Published in final edited form as:

Nat Rev Immunol. 2013 March ; 13(3): 199–206. doi:10.1038/nri3398.

Effector-triggered versus pattern-triggered immunity: how animals sense virulent pathogens

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Abstract

A fundamental question of any immune system is how it can discriminate between pathogens and non-pathogens. Here, we discuss that this can be mediated by a surveillance system distinct from pattern recognition receptors that recognize conserved microbial patterns and can be based instead on the host's ability to sense perturbations in host cells induced by bacterial toxins or 'effectors' that are exclusively encoded by virulent microorganisms. Such 'effector-triggered immunity' was previously thought to be restricted to plants, but recent data indicate that animals also use this strategy.

Introduction

During an infection, bacterial products are detected by an array of Toll-like receptors (TLRs) or other pattern recognition receptors (PRRs) [G], leading to the activation of immune signalling pathways and defence mechanisms that co-operate to protect the host. When this theory was initially proposed by Janeway in 1989¹, the full repertoire of microbial ligands that engage these pattern-recognition pathways was unknown but they were collectively referred to as pathogen-associated molecular patterns (PAMPs) [G]. The elegant system of recognition of PAMPs by PRRs predicted by Janeway has now been demonstrated in many systems and has served as a guiding principle for how the host recognizes and responds to pathogen attack². As a consequence of extensive work carried out over the past 15 years, we now know that the TLRs specifically recognize and respond to microbial molecules, such as lipopolysaccharide and flagellin that decorate Gram-negative bacteria, and lipopeptides that are essential constituents of the Gram-positive bacterial cell wall³. However, as an increasing number of PRR ligands was identified, it became evident that these ligands are not unique to pathogenic bacteria, but are also found in the non-pathogenic and commensal bacterial strains that make up our microbiota and with whom we have a beneficial and often mutualistic relationship⁴. Thus, it became apparent

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that PAMP was a misnomer and that these ligands are now described more appropriately as microbe-associated molecular patterns (MAMPs) [G].

The realization that the ligands recognized by PRRs are in fact often shared by pathogens and commensals has recently prompted some in the field of host-pathogenesis to revisit the question of how the host responds to virulent microorganisms. In particular, it has raised the question of how the host augments the immune response specifically to pathogens while remaining tolerant to beneficial commensal bacteria^{5,6}. Several interesting hypotheses have been proposed. One idea that emerged in the 1990s was the Danger Theory, proposed by Matzinger as a parallel system to the PAMP-PRR model of microbial recognition. Matzinger suggested that the immune system does not recognize pathogens *per se*, but rather recognizes the damage they cause⁷. In this model no distinction is made as to how the damage occurs and the model implies that it could be elicited either by sterile injury or infectious agents. Matzinger later proposed that there might be specific damage-associated molecular patterns (DAMPs) [G], and that these provide contextual cues to the immune system that there is a threat to the host^{8,9}. The Danger hypothesis is now well accepted in the context of sterile inflammation [G], and several self-derived immune elicitors have now been identified, such as uric acid and high-mobility group box 1 (HMGB1), both of which are released by damaged cells^{10,11}. However, whether DAMPs contribute to immune activation during infection is less clear and, moreover, it is not known whether these DAMP-triggered immune responses are beneficial to the host or only serve to cause immune pathology.

Another possible mechanism of discrimination between pathogen and non-pathogen suggested by Vance, Isberg and Portnoy is the importance of the location of recognition¹². At a subcellular level, the ‘patterns-of-pathogenesis’ hypothesis was suggested by the observation that the most robust immune responses are induced by cyto-invasive pathogens such as *Listeria* spp. that breach the immunological sanctity of the cytosol, which is normally devoid of bacterial products, and trigger a strong immune response¹³. In a somewhat analogous manner, immune responses can also vary if bacteria are recognized in different tissue compartments. As an example, the immune response elicited by bacteria that are in the lumen of the gut — and hence interacting with the apical surface of the epithelial monolayer — is less vigorous than if the epithelial monolayer is breached and the basal membrane exposed to bacterial products¹⁴. In this latter case, the discrimination is achieved by the sequestration of TLRs from the apical membrane into endosomes or to the basal aspect of the cell¹⁴, such that these TLRs can only interact with pathogenic invasive microorganisms that traverse the monolayer.

A third model to explain how a host distinguishes pathogens from commensals is that the host monitors bacterial viability; an increase in viability serves as a cue that a potential infection is not being controlled and that the immune response needs to be amplified¹⁵. These and other ideas describing how the immune response is regulated proportionate to the level of perceived threat have been the focus of excellent recent commentaries^{12,16,17} and will not be explored further here. Instead, we focus on one particular system that allows the host to distinguish pathogen from non-pathogen, which has been termed effector-triggered immunity (ETI) [G] (Figure 1). This mechanism of immune activation is probably of

particular relevance in non-professional immune cells, such as epithelial cells, that don't display the full repertoire of PRRs. Although the remainder of this article discusses only ETI, it is important to note that it is likely that several, if not all of these models and pathways co-operate with conventional PRRs to ultimately define the level of response to a pathogenic microorganism. For simplicity, we will focus broadly on the idea of discriminating 'pathogen' from 'non-pathogen'. We acknowledge that microbes should be considered as a spectrum that includes commensal, opportunist, mutualist and pathogens depending on their state and the state of the host but, due to space constraints, we will not discuss here the potential role of effectors in these host-microbe interactions.

The origins of effector-triggered immunity

The idea of ETI grew out of the work of the plant immunologist, Flor, who originally described an idea he termed "gene-for-gene resistance" in the 1940s¹⁸. This hypothesis was based on the simple observation that for an identical microbial infection some plants are susceptible and others are resistant¹⁸. Based on this observation, Flor proposed a one to one relationship between a pathogen avirulence gene (*avr* gene) [G] and a plant resistance gene (*r* gene) [G]. According to this theory, resistant plants have in their genome an *r* gene that is required for the recognition of a specific *avr* gene encoded by the pathogen. Recognition of the *avr* gene by the *r* gene was hypothesized to be sufficient to trigger a signalling cascade leading to plant immunity and a resistant phenotype¹⁹. This theory was a provocative model that intrigued the field of modern plant immunity for more than 50 years. The discovery of the first *avr* genes and the corresponding *r* genes in the 1980s validated Flor's hypothesis²⁰. Various theories were proposed in an attempt to explain the molecular details, but after it became apparent that many *avr* genes encode bacterial effectors, plant immunologists settled on the term effector-triggered immunity (ETI)^{21,22}. ETI is defined as the detection of microbial effectors that triggers a protective immune response for the host. Various direct and indirect mechanisms of effector recognition have been suggested (Box 1). One particularly intriguing idea was an early proposition by Dangl and Jones termed the Guard Hypothesis²³. In this model, the products of *r* genes monitor or 'guard' those crucial cellular processes that are often targeted and modified by the toxins secreted by pathogens. Although the Guard hypothesis has gained the most widespread recognition, it is somewhat restrictive in its purest application, and hence we use the more encompassing term of ETI.

While it has been speculated that ETI might play a role in animals, several recent papers^{24–30} have provided some of the first mechanistic descriptions of this in animal hosts. This has prompted us to review the evidence that suggests the existence of alternative mechanisms of immune activation that are distinct from PRRs and to consider how ETI might apply to pathogen recognition in metazoans. Like the plant system, the model of effector-triggered immunity in animals suggests that the 'pathogenic potential' of a microorganism is sensed because it expresses toxins that are absent from commensal and non-pathogenic bacteria. Unlike plants, there is currently no evidence that animals directly recognize these pathogen effectors; all the known examples suggest that bacterial effectors are recognized indirectly because of the consequences of their activity on cellular homeostasis or the alterations they cause in their specific cellular targets (described in more detail below).

The complex interplay between bacterial effectors and the immune system

Pathogenesis can be considered as the ability of a microorganism to multiply and do damage to its host^{31,32}. To facilitate replication, pathogens secrete toxins and bacterial effectors, many of which enter the host cytosol through an array of bacterial secretion systems or, in the case of some toxins, by translocating across the plasma membrane³³. Although the term ‘effector’ has been used to specifically describe bacterial proteins that are introduced directly into the host cell by a type III secretion system [G], for the purposes of this article we use the term ‘effector’ more loosely to describe any bacterial toxin with intracellular targets, regardless of its mechanism of delivery. Once pathogen effectors gain access to the cytoplasm they interact with host proteins and, in many cases, efficiently inhibit various aspects of the immune response including phagocytosis, cell motility and immune signalling pathway activation^{34,35}. These effectors are often required for the microorganism to establish itself within its host and hence were often collectively referred to as ‘virulence’ factors. So far, large numbers of bacterial effectors have been identified that have an immune-inhibitory activity³⁶, probably because this has been a focus of the field. Although the idea that effectors primarily mediate immune evasion is logically appealing, studies in plants and recent work in animals have shown that some pathogen effectors actually elicit an immune response (Table 1), and that this can be protective for the host. Although perhaps counterintuitive if considered solely from an immune perspective, as discussed below, these responses can be understood when considered in the context co-evolved host-pathogen dynamics. In plants the molecular details of how this ETI occurs are beginning to emerge, and it is clear that pathogen effectors can be recognized either directly or indirectly through their action on host components (Box 1). Additionally, although not the focus of this review, there are examples of secreted toxins that act extracellularly and can induce a defense reaction³⁷.

Although ETI has a significant role in determining the outcome of the host–pathogen interaction in plants, the discovery of *Arabidopsis thaliana* FLS2 as a PRR that recognizes bacterial flagellin as a MAMP indicated that, like animals, plants also use PRR-mediated sensing³⁸. Indeed, FLS2 has marked similarities to TLR5, which detects bacterial flagellin in mammals. To conform with the ETI terminology, plant immunity triggered by the recognition of MAMPs by PRRs has been termed pattern-triggered immunity (PTI) [G] (also referred to as PRR-, PAMP- and MAMP-triggered immunity)^{21,22,39}. Developing further on these observations, recent efforts have built an integrated framework of plant immunity that incorporates both ETI and PTI and have generated models that describe the co-operation between these two arms of the plant immune response. One particular model, the ‘Zig-Zag’ model [G], integrates the quantitative output of the two arms of the plant immune system for optimal defence and illuminates the molecular dialogue and co-evolution between ETI and PTI that occurs in plants during pathogen–host interactions²².

Examples of effectors that trigger immune responses in metazoans

The identification of PTI as well as ETI in plants raised the possibility that there are more similarities between how plants and animals orchestrate microbial detection than were previously appreciated^{38,40,41}. Extrapolating from this, it seemed plausible that ETI also

occurs in metazoans but might have been overlooked because the field was highly focused on PRRs as the primary mechanism of immune surveillance.

Modification of signaling molecules

For example, we recently showed that the *Escherichia coli* effector CNF1 elicits a vigorous ETI response in *Drosophila melanogaster*²⁴. As with the other effectors described herein, the host does not directly detect the presence of CNF1; rather, CNF1 is sensed after it modifies a host protein, the Rho GTPase Rac2. CNF1 causes the deamidation of glutamine 61 of *D. melanogaster* Rac2, converting it to glutamic acid and locking the protein in an active GTP-bound state. The active form of Rac2 triggers membrane ruffling to facilitate bacterial entry across the epithelial barrier. However, this modified Rac2 also interacts with a proximal immune adaptor protein IMD to induce activation of the IMD innate immune signalling pathway [G]. Notably, flies lacking the PRRs that recognize *E. coli* (PGRP-LC and PGRP-LE) still responded to intoxication with CNF1 indicating that CNF1 is sufficient to induce a defence response in the absence of PRR ligation. Importantly, this response to CNF1 was protective and could confer resistance to bacterial challenge. Observations in mammalian epithelial cells have confirmed that a similar CNF1-mediated ETI response occurs after the modification of human Rac2 and its interaction with the IMD homologues RIP1 and RIP2, which drive NF- κ B activation (Figure 2). Taken together, these data provide not only an example of ETI in metazoans but also a credible molecular mechanism for how it might occur; they thus helped to establish ETI as a sensing system that is not limited to plants but also found in animals.

Translation inhibition

Additional studies support the existence of ETI in animals. Several examples have emerged from studies of pathogen effectors that disrupt protein translation^{27–30,42}. Translation is an essential cellular process that is of particular importance during microbial infection when chemokines, cytokines and antimicrobial peptides need to be produced rapidly and at high levels by host cells. Many bacteria and viruses have evolved strategies to disrupt host translation — for example, a quintet of effectors from *Legionella pneumophila* (lgt1, lgt2, lgt3, sidI and sidL) have been shown to have this activity²⁷. Interestingly, these effectors were recently shown to be sufficient to induce mitogen-activated protein (MAP) kinase activation and to augment ongoing nuclear factor- κ B (NF- κ B) signalling²⁸ (Figure 2). In the latter case, this was attributed to blocking the translation of inhibitor of NF- κ B (I κ B); as I κ B is a negative regulator of the TLR pathway, its loss is predicted to result in prolonged and increased NF- κ B signalling and cytokine expression downstream of TLR ligation (Figure 2). These data are in keeping with earlier observations showing that the response to *L. pneumophila* was highly dependent on the *Legionella* Dot/Icm secretion system that delivers effectors into the host cells^{43,44}. Intriguingly, although the molecular details of how this occurs were not exhaustively explored, it was shown that the host response was triggered independently of the TLR and NOD2 PRR pathways, and hence was consistent with it being an ETI response⁴³.

Recent studies in *Caenorhabditis elegans* have revealed the contribution of ETI induced by translation inhibition during *in vivo* infection using a different system^{29,30,42}. These studies

used *Pseudomonas aeruginosa*, which can be a highly virulent pathogen in many species, including *C. elegans*. It has been known for several years that *C. elegans* mounts a robust transcriptional response when infected by *P. aeruginosa* as a consequence of the activation of several pathways, including the worm equivalent of the p38 MAP kinase pathway (the *pmk-1* pathway). However, investigators had failed to identify a bona fide PRR–MAMP recognition system in *C. elegans* that responds to *P. aeruginosa*. Two recent papers showed that a major component of the response to *P. aeruginosa* is triggered not by the interaction of a PRR with a MAMP, but rather in response to a *P. aeruginosa* exotoxin, ToxA^{29,30}, which blocks the elongation of polypeptide chains through the ribosylation of elongation factor 2 (EF2) and functions to prevent translation of the crucial host defence molecules that are induced rapidly by the innate immune response. Importantly, ToxA is not recognized per se. Instead, the activity of ToxA that inhibits protein synthesis by blocking the activity of EF2 is sensed. This translational inhibition in *C. elegans* paradoxically induces ZIP-2 translation, which then induces an immune transcriptional response. Interestingly, the immune response elicited by ToxA also protected the worms from ToxA-mediated toxicity.

Pore-forming toxins

Several other effectors might be considered in this framework as elicitors of ETI (Table 1), a particularly interesting group of which is the pore-forming toxins. These are a diverse group of proteins with divergent structures, peptide sequences and target cells that have a common function of generating pores in the plasma or endosomal membrane. When present in high concentrations, pore-forming toxins cause severe membrane disruption, leakage of intracellular contents and rapid cellular death due to lysis. However, at lower concentrations the host cells can respond to the damage generated by pore-forming toxins and activate ETI responses in both *C. elegans* and mammals^{45–48}. Although the precise molecular mechanisms remain unclear, it seems that the efflux of K⁺ and the influx of Ca²⁺ ions caused by pore-forming toxins has a major role in the immune activation (Figure 2). In mammals, this results in two types of response: an antimicrobial response and a repair response. These two complementary outcomes are mediated by the activation of MAP kinases and stress response pathways^{48–51} and the NLPR3 inflammasome, which results in secretion of IL-1 β and IL-18^{46,47} and regulates sterol regulatory element binding proteins (SREBPs) that orchestrate membrane repair⁴⁷. Repair can also be achieved through other pathways such as endocytosis/exocytosis of damaged membrane and the imbedded toxins^{52,53}. An interesting point raised by these studies of pore-forming toxins is that the ETI is not non-specific, but rather can elicit pathways that are appropriate to counteract the initiating insult (Figure 2). As an example, pore formation induces a membrane repair response that is likely to be important for facilitating tolerance to the bacterial burden and maintaining the fitness of the host⁵⁴. In contrast, the response to other microbial toxins induces NF- κ B signaling pathways and cytokine secretion that recruits neutrophils and activates cellular defences.

Together, these examples of responses of phylogenetically distant hosts (*C. elegans*, *D. melanogaster* and mammals) to disparate bacterial species (*E. coli*, *L. pneumophila* and *P. aeruginosa*) suggest that ETI is not restricted to plants but is actually an evolutionarily conserved strategy used by all species to sense and respond to virulent pathogens.

Highly evolved pathogens manipulate ETI to promote infection and dissemination

Whereas many bacteria prefer to evade the host immune response and to effectively colonize by stealth, other bacteria take the opposite approach and use the inflammatory response for their benefit. As an example, *Salmonella* and *Shigella* spp. deliberately activate immune signalling and inflammation in the gut as a means of disrupting the epithelial barrier and eradicating the commensal bacteria to help establish themselves in a particular niche. Furthermore, this severe intestinal inflammation leads to diarrhea, which has a pivotal role in the dissemination of the bacteria between hosts. Although it is unclear exactly how these enteropathogens trigger such florid inflammation in the gut, work in *Salmonella* spp. provides some evidence that this may be, at least in part, due to the entry of pro-inflammatory effectors into host cells in the gut. Supporting this proposition, at least two studies have suggested that *Salmonella typhimurium* effectors can induce ETI responses *in vivo*. This was first speculated to explain observations made *in vivo* in a *Drosophila* model system, when it was noted that the mutant bacteria that lacked the type III secretion system unexpectedly grew to higher concentrations and were less effectively cleared from the fly²⁶. The ability of *Salmonella* effectors to induce immune responses was then confirmed in mammals²⁵ and the molecular mechanism of this ETI explored. A trio of *Salmonella typhimurium* effectors that are delivered by the type III secretion system — SopE, SopE2 and SopB — have been shown to induce ETI responses in mammalian cells. SopE and SopE2 are guanidyl nucleotide exchange factors that seem to be partially redundant, and SopB functions as a phosphoinositide phosphatase that activates Rho-family GTPases and induces the activation of NF- κ B signalling. The immune activation induced by SopE, SopE2 and SopB seems to be largely dependent on the Rho GTPase CDC42. However, CDC42 depletion does not completely abrogate immune activation raising the possibility that additional GTPases might also be involved²⁵. As confirmation of the important contribution of ETI, infection of cultured epithelial cells with triple mutant *S. typhimurium* lacking these three effectors elicits a similar host transcriptional response to that elicited by bacteria that lack the entire type III secretion system. This suggests that SopE, SopE2 and SopB are the main *S. typhimurium* effectors responsible for induction of ETI in the epithelium. A similar pro-inflammatory function has been attributed to the *Yersinia pseudotuberculosis* effector YopJ, which both inhibits MAP2 and MAP3 kinases and simultaneously activates caspase-1^{55–58}. Caspase-1 activation in this context disrupts the intestinal barrier and has been suggested to promote infection and bacterial dissemination. Together these recent examples suggest that highly evolved pathogens have learnt to use ETI to their advantage; as the inflammatory response in these cases favours the pathogen and not the host, we would suggest it might be better termed effector-triggered immune-pathology (ETIP) [G]. Indeed ETIP that occurs in late stages of infection might be integral to dissemination of the pathogen within a population (Figure 3).

Common themes in the molecular details of ETI

In most cases, the mechanisms responsible for ETI in metazoans remain incompletely understood and, with the exception of CNF1, the molecular details remain speculative. For

this reason, it is hard to generalize beyond identifying features of ETI that are common between animals and plants (Box 1). As an example, for the majority of pathogen effectors, eliminating their enzymatic activity disrupts their ability to induce an immune response. This observation suggests that recognition of the effector is indirect and triggered after sensing the consequence of the effector rather than direct recognition of the effector *per se*. Despite often targeting similar host pathways and having similar functional outcomes, bacterial effectors are often structurally very different. For this reason, a system of direct recognition would necessitate the evolution of a huge array of receptors able to accommodate such a structurally diverse set of ligands. Instead, through indirect recognition the host is able to monitor for perturbations in a few key cellular processes or proteins and, irrespective of how these modifications occur, mount an immune response. Thus, the repertoire of host sensing mechanisms required for indirect recognition of bacterial effectors could be quite parsimonious while remaining responsive to a large spectrum of pathogens. Indeed, to minimize the ‘cost’ to the host, the recognition machinery is probably limited to monitor only those cellular processes considered essential. Thus, the potential for not only direct but also indirect recognition of virulent bacteria is one of the most versatile features of ETI as a proposed mechanism of immune surveillance.

Conclusion and Perspective

Prompted by compelling studies that demonstrated the important role of ETI in plants, we and others have investigated whether this phenomena also occurs in metazoans. We recently showed that an *E. coli* effector can elicit a vigorous response in an animal host (*D. melanogaster*). This work, along with studies of *Salmonella*^{25,26}, *Legionella*^{27,28} and *Pseudomonas*^{29,30} helped to establish a precedent for ETI as playing an integral role in modifying how animals respond to a variety of potentially pathogenic microbes. This growing body of evidence has led us to revise our thinking about mechanisms of pathogen recognition in animals to accommodate ETI as an important system of immune surveillance that functions in a synergistic and/or parallel manner with pattern-triggered immunity. This provides a fresh perspective on the recognition of virulent bacteria and our future challenge will be to understand the molecular details of how ETI is induced by different effectors as well as its relative importance in the host response to different pathogens. Once equipped with that information, it is possible that therapeutic strategies could be developed to induce beneficial ETI responses, such as stimulating mucosal defences or developing mucosal adjuvants. Conversely, understanding how highly evolved pathogens such as *Salmonella* and *Shigella* spp. use ETI to their advantage will provide new insights into pathogenesis and help to identify anti-infectives that target this particular virulence strategy.

Acknowledgments

The authors would like to thank E. Lemichez, F. Ausubel, A. Lacy-Hulbert and J. Irazoqui for their helpful discussion and critical reading of the manuscript. N.P. was supported by NIH/NIAID/NERCE (U54 AI057159), L.B. by Ligue Nationale Contre le Cancer, by institutional funding from INSERM, grants from Fondation Infectiopôle Sud and Fondation ARC pour la Recherche sur le Cancer (ARC SFI 20111203659 and ARC SFI 20121205382) and L.M.S. by grants from NIH/NIAID.

Definitions

Pattern-recognition receptors (PRRs)	Proteins expressed by innate immune cells that detect molecules associated with microbial pathogens or cellular stress
Pathogen-associated molecular patterns (PAMPs)	Originally described the term given to the immune elicitors from bacteria, but not mammalian cells, that bind PRRs and drive immune responses. 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. This term has now been superseded by MAMPs
Microorganism-associated molecular patterns (MAMPs)	After the realization that PAMPs are found in both pathogens and non-pathogens, the term MAMPs was suggested as a more appropriate term for the microbial ligands that bind PRRs
Effector-triggered immunity (ETI)	The detection of and response to microbial effectors that enter the host cell and trigger a protective immune response by the host
Damage-associated molecular patterns (DAMPs)	Self-derived immune elicitors that indicate danger to the host. As a result of cellular stress, cellular damage and non-physiological cell death, DAMPs are released from the degraded stroma (for example, hyaluronate), from the nucleus (for example, high-mobility group box 1 protein, HMGB1) and from the cytosol (for example, ATP, uric acid, S100 calcium-binding proteins and heat-shock proteins). Such DAMPs are thought to elicit local inflammatory reactions
Sterile inflammation	Inflammation triggered by the danger signals induced by chemical or physical stress, in the absence of infection
Avirulence gene (<i>avr</i> gene)	The original plant terminology for bacterial effectors that induced ETI responses in the resistant host
Resistance gene (<i>r</i> gene)	The original plant terminology for the host gene product required for the direct or indirect sensing of an effector that elicits the protective immune response
Type III secretion system	A specialized molecular machine present in some bacteria that allows translocation of bacterial proteins into host cells
Pattern-triggered immunity (PTI)	The immune response elicited after ligation of a PRR by its microbial ligand. A more appropriate term would in fact be MAMP-triggered immunity, for the reasons described above
‘Zig-Zag’ model	Model proposed to illustrate the quantitative output of the plant immune system and to explain the co-evolution of plant resistance genes and pathogen effectors

IMD innate immune signalling pathway	One of two innate immune NF- κ B signaling pathways in <i>Drosophila</i> which responds to DAP type peptidoglycan from Gram-negative, and some Gram-positive, bacteria leading to rapid and robust production of antimicrobial peptides
Effector-triggered susceptibility (ETS)	Immune suppression or evasion caused by bacterial effectors that enter the host-cell and disrupt defense pathways
Effector-triggered immune-pathology (ETIP)	Effectors that elicit an over-exuberant immune response that benefits the pathogen and is to the detriment of the host

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Box 1: Potential host strategies for sensing pathogen effectors

Susceptible strains (Red box)

During the infection of a susceptible host that lacks resistance to a particular pathogen, the activity of bacterial effectors (grey hexagon) on their target protein (light grey circle) goes unchecked and leads the host to be susceptible to infection with that specific pathogen.

Resistant hosts (green and blue boxes)

In a resistant host, pathogen effectors can be sensed directly (**green box**) or indirectly (**blue boxes**) and result in a protective immune response.

Receptor-ligand model

Direct recognition occurs when a pathogen effector is directly recognized by a host receptor. Evidence of direct recognition of pathogen effectors in animals is currently lacking.

Guard hypothesis

In the guard model, certain key cellular targets are 'guarded' by a specific R protein and an immune response is evoked if they are perturbed. In model 1, engagement of the host target by the pathogen effector results in modification of the target (brown box). This modification is recognized by the R protein to signal an immune response. In an alternative model (model 2), the R protein guard and the host target 'guardee' exist together in the healthy state and the response is repressed. During infection, the modification of the host target protein (guardee) by the pathogen effector liberates the R protein (guard) allowing it to engage signalling pathways and induce a defence response.

Decoy model

The decoy model suggests that the host has deliberately evolved proteins that are decoys for the targets of pathogen effectors and function as sentinels for the presence of these effectors. These decoys could arise through gene duplication events or could be splice-variants of the normal target. However, decoys are generally thought to have no other primary function within the cell.

Bait-and-switch model

In this case, the pathogen effector is not sensed after binding its true target but rather is 'baited' by a decoy protein that engages an R protein whose function switches to induce signalling once the pathogen effector is bound.

(Figure adapted from Hann and Boller⁵⁹).

Figure 1. An integrated model of the detection of virulent bacteria

a, During a bacterial infection, microorganism-associated molecular patterns (MAMPs) ligate pattern recognition receptors (PRRs) and induce immune signalling pathways and pattern-triggered immunity (PTI). **b,** Secreted effector proteins from evolved bacteria suppress the immune response induced by PRRs to promote infection. **c,** Resistant hosts can sense the presence of effectors and activate effector-triggered immunity (ETI). ETI can occur independently of the primary immune signalling pathways or by amplifying the ongoing PTI. **d,** In non-immune cells that do not express a full complement of PRRs (such as epithelial cells), detection of pathogenic bacteria may occur primarily through ETI response pathways.

Figure 2. The outputs of effector-triggered immune responses are specific to the initiating type of damage

Different ETI responses are associated with different outputs that can specifically counteract the original insult. **Left**, After translocation into the cytoplasm, *Escherichia coli*-derived CNF1 deamidates RAC, which disrupts its intrinsic GTPase activity and locks it in an active GTP-bound state. Rac then interacts with RIP to induce immune signalling and nuclear factor- κ B (NF- κ B) activation that results in a robust antibacterial response. **Center**, Translation inhibition cause by *Legionella pneumophila* effectors induces a stress response through mitogen-activated protein kinase (MAPK) and associated pathways and amplifies NF- κ B activation by causing loss of the NF- κ B inhibitor, I κ B. **Right**, Bacterial pore-forming toxins cause potassium efflux and decreased intracellular potassium concentrations. This low potassium is sensed through an unknown mechanism and induces a stress response (a p38 MAPK signalling pathways in mammals and JNK MAPK in *C. elegans*), and activation of the NLRP3 inflammasome. Inflammasome activation is associated with both an antimicrobial IL-1 β response and cell autonomous membrane repair through SREBP. The figure focuses primarily on the mechanisms that have been shown in mammals and does not represent the totality of pathways demonstrated in all species. As an example, ETI in response to translational inhibition in worms is mediated by ZIP2 and is not shown.

Figure 3. The co-evolution of pathogenic bacteria with the host's immune response

In the absence of an immune response bacteria quickly overwhelm their host. To contain these infections the host has evolved pattern-triggered immunity such as TLRs. To deal with the threat from the host, bacteria become more virulent by evolving inhibitory effector proteins, which disrupt host immune defenses. This effector-triggered susceptibility [G] promotes infection. In the next step of co-evolution, the host evolves the ability to detect the presence of effector proteins and induce effector-triggered immunity. In a final evolutionary step, hyper-virulent bacteria, such as *Shigella* spp. and *Salmonella typhimurium* have acquired the ability to deliberately activate immune signalling via activating effector proteins. This inflammatory response is associated with effector-triggered immune-

pathology, which promotes both the colonization of the bacteria and aids their dissemination.

Table 1

Bacterial effectors that trigger defense responses

Effector	Species	Modifies	Immune response	References
CNF1	<i>Escherichia coli</i>	Rac2	Activates NF- κ B Signaling	Boyer, 2011 ²⁴ .
Igt1, Igt2, Igt3, sidI, sidL	<i>Legionella pneumophila</i>	Translational Inhibitor	Activates NF- κ B and MAP kinase signaling	Fontana, 2011; Fontana, 2012 ^{27,28}
Exotoxin A (ToxA)	<i>Pseudomonas spp.</i>	E2F	Activates MAP kinase signaling	McEwan, 2012; Dunbar, 2012 ^{29,30}
Pore forming toxins	Widely distributed	Cytosolic ion concentration	Activates NLRP3 inflammasome, p38, JNK and SREBPs	Huffman, 2004; Gurcel, 2006; Porta, 2011; Bebien, 2012 ⁴⁷⁻⁵⁰
SopE, SopE2, SopB	<i>Salmonella spp.</i>	Rho GTPases (mainly Cdc42)	Activates NF- κ B signaling	Bruno, 2009 ²⁵
YopJ	<i>Yersinia spp.</i>	MAP2 and MAP3 kinases	Inhibits NF- κ B signaling	Paquette, 2012; Meinzer, 2012; Mittal, 2006; Mukherjee, 2006 ⁵⁵⁻⁵⁸
		RICK	Activates NLRP3 inflammasome	Meinzer, 2012 ⁵⁵