

# Effector triggered immunity

## Activation of innate immunity in metazoans by bacterial effectors

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Pathogenic bacteria produce virulence factors called effectors, which are important components of the infection process. Effectors aid in pathogenesis by facilitating bacterial attachment, pathogen entry into or exit from the host cell, immunoevasion, and immunosuppression. Effectors also have the ability to subvert host cellular processes, such as hijacking cytoskeletal machinery or blocking protein translation. However, host cells possess an evolutionarily conserved innate immune response that can sense the pathogen through the activity of its effectors and mount a robust immune response. This “effector triggered immunity” (ETI) was first discovered in plants but recent evidence suggest that the process is also well conserved in metazoans. We will discuss salient points of the mechanism of ETI in metazoans from recent studies done in mammalian cells and invertebrate model hosts.

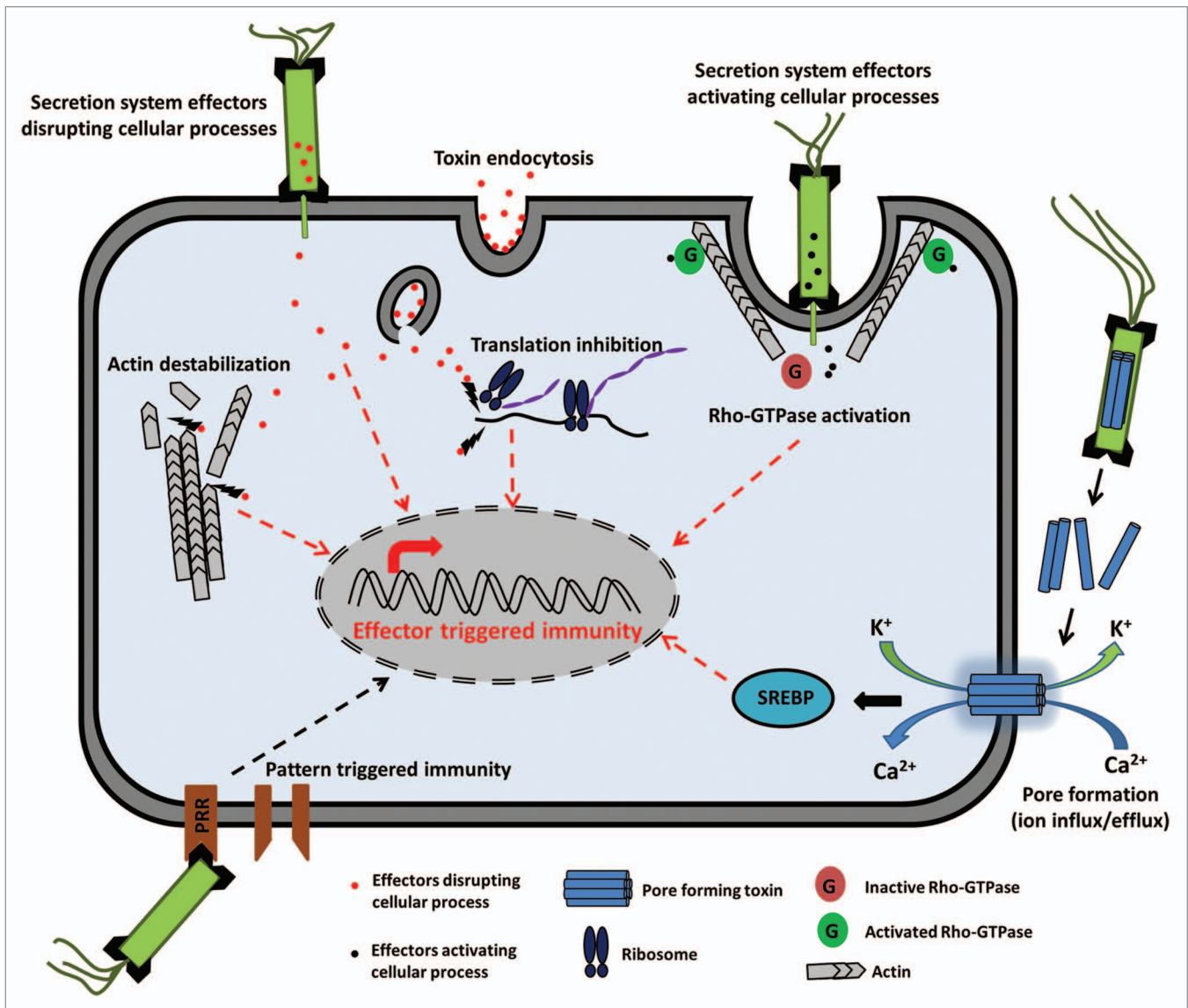
The immune system consists of two evolutionarily divergent arms: the sophisticated and specific adaptive immunity and the more generic innate immunity. Although the adaptive immune system confers long lasting and protective immunity, it takes several weeks to develop a sustained response and the majority of organisms lack this acquired immune system.<sup>1</sup> The innate immune system on the other hand, involves a population of cells and signaling pathways that constitutively function to respond rapidly to pathogens at the site of infection. Innate immune system thus forms the first line of defense, suppressing pathogens or keeping them at bay before the adaptive immune system takes over. This primitive form of immunity is present across multicellular organisms as disparate as nematodes, flies and vertebrates.<sup>2</sup> However, not all innate immunity signaling pathways are conserved among metazoans, the nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) pathway is conserved in vertebrates and flies but not worms whereas the mitogen-activated protein kinases (MAPK)

pathway is conserved among all the three. The focus of this review will be on innate immunity, particularly on “effector triggered immunity” (ETI), a process by which bacterial toxins or secreted proteins initiate a protective immune response in the host. During infection, pathogens secrete a broad array of virulence factors called “effector proteins”, which subvert the host cellular processes, including hijacking cytoskeletal machinery, blocking translation and suppressing the immune response.<sup>3,4</sup> Non-professional immune cells such as epithelial cells depend on ETI to respond quickly and robustly to pathogens, especially since these cells are constantly exposed to a barrage of microbes including those that form the microbiota. In contrast, professional immune cells like macrophages, which normally reside inside tissues, are less dependent on ETI and respond against all microbes that violate the sanctity of the tissues, irrespective of whether or not they are pathogenic. However, studies done on macrophages infected with *Legionella pneumophila* also suggest that TLR signals and ETI activation possibly work in concert as a two-signal infection response, which leads to transcriptional upregulation of cytokines and activation of the adaptive immune system.<sup>5,6</sup>

### Detection of Pathogens by the Host

The cells of the innate immune system rely on their pattern recognition receptors (PRR) to recognize conserved pathogen-associated molecular patterns (PAMPs) and microbe-associated molecular patterns (MAMPs) such as microbial nucleic acids, lipoproteins, and carbohydrates that are expressed only in pathogens and not in the host.<sup>7</sup> PRRs can be categorized into four families, Toll-like receptors (TLRs), C-type lectin receptors (CLRs), (RIG)-I-like receptors (RLRs), and NOD-like receptors (NLRs).<sup>8</sup> TLRs, the best characterized receptors among the PRRs, are transmembrane proteins that recognize lipoprotein, lipopolysaccharide, double stranded RNA, and other ligands associated with diverse pathogens such as bacteria, viruses, and protozoa.<sup>9,10</sup> RLRs and NLRs are localized to the cytoplasm and recognize viral nucleic acids and bacterial peptides. PRRs can also recognize cellular damage by binding with products of cellular and tissue degradation, or damage-associated molecular

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**Figure 1.** Effector triggered immunity (ETI). ETI can be triggered by toxins that are either directly injected into the host by bacterial secretion systems or internalized from the extracellular environment by endocytosis. Effectors are directly capable of triggering an immune response through transcriptional regulation. Effectors can also disrupt cellular processes such as protein translation and cytoskeletal remodeling, which will trigger an immune response. Some bacterial effectors activate Rho-GTPases, which facilitate bacterial entry and can also trigger ETI. Pore-forming toxins form membrane channels, and the resulting influx/efflux of ions also triggers a protective response.

patterns (DAMPs).<sup>11</sup> Damaged or necrotic cells release factors such as high mobility group box-1 (HMGB1), serum amyloid A (SAA), and S100A8, which initiate an immune response by engaging TLRs.<sup>12-14</sup> DAMPs trigger formation of inflammasomes, which are multimeric protein complexes consisting of caspase 1. Inflammasome formation results in caspase 1 activation, followed by the activation of cytokines IL-1 $\beta$  and IL-18, which induce inflammation.<sup>15</sup> Binding with ligands activates the PRRs, which oligomerize and trigger a defense response including activation of NF $\kappa$ B, IRF, and MAPK pathways, signaling the presence of an infection (Fig. 1). This signaling cascade leads to secretion of antimicrobial peptides and attracts cells of the innate and adaptive immune system.<sup>16</sup>

Beneficial microbes, including commensal bacteria, also possess MAMPs. Therefore, mounting an immune response specifically against harmful pathogens is dependent on the recognition of both the pathogen and the associated host cell damage caused by the pathogen, through MAMPs and DAMPs respectively.<sup>11</sup> A decision checkpoint used by phagocytes before amplifying an immune response is the detection of live intracellular bacteria. Following phagocytosis, bacterial mRNA is released only by live bacteria, which is detected by cytosolic PRRs, signaling microbial life to the innate immune system.<sup>17</sup> Non-professional immune cells such as intestinal epithelial cells, which are constantly exposed to microbes, detect the presence of pathogens through their cytosolic PRRs and by a polarized distribution of PRRs at

the apical and basolateral surfaces. Activation of the PRRs in the cytosol or the basolateral surface will indicate an epithelial cell, or cell surface breach, and attract professional immune cells.<sup>18</sup> Pathogens have evolved multiple strategies to avoid detection by modifying MAMPs or subverting PRR signaling.<sup>19</sup> Therefore, a rapid immune defense response can be initiated from also monitoring for perturbations in a few core pathways and essential cellular activities, which enables the host to indirectly sense the pathogen instead of evolving specific PRRs for each pathogen or damage-associated molecule.

## Effector Triggered Immunity

The defense phenomenon ETI was first observed in plants and our understanding of this phenomenon has evolved starting from the “gene-for-gene theory”, which describes the association between plants and their pathogens through the interaction of pathogen-derived avirulence (Avr) genes and plant derived resistance (R) genes.<sup>20</sup> Bacterial effectors are secreted by six distinct secretion systems classified as Type I–VIII.<sup>21,22</sup> Pathogenic bacteria deliver their effectors into the plant cells through the type III secretion systems (TTSS) to interfere with plant PAMP-triggered immunity (PTI) and facilitate pathogen survival and dispersal. Plants respond to these challenges by activation of ETI, which triggers release of antimicrobial molecules and hydrolytic enzymes and causes encasement of pathogens and deposition of callus at the infection site.<sup>23</sup> For many years, ETI was also speculated to exist in animals, but the cellular mechanisms of ETI activation in metazoans have only just been identified in recent decades.<sup>5,24–34</sup> In contrast to the fairly straightforward system for ETI activation in plants, ETI in metazoans can be more broadly defined to encompass host response to cellular damage and to bacterial virulence factors (including effectors) that manipulate central host processes, independent of PAMP and MAMP recognition. ETI in metazoans is triggered by the activation of MAPK and NFκB signaling pathways and there are several excellent reviews describing the downstream effect of this activation.<sup>35–37</sup>

## Blockade of Protein Translation

During an infection, bacterial pathogens cause damage to host cells either directly through their toxins or indirectly by eliciting an adverse immune reaction. The host responds to these challenges by initiating a damage response to maintain cellular integrity and an immune response to restrict bacterial growth.<sup>38,39</sup> Bacterial effectors and toxins can blunt these responses by blocking several steps of the host translation machinery.<sup>40</sup> However, blockade of protein translation activates ETI and augments the initial immune response (Fig. 1). *Legionella pneumophila* invades macrophages by translocating over 200 effectors into the host cell through the type IV secretion system to create an intracellular niche ideal for the pathogen to survive and replicate.<sup>41,42</sup> The effectors Lgt1, Lgt2, Lgt3, SidI, and SidL inhibit host translation through inactivation of the host elongation factor eEF1a,

which activates the NFκB pathway and promotes transcription of stress response genes and pro-inflammatory cytokines.<sup>32</sup> Infection of macrophages with *L. pneumophila* also activates the MAPK pathway.<sup>43</sup> Further insights into the effect of translational inhibition on innate immunity was gained from studies done in *Caenorhabditis elegans*, which has emerged as a popular model for studying host-pathogen relationship and drug discovery.<sup>44,45</sup> RNAi-mediated disruption of translation and other essential processes in *C. elegans* was reported to induce expression of innate immune response genes.<sup>46</sup> In *C. elegans* infected with *Pseudomonas aeruginosa*, bacterial exotoxin ToxA is internalized by host cells through endocytosis. ToxA blocks translation by inhibiting the host elongation factor 2 (EF-2), triggering expression of the transcription factor ZIP-2, which elicits a protective transcriptional response.<sup>30,31,47</sup>

In contrast to the above studies, intracellular pathogens such as *Shigella flexneri* and *Salmonella Typhimurium* are not known to translocate toxins that directly block host translation. Instead, they cause an overall downregulation of protein synthesis. Infection of epithelial cells with *S. flexneri* triggers acute amino acid starvation. The resulting induction of the amino acid stress pathway triggers the activation of the GCN2, eIF2α, and ATF3-dependent reprogramming of the transcriptional response in response to invasive pathogens.<sup>48</sup> Infection with *S. Typhimurium* also triggers a similar, albeit transient amino acid stress response, suggesting that the pathogen has evolved strategies to subvert host metabolic stress response pathways. Amino acid starvation can also trigger autophagy, a highly conserved cellular process that is turned on during starvation stress.<sup>49</sup> Autophagy functions to recycle damaged cellular organs and complexes in order to maintain levels of essential nutrients such as amino acids.<sup>50</sup> Activation of autophagy response against bacteria (xenophagy), and the subsequent activation of the amino acid starvation pathways, represents an important link between innate immunity and metabolic pathways.

## Reorganization of the Host Cytoskeleton

The eukaryotic cytoskeleton plays a pivotal role in several cellular processes, including endocytosis, adhesion, migration, phagocytosis, and formation of the immunological synapse.<sup>51–54</sup> Genetic mutations, such as those causing Wiskott Aldrich syndrome, affect cytoskeletal regulation and lead to immune deficiency due to impaired function of phagocytes.<sup>55–58</sup> Pathogenic bacteria have evolved multiple strategies to manipulate the host cytoskeleton to facilitate intercellular entry and tissue invasion.<sup>59</sup> In addition, pathogens have evolved effectors that manipulate the host cytoskeleton to facilitate evasion from the host immune response. In fact, a large number of bacterial effectors have been identified that have an immune inhibitory activity.<sup>60</sup> However, when pathogens interfere with the host cytoskeleton for the purpose of immune-evasion, paradoxically, they can also trigger an immune response against the pathogen (Fig. 1). For example, the type III secretion system in pathogenic *Yersinia* spp. translocates the Yop effectors across the eukaryotic plasma membrane and

into the cytosol, where they disrupt key functions of the host cell.<sup>61</sup> YopJ inhibits MAPK kinases and MAPKK kinases and also activates Caspase-1, which disrupts the intestinal barrier and promotes dissemination of the bacteria.<sup>28</sup> YopE paralyzes the phagocytic functions in macrophages by disrupting the actin microfilament structure.<sup>62</sup> The treatment of cultured intestinal epithelial cells with cytochalasin D or latrunculin B, which disrupts the actin cytoskeleton, can cause activation of the p38 MAP kinase and NF $\kappa$ B pathways, showing that subversion of the host actin cytoskeleton components can activate an immune response.<sup>63</sup> This was further evident from studies on the effect of *Clostridium difficile* toxin A on colonic CaCo-2 cells.<sup>64</sup> Toxin A causes disruption of the actin cytoskeleton by monoglucosylation of Rho-GTPases Rho, Rac, and Cdc42, which triggers transcriptional upregulation of the p38 MAP kinase pathway. Additionally, *Salmonella* Typhimurium binds to the surface of epithelial cells and uses type III secretion system effectors to cause a burst of actin polymerization, which induces membrane ruffling and facilitates bacterial entry into the host cell.<sup>65</sup> The *Salmonella* effectors SopE, SopE2, and SopB trigger these events by activating the Rho-GTPases of the host cell in a redundant manner, which in turn can cause a defense response by activating the MAPK and NF $\kappa$ B signaling cascade.<sup>29</sup> The CNF1 toxin produced by *E. coli* is another example of a bacterial protein, which activates Rho-GTPase and can elicit activation of immune pathways.<sup>66</sup> Interestingly, studies done in *Drosophila* and mammalian cells suggest that CNF1 is not injected into the host cell but internalized by receptor-mediated endocytosis into the cytosol, where it covalently modifies the Rho-GTPase Rac 2, triggering protective immunity by activating the Rip kinase signaling pathway.<sup>34,67</sup> Enteropathogenic *E. coli* (EPEC) and enterohemorrhagic *E. coli* (EHEC) use the type III secretion system to translocate the WxxxE effectors Map, EspM, and EspT into the host cell. These effectors subvert the actin cytoskeleton by mimicking guanine nucleotide exchange factors (GEFs) for activation of Rho-GTPases, which in turn triggers the MAPK and NF $\kappa$ B pathways.<sup>68</sup>

### Pore Forming Toxins

Bacterial pathogens produce virulence factors called pore-forming toxins (PFTs), which attack the cellular membranes of eukaryotic cells. Host proteases recognize and cleave PFTs, which aggregate into oligomeric structures that insert into the membrane to form ionic pores (Fig. 1).<sup>69</sup> At high concentrations, PFTs cause rapid death of the host cell due to membrane disruption, leakage of intracellular contents and lysis. However, when PFTs are present in low, sublytic concentration, host cells respond to the damage by activating the ETI response, as seen from studies done in *C. elegans* and mammalian cells.<sup>25,26,70,71</sup> Exposure to PFTs can lead to the activation of NF $\kappa$ B and MAPK pathways, in addition to the unfolded protein response (UPR) and increased autophagy. In epithelial cells, osmotic stress induced by membrane destabilization leads to phosphorylation of p38 MAP

kinase. Relieving the osmotic stress by addition of dextran or cellulose can block phosphorylation and activation of p38 MAP kinase.<sup>72</sup> The mechanism of activation of immune pathways possibly involves the efflux of potassium ions, as well as an influx of calcium ions.<sup>73,74</sup> Treatment of chinese hamster ovary (CHO) cells with aerolysin, a toxin produced by *Aeromonas hydrophila*, induces K<sup>+</sup> efflux, which subsequently leads to activation and assembly of caspase-1 inflammasome.<sup>26</sup> Caspase-1 then induces the activation of the sterol regulatory element binding proteins (SREBPs), which in turn activate the MAPK pathway.<sup>75</sup> A role for K<sup>+</sup> efflux was similarly proposed for activation of p38 MAPK in HaCaT cells treated with *Staphylococcus aureus*  $\alpha$ -toxin, in which case the immune activation could be neutralized by high concentrations of extracellular K<sup>+</sup>.<sup>73</sup> Mammalian cells subjected to mechanical stress, or toxin-mediated plasma membrane insult cause Ca<sup>2+</sup> influx, which triggers a rapid repair process.<sup>76</sup> The repair mechanism possibly involves both removal of the damaged area and resealing the membrane by a combination of endocytosis and exocytosis. *C. elegans* exposed to low doses of the *Bacillus thuringiensis* toxin Cry5B, resulted in transcriptional upregulation of the genes in the JNK and MAPK pathways.<sup>25</sup> The involvement of these pathways in host defense was further demonstrated when *C. elegans* strains defective in these pathways were more susceptible than the wild type strain to exposure to low doses of Cry5B. A similar response was seen when nematodes were treated with the *B. thuringiensis* toxin Cry21A. The p38 MAPK pathway is also induced by sublytic concentrations of the cholesterol binding cytolysin (CDC) PFTs such as anthrolysin O (*Bacillus anthracis*), vaginolysin (*Gardnerella vaginalis*), pneumolysin (*Streptococcus pneumoniae*), and streptolysin O (*Streptococcus aureus*).<sup>77-80</sup> CDC toxins bind to the cholesterol in the eukaryotic cell membrane to form oligomeric structures that form membrane pores.

### Conclusion

Unlike the adaptive immune system, the innate immune system does not need specialized immune cells to mount a protective response. Microbes and their components have a unique molecular signature, which allows early and rapid detection through pattern recognition receptors. However, a system that mounts an antimicrobial response based solely on the molecular signature of microbial components is not only inefficient, but also runs the risk of killing the beneficial commensals that reside in our body. In this respect, effector-triggered immunity (ETI) is an ideal compensatory mechanism that relies primarily on detecting the damage inflicted on the host cell from the microbe, which allows distinguishing between pathogen and commensal. The examples listed in this review provide clear evidence that ETI is a key component of the innate immune response, which is supported by the fact that it is evolutionary conserved across phylogeny in divergent species such as plants, nematodes, and mammals.

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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