Salmonella enterica*. *Proc. Natl. Acad. Sci. USA* **99**, 8784–8789 (2002).
17. Hajjar, A. M., Ernst, R. K., Tsai, J. H., Wilson, C. B. & Miller, S. I. Human Toll-like receptor 4 recognizes host-specific LPS modifications. *Nature Immunol.* **3**, 354–359 (2002).
18. Bowie, A. *et al.* A46R and A52R from vaccinia virus are antagonists of host IL-1 and toll-like receptor signaling. *Proc. Natl. Acad. Sci. USA* **97**, 10162–10167 (2000).
19. Neish, A. S. *et al.* Prokaryotic regulation of epithelial responses by inhibition of IκBα ubiquitination. *Science* **289**, 1560–1563 (2000).
20. Zasloff, M. Antimicriobial peptides of multicellular organisms. *Nature* **415**, 389–394 (2002).
21. Guina, T., Yi, E. C., Wang, H., Hackett, M. & Miller, S. I. A PhoP-regulated outer membrane protease of *Salmonella enterica* serovar typhimurium promotes resistance to α-helical antimicobial peptides. *J. Bacteriol.* **182**, 4077–4086 (2000).
22. Stumpe, S., Schmid, R., Stephens, D. L., Georgiou, G. & Bakker, E. P. Identification of OmpT as the protease that hydrolyses the antimicrobial peptide protamine before it enters growing cells of *Escherichia coli*. *J. Bacteriol.* **180**, 4002–4006 (1998).
23. Shafer, W. M., Qu, X.-D., Waring, A. J. & Lehrer, R. I. Modulation of *Neisseria gonorrhoeae* susceptibility to vertebrate antibacterial peptides due to a member of the resistance/ nodulation/division efflux pump family. *Proc. Natl. Acad. Sci. USA* **95**, 1829–1833 (1998).
24. Schmidtchen, A., Frick, I.-M. & Björck, L. Dermatan sulphate is released by proteinases of common pathogenic bacteria and inactivates antibacterial α-defensins. *Mol. Microbiol.* **39**, 708–713 (2001).
25. Matsuzaki, K., Fukui, M., Fujii, N. & Miyajima, K. Interactions of an antimicrobial peptide, tachyplesin I, with lipid membranes. *Biochem. Biophys. Acta* **1070**, 259–264 (1991).
26. Peschel, A., Vuong, C., Otto, M. & Gotz, F. The D-alanine residues of *Staphylococcus aureus* teichoic acids alter the susceptibility to vancomycin and the activity of autolytic enzymes. *Antimicrob. Agents Chemother.* **44**, 2845–2847 (2000).
27. Peschel, A. *et al. Staphylococcus aureus* resistance to human defensins and evasion of neutrophil killing via the novel virulence factor *mprF* is based on modification of membrane lipids with L-lysine. *J. Exp. Med.* **193**, 1067–1076 (2001).
28. Guo, L. *et al.* Lipid A acylation and bacterial resistance against vertebrate antimicrobial peptides. *Cell* **95**, 189–198 (1998).
29. Groisman, E. A., Parra-Lopez, C., Salcedo, M., Lipps, C. J. & Heffron F. Resistance to host antimicrobial peptides is necessary for *Salmonella* virulence. *Proc. Natl. Acad. Sci. USA* **89**, 11939–11943 (1992).
30. Brodsky, I. E., Ernst, R. K., Miller, S. I. & Falkow, S. *mig-14* is a *Salmonella* gene that plays a role in bacterial resistance to antimicrobial peptides. *J. Bacteriol.* **184**, 3203–3213 (2002).
31. Nizet, V. *et al.* Innate antimicrobial peptide protects the skin from invasive bacterial infection. *Nature* **414**, 454–457 (2001).
32. Gunn, J. S., Ryan, S. S., Van Velkinburgh, J. C., Ernst, R. K. & Miller, S. I. Genetic and functional analysis of a PmrA-PmrB-regulated locus necessary for lipopolysaccharide modification, antimicrobial peptide resistance, and oral virulence of *Salmonella enterica* serovar typhimurium. *Infect. Immun.* **68**, 6139–6146 (2000).
33. Schutte, B. C. *et al.* Discovery of five conserved β-defensin gene clusters using a computational search strategy. *Proc. Natl. Acad. Sci. USA* **99**, 2129–2133 (2002).
34. Wilson, C. L. *et al.* Regulation of intestinal α-defensin activation by the metalloproteinase matrilysin in innate host defense. *Science* **286**, 113–117 (1999).
35. Ghosh, D. *et al.* Paneth cell trypsin is the processing enzyme for human defensin-5. *Nature Immunol.* **3**, 583–590 (2002).
36. Diamond, G., Kaiser, V., Rhodes, J., Russell, J. P. & Bevins, C. L. Transcriptional regulation of β-defensin gene expression in tracheal epithelial cells. *Infect. Immun.* **68**, 113–119 (2000).
37. Islam, D. *et al.* Downregulation of bactericidal peptides in enteric infections: a novel immune escape mechanism with bacterial DNA as a potential regulator. *Nature Med.* **7**, 180–185 (2001).
38. Lindmark, H. *et al.* Enteric bacteria counteract lipopolysaccharide induction of antimicrobial peptide genes. *J. Immunol.* **167**, 6920–6923 (2001).
39. Dale, B. A. *et al.* Localized antimicrobial peptide expression in human gingiva. *J. Periodontal. Res.* **36**, 285–294 (2001).
40. Ayabe, T. *et al.* Secretion of microbicidal α-defensins by intestinal Paneth cells in response to bacteria. *Nature Immunol.* **1**, 113–118 (2000).
41. Friebel, A. *et al.* SopE and SopE2 from *Salmonella typhimurium* activate different sets of RhoGTPases of the host cell. *J. Biol. Chem.* **276**, 34035–34040 (2001).
42. Galan, J. E. & Zhou, D. Striking a balance: modulation of the actin cytoskeleton by *Salmonella*. *Proc. Natl. Acad. Sci. USA* **97**, 8754–8761 (2000).
43. Mulvey, M. A. *et al.* Induction and evasion of host defenses by type 1-piliated uropathogenic *Escherichia coli*. *Science* **282**, 1494–1497 (1998).
44. High, N., Mounier, J., Prevost, M. C. & Sansonetti, P. J. IpaB of *Shigella flexneri* causes entry into epithelial cells and escape from the phagocytic vacuole. *EMBO J.* **11**, 1991–1999 (1992).
45. Goldberg, M. B. & Theriot, J. A. *Shigella flexneri* surface protein IcsA is sufficient to direct actin-based motility. *Proc. Natl. Acad. Sci. USA* **92**, 6572–6576 (1995).
46. Girardin, S. E. *et al.* CARD4/Nod1 mediates NF-κB and JNK activation by invasive *Shigella flexneri*. *EMBO Rep.* **2**, 736–742 (2001).
47. Hugot, J. P. *et al.* Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. *Nature* **411**, 599–603 (2001).
48. Ogura, Y. *et al.* A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. *Nature* **411**, 603–606 (2001).
49. Weinrauch, Y. & Zychlinsky, A. The induction of apoptosis by bacterial pathogens. *Annu. Rev. Microbiol.* **53**, 155–187 (1999).

50. Jones, B. D., Ghori, N. & Falkow, S. *Salmonella typhimurium* initiates murine infection by penetrating and destroying the specialized epithelial M cells of the Peyer's patches. *J. Exp. Med.* **180**, 15–23 (1994).
51. Underhill, D. M. & Ozinsky, A. Phagocytosis of microbes: complexity in action. *Annu. Rev. Immunol.* **20**, 825–852 (2002).
52. Grassme, H. *et al.* CD95/CD95 ligand interactions on epithelial cells in host defense to *Pseudomonas aeruginosa*. *Science* **290**, 527–530 (2000).
53. Hersh, D. *et al.* The *Salmonella* invasin SipB induces macrophage apoptosis by binding to caspase-1. *Proc. Natl. Acad. Sci. USA* **96**, 2396–2401 (1999).
54. Zychlinsky, A. *et al.* IpaB mediates macrophage apoptosis induced by *Shigella flexneri*. *Mol. Microbiol.* **11**, 619–627 (1994).
55. Monack, D. M. *et al. Salmonella* exploits caspase-1 to colonize Peyer's patches in a murine typhoid model. *J. Exp. Med.* **192**, 249–258 (2000).
56. Ruckdeschel, K., Mannel, O. & Schrottner, P. Divergence of apoptosis-inducing and preventing signals in bacteria-faced macrophages through myeloid differentiation factor 88 and IL-1 receptor-associated kinase members. *J. Immunol.* **168**, 4601–4611 (2002).
57. Black, D. S. & Bliska, J. B. The RhoGAP activity of the *Yersinia pseudotuberculosis* cytotoxin YopE is required for antiphagocytic function and virulence. *Mol. Microbiol.* **37**, 515–527 (2000).
58. Fallman, M. *et al. Yersinia pseudotuberculosis* inhibits Fc receptor-mediated phagocytosis in J774 cells. *Infect. Immun.* **63**, 3117–3124 (1995).
59. Dramsi, S. & Cossart, P. Listeriolysin O: a genuine cytolysin optimized for an intracellular parasite. *J. Cell. Biol.* **156**, 943–946 (2002).
60. Eriksson, S. *et al. Salmonella typhimurium* mutants that downregulate phagocyte nitric oxide production. *Cell. Microbiol.* **2**, 239–250 (2000).
61. Uchiya, K. *et al.* A *Salmonella* virulence protein that inhibits cellular trafficking. *EMBO J.* **18**, 3924–3933 (1999).
62. Vazquez-Torres, A. *et al. Salmonella* pathogenicity island 2-dependent evasion of the phagocyte NADPH oxidase. *Science* **287**, 1655–1658 (2000).
63. Chakravortty, D., Hansen-Wester, I. & Hensel, M. *Salmonella* pathogenicity island 2 mediates protection of intracellular *Salmonella* from reactive nitrogen intermediates. *J. Exp. Med.* **195**, 1155–1166 (2002).
64. Fang, F. C. *et al.* Virulent *Salmonella typhimurium* has two periplasmic Cu, Zn-superoxide dismutases. *Proc. Natl. Acad. Sci. USA* **96**, 7502–7507 (1999).
65. Sturgill-Koszycki, S. *et al.* Lack of acidification in *Mycobacterium* phagosomes produced by exclusion of the vesicular proton-ATPase. *Science* **263**, 678–681 (1994).
66. Horstmann, R. D., Sievertsen, H. J., Knobloch, J. & Fischetti, V. A. Antiphagocytic activity of streptococcal M protein: selective binding of complement control protein factor H. *Proc. Natl. Acad. Sci. USA* **85**, 1657–1661 (1988).
67. Brown, E. J., Joiner, K. A., Gaither, T. A., Hammer, C. H. & Frank, M. M. The interaction of C3b bound to pneumococci with factor H (β1H globulin), factor I (C3b/C4b inactivator), and properdin factor B of the human complement system. *J. Immunol.* **131**, 409–415 (1983).
68. Wurzner, R. Evasion of pathogens by avoiding recognition or eradication by complement, in part *via* molecular mimicry. *Mol. Immunol.* **36**, 249–60 (1999).
69. van Heyningen, T. K., Collins, H. L. & Russell, D. G. IL-6 produced by macrophages infected with *Mycobacterium* species suppresses T cell responses. *J. Immunol.* **158**, 330–337 (1997).
70. Giacomini, E. *et al.* Infection of human macrophages and dendritic cells with *Mycobacterium tuberculosis* induces a differential cytokine gene expression that modulates T cell response. *J. Immunol.* **166**, 7033–7041 (2001).
71. Toossi, Z., Gogate, P., Shiratsuchi, H., Young, T. & Ellner, J. J. Enhanced production of TGF-β by blood monocytes from patients with active tuberculosis and presence of TGF-β in tuberculosis granulomatous lung lesions. *J. Immunol.* **154**, 465–473 (1995).
72. Moore, K. W., O'Garra, A., de Waal Malefyt, R., Vieira, P. & Mosmann, T. R. Interleukin-10. *Annu. Rev. Immunol.* **11** 165–190 (1993).
73. Zuany-Amorim, C. *et al.* Suppression of airway eosinophilia by killed *Mycobacterium vaccae*-induced allergen-specific regulatory T-cells. *Nature Med.* **8**, 625–629 (2002).
74. McGuirk, P., McCann, C. & Mills, K. H. G. Pathogen-specific T regulatory 1 cells induced in the respiratory tract by a bacterial molecule that stimulates interleukin 10 production by dendritic cells: a novel strategy for evasion of protective T helper type 1 responses by *Bordetella pertussis*. *J. Exp. Med.* **195**, 221–231 (2002).
75. Sing, A., Roggenkamp, A., Geiger, A. M. & Heesemann, J. *Yersinia enterocolitica* evasion of the host innate immune response by V antigen-induced IL-10 production of macrophages is abrogated in IL-10-deficient mice. *J. Immunol.* **168**, 1315–1321 (2002).
76. Ruckdeschel, K. *et al. Yersinia enterocolitica* impairs activation of transcription factor NF-κB: involvement in the induction of programmed cell death and in the suppression of the macrophage tumor necrosis factor α production. *J. Exp. Med.* **187**, 1069–1079 (1998).
77. Schesser, K. *et al.* The YopJ locus is required for *Yersinia*-mediated inhibition of NF-κB activation and cytokine expression: YopJ contains a eukaryotic SH2-like domain that is essential for its repressive activity. *Mol. Microbiol.* **28**, 1067–1079 (1998).
78. Orth, K. *et al.* Inhibition of the mitogen-activated protein kinase kinase superfamily by a *Yersinia* effector. *Science* **285**, 1920–1923 (1999).
79. Kayal, S. *et al.* Listeriolysin O-dependent activation of endothelial cells during infection with *Listeria monocytogenes*: activation of NF-κB and upregulation of adhesion molecules and chemokines. *Mol. Microbiol.* **31**, 1709–1722 (1999).
80. Kayal, S. *et al.* Listeriolysin O secreted by *Listeria monocytogenes* induces NF-κB signalling by activating the IκB kinase complex. *Mol. Microbiol.* **44**, 1407–1419 (2002).
81. Wick, M. J., Harding, C. V., Twesten, N. J., Normark, S. J. & Pfeifer, J. D. The phoP locus influences processing and presentation of *Salmonella typhimurium* antigens by activated macrophages. *Mol. Microbiol.* **16**, 465–476 (1995).
82. Niedergang, F., Sirard, J.-C., Blanc, C. T. & Kraehenbuhl, J.-P. Entry and survival of *Salmonella typhimurium* in dendritic cells and presentation of recombinant antigens do not require macrophage-specific virulence factors. *Proc. Natl. Acad. Sci. USA* **97**, 14650–14655 (2000).
83. Molinari, M. *et al.* Selective inhibition of Ii-dependent antigen presentation by *Helicobacter pylori* toxin VacA. *J. Exp. Med.* **187**, 135–140 (1998).
84. Ferrari, G., Naito, M., Langen, H. & Pieters, J. A coat protein on phagosomes involved in the intracellular survival of mycobacteria. *Cell* **97**, 435–447 (1999).
85. Ullrich, H.-J., Beatty, W. L. & Russell, D. G. Interaction of *Mycobacterium avium*-containing phagosomes with the antigen presentation pathway. *J. Immunol.* **165**, 6073–6080 (2000).
86. Ramachandra, L., Noss, E., Boom, H. W. & Harding, C. V. Processing of *Mycobacterium tuberculosis* antigen 85B involves intraphagosomal formation of peptide-major histocompatibility class II complexes and is inibited by live bacilli that decrease phagosome maturation. *J. Exp. Med.* **194**, 1421–1432 (2001).
87. Stenger, S., Niazi, K. R. & Modlin, R. L. Down-regulation of CD1 on antigen-presenting cells by infection with *Mycobacterium tuberculosis*. *J. Immunol.* **161**, 3582–3588 (1998).
88. Hmama, Z., Gabathuler, R., Jefferies, W. A., de Jong, G. & Reiner, N. E. Attenuation of HLA-DR expression by mononuclear phagocytes infected with *Mycobacterium tuberculosis* is related to intracellular sequestration of immature class II heterodimers. *J. Immunol.* **161**, 4882–4893 (1998).
89. Wojciechowski, W., DeSanctis, J., Skamene, E. & Radzioch, D. Attenuation of MHC class II expression in macrophages infected with *Mycobacteirum bovis* Bacillus Calmette-Geurin involves class II transactivator and depends on the Nramp1 gene. *J. Immunol.* **163**, 2688–2696 (1999).
90. Noss, E. H. *et al.* Toll-like receptor 2-dependent inhibition of macrophage class II MHC expression and antigen processing by 19-kDa lipoprotein of *Mycobacterium tuberculosis*. *J. Immunol.* **167**, 910–918 (2001).
91. Ting, L.-M., Kim, A. C., Cattamanchi, A. & Ernst, J. D. *Mycobacterium tuberculosis* inhibits IFN-γ transcriptional responses without inhibiting activation of STAT1. *J. Immunol.* **163**, 3898–3906 (1999).
92. Zhong, G., Fan, T. & Liu, L. *Chlamydia* inhibits interferon-γ–inducible major histocompatibility complex class II expression by degradation of upstream stimulatory factor 1. *J. Exp. Med.* **189**, 1931–1937 (1999).
93. Zhong, G., Liu, L., Fan, T., Fan, P. & Ji, H. Degradation of transcription factor RFX5 during the inhibition of both constitutive and interferon γ-inducible major histocompatibility complex class I expression in *Chlamydia*-infected cells. *J. Exp. Med.* **191**, 1525–2534 (2000).
94. van den Elsen, P. J., Peijnenburg, A., van Eggermond, M. C. & Gobin, S. J. Shared regulatory elements in the promoters of MHC class I and class II genes. *Immunol. Today* **19**, 308–312 (1998).
95. Wang, J. *et al.* Negative selection of T cells by *Helicobacter pylori* as a model for bacterial strain selection by immune evasion. *J. Immunol.* **167**, 926–934 (2001).
96. Yao, T., Mecsas, J., Healy, J. I., Falkow, S. & Chien, Y.-H. Suppression of T and B lymphocyte activation by a *Yersinia pseudotuberculosis* virulence factor, YopH. *J. Exp. Med.* **190**, 1343–1350 (1999).
97. Persson, C., Carballeira, N., Wolf-Watz, H. & Fällman, M. The PTPase YopH inhibits uptake of *Yersinia*, tyrosine phosphorylation of p130Cas and FAK, and the associated accumulation of these proteins in peripheral focal adhesions. *EMBO J.* **16**, 2307–2318 (1997).
98. Boulton, I. C. & Gray-Owen, S. D. *Neisserial* binding to CEACAM1 arrests the activation and proliferation of $CD4^+$ T lymphocytes. *Nature Immunol.* **3**, 229–236 (2002).
99. Nassif, X., Pujol, C., Morand, P. & Eugene, E. Interactions of pathogenic *Neisseria* with host cells. Is it possible to assemble the puzzle? *Mol. Microbiol.* **32**, 1124–1132 (1999).
100. Shiloh, M. U. *et al.* Phenotype of mice and macrophages deficient in both phagocytic oxidase and inducible nitric oxide synthase. *Immunity* **10**, 29–38 (1999).
101. Hooper, L. V. *et al.* Molecular analysis of commensal host-microbial relationships in the intestine. *Science* **291**, 881–884 (2001).