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► **To cite this version:**

Thomas Pradeu, Bart P.H.J. Thomma, Stephen Girardin, Bruno Lemaitre. The conceptual foundations of innate immunity: Taking stock 30 years later. *Immunity*, 2024, 57 (4), pp.613-631. 10.1016/j.immuni.2024.03.007 . hal-04549343

**HAL Id: hal-04549343**

**<https://hal.science/hal-04549343>**

Submitted on 18 Apr 2024

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Final published version in *Immunity*: <https://doi.org/10.1016/j.immuni.2024.03.007>

*Immunity*, 30<sup>th</sup> anniversary issue (2024)

## The conceptual foundations of innate immunity:

### Taking stock 30 years later

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### Summary

While largely neglected over decades where adaptive immunity captured most of the attention, innate immune mechanisms have become central to our understanding of immunology<sup>1–3</sup>. Innate immunity provides a first barrier to infection in vertebrates, and is the sole mechanism of host defense in invertebrates and plants<sup>2–4</sup>. Innate immunity also plays a critical role in maintaining homeostasis, shaping the microbiota<sup>5</sup>, and in disease contexts such as cancer, neurodegeneration, metabolic syndromes, and aging. The emergence of the field of innate immunity has led to an expanded view of the immune system, which is no longer restricted to vertebrates and concerns instead all metazoans, plants, and even prokaryotes. The study of innate immunity has given rise to new concepts and language. Here we review the history and definition of the core concepts of innate immunity, discussing their value and fruitfulness in the long run.

### Introduction

Immunology has undergone a conceptual, experimental, and medical revolution in the 1990s with the recognition of the central importance of innate immunity<sup>6</sup>. While it had long been thought that only jawed vertebrates possess an immune system, it has become apparent that an immune system is common to virtually all living organisms, from prokaryotes to protists, fungi, plants, and mammals<sup>7–10</sup>. This has major consequences for what we mean by ‘immunity’, how we conceive the evolutionary origins of immune systems<sup>11</sup>, and therapeutic manipulations of the immune system<sup>12–14</sup>. Investigations of plant and invertebrate immunity have been already abundant before the 1990s<sup>15,16</sup>, but have remained relatively disconnected from mainstream immunology, which have focused on vertebrates and the lymphocytic compartment. This situation changed in the 1990s, due to various factors, including better mechanistic knowledge of invertebrate immunity<sup>17</sup>, the recognition that innate immune mechanisms also play a central role in vertebrates and share similarities with invertebrate immune modules<sup>18</sup>, and the concept that adaptive immunity is under the control of innate immunity<sup>6,19</sup>.

The emergence of the field of innate immunity has been associated with a new view of immunity and the advent of an additional language. Terms such as ‘pattern recognition receptors’ (PRRs), ‘pathogen-associated molecular patterns’ (PAMPs), ‘damage-associated molecular patterns’ (DAMPs), and many others, have appeared during this period. Despite their popularity, the meaning of these terms is not always clear. Scientific terms are often ambiguous and surrounded by a halo of tacit knowledge. Too much terminological flexibility can lead to confusion, but too much constraint can limit creativity. It is therefore crucial to examine the meaning of these terms with hindsight and determine the extent to which they stand the test of time.

An innate immune system must recognize pathogens, potentially through dedicated receptors, then integrate that information via signaling pathways to ultimately launch a response that targets the pathogens. Specificity is key at each of these three steps. Another central feature of any immune system is the ability to deal with pathogens of various natures and that can infect the host via different routes. This ability requires complex crosstalk between local and systemic immune responses. Moreover, rare versus recurrent infections across an organism's life stages require different types of reactions, engaging immune responses that can be constitutive or inducible, and can have long-term memory-like effects. Finally, an immune system must avoid pathological autoimmunity, and regulate and keep a balanced microbiota. Over the last thirty years, studies have shown that an innate immune system can achieve all these 'hallmarks' of immunity, and have identified core mechanisms underlying these innate immune responses across different species.

Our goal here is to discuss important concepts (**Table 1**) used in the study of innate immunity. We review the history of these terms, define them, and discuss their range of use. We also highlight how the emergence of the field of innate immunity has not just dramatically expanded our conception of which organisms possess an immune system, but has revealed common immunological principles across kingdoms.

### Innate immune recognition

The capacity to recognize infectious agents is a key feature of all immune systems. Six sometimes-overlapping mechanisms of innate sensing have been proposed over the years (**Figure 1**).

Historically, the related concepts of pattern-recognition receptors (PRRs) and pathogen-associated molecular patterns (PAMPs) were formulated by Janeway in 1989<sup>6</sup>. Janeway speculated that, before the emergence of adaptive immunity based on gene rearrangement, what he called "primitive effector cells" possessed PRRs able to sense "general structural patterns in molecules found in many microorganisms" – for instance complex cell wall carbohydrates or lipopolysaccharide (LPS)<sup>6</sup>. Before Janeway's paper, various recognition molecules (later considered as 'PRRs') had already been identified, such as mammalian CD14 (sensing LPS) or Mannose Binding Lectin (upstream of Complement), Limulus clotting factor C (sensing LPS), or insect  $\beta$ -Glucan Binding protein<sup>20,21</sup>. Important work, distinct from mainstream immunology, had identified many bacterial cell wall components with the ability to stimulate immunity (e.g., LPS and muramyl dipeptide)<sup>22</sup>. A central value of Janeway's article was to conceptualize the importance of these PRRs in initiating all immune responses, including, via co-stimulation, adaptive immune responses. The characterization of many PRRs and their ability to induce co-stimulatory signals in dendritic cells, notably the Toll-like receptors (TLRs) at the end of the 1990s, confirmed this speculation<sup>18</sup>.

PRRs have been defined as non-clonal and as having broad pathogen-specificity. In 1997, Medzhitov and Janeway further refined the concept of PRRs by describing the properties of their ligands, the PAMPs. PAMPs are highly conserved molecular motifs shared across many pathogens and generally absent from the host<sup>19</sup>. Thus, PRRs are not involved in the recognition of a specific pathogen, but instead a class of pathogens that display a given PAMP (e.g., LPS for Gram-negative bacteria). Later studies have shown that PRRs do not only recognize pathogens, as symbiotic interactions use the same 'PRR-PAMP language', which led to the conceptual proposal that the term 'microbe-associated molecular patterns' (MAMPs) should replace the original concept of PAMPs<sup>23</sup>. Echoing that idea, the two terms are increasingly used interchangeably<sup>24,25</sup>, and this is also the convention that we will use here.

There are different types of PRRs: cytoplasmic, transmembrane, and secreted. Some can directly bind to microbes while most sense indirectly MAMPs released by microbes. Over the years, a plethora of receptors fulfilling the concept of PRRs have been identified and, in some cases, structural studies have revealed clear binding of a MAMP to its receptor. The concepts of PRRs and MAMPs has turned out to be of broad relevance, notably when PRRs were found in plants through the identification in *Arabidopsis* of the surface receptor FLS2, which recognizes flagellin<sup>26–28</sup>. This discovery was followed by a suite of new PRRs<sup>29</sup> in plants. Other examples of PRRs include TLRs, Nucleotide-binding domain Leucine-rich Repeat containing-like receptors (NLRs), RIG-I-like receptors, AIM2-like receptors, Protein kinase R and cGAS in mammals, as well as PGRP and  $\beta$ GRP in insects. Some of these have deep evolutionary origins, such as cGAS which is also found in bacteria<sup>10</sup>, or LRR-TIR proteins found in both plants and animals<sup>30,31</sup>.

MAMPs are not just tags identifying the class of an infectious agent, they also convey contextual information related to pathogenesis, such as location or division rate. For instance, in Gram-negative bacteria, peptidoglycan is hidden by an external layer of LPS and released mostly when bacteria divide. Thus, the detection of fragments of peptidoglycan by PGRPs in arthropods, by the NLR proteins NOD1 and NOD2 in mammals, or by LysM receptors in plants may allow specific sensing of 'dividing bacteria', a signature of

threatening bacterial pathogens. In support of this, Gram-negative bacteria that divide very slowly are invisible to the fly immune system<sup>3</sup>. Similarly, the mammalian intracellular ALPK1-TIFA pathway<sup>32,33</sup> may be more readily activated by live bacteria than dead ones since the MAMPs detected are heptose phosphates produced as transient intermediates of LPS biosynthesis, found only in live bacteria. More generally, MAMPs associated with live microorganisms have been conceptualized as vita-MAMPs<sup>34</sup>, and may include other MAMPs such as bacterial RNA<sup>35</sup> and cyclic-di-adenosine monophosphate (c-di-AMP)<sup>36</sup>.

Despite its efficacy, the PRR detection system has some limitations. MAMPs are not as invariant as initially thought. Modifications of MAMPs, such as amidation or acetylation of peptidoglycan or acylation of LPS, allow some pathogens to escape immune recognition<sup>37,38</sup>. Some microbes also seem to lack most or all classical MAMPs, and therefore remain hidden to the host. Most of these microbes are symbionts with slow division rates. Moreover, the PRR-PAMP system is not especially suited to pathogens that are phylogenetically close to their hosts. This is why, for instance, detecting eukaryotic parasites may pose a challenge to metazoans, due to the lack of distinguishing MAMPs that allow recognition.

In contrast to PAMP-triggered immunity (PTI) that relies on the detection of structural patterns of microbes, effector-triggered immunity (ETI) is based on the detection of pathogen-encoded virulence factors ('effectors') or their activities. This concept, initially elaborated in plants, is now of broad scope. Historically, the 'gene-for-gene' concept in plants has pointed to strong specificity between an 'avirulence factor' and its cognate receptor<sup>39</sup>. It was later shown that the intrinsic function of these avirulence factors is instead in virulence, as they are secreted by pathogens to mediate host colonization, frequently through suppression of PRR-mediated PTI. However, over evolutionary time many of these effectors were picked up by host receptors (mostly intracellular) to activate ETI. The interplay between pathogen- and host-encoded factors has been brought together in the 'zig-zag model'<sup>40</sup>. This model embraces an earlier seminal hypothesis stating that effectors can either be recognized directly, as MAMPs are, or indirectly by receptors that monitor the integrity of host cellular targets of effector action, also known as the 'guard hypothesis'<sup>41</sup>: plant immune receptors act as 'guards' that recognise when host cellular targets ('guardees') have been modified by pathogen virulence factors<sup>42</sup>. Many receptors that recognize effectors or effector activity have been documented in plants (>200 in *Arabidopsis*).

ETI is in one sense more specific than pattern-recognition, insofar as it detects pathogens based on their virulence, so it is not triggered by symbionts and other non-pathogenic microbes. Simultaneously, ETI is often considered parsimonious, or even non-specific, as the host does not have to recognize specific patterns for each pathogen, but instead monitors key host processes targeted by many different pathogens<sup>43</sup>.

Although it originated in plants, the concept of ETI has more recently been extended to animals<sup>43-45</sup>. Many entomopathogenic bacteria and fungi use proteases to penetrate into host tissues. In *Drosophila*, some host serine proteases Persephone and Haya can be activated by pathogen proteases, resulting in activation of the Toll immune pathway<sup>46</sup>. Several authors<sup>45</sup> suggest that inflammasomes provide another example of ETI, by sensing microbial effectors that perturb cells. This is the case of the NLR protein NLRP1, which may serve as a "guard" protein, similar to plant guards. Indeed, NLRP1's N-terminal region serves as a "bait" for microbial-derived proteases and E3 ligases, as its degradation liberates a C-terminal fragment that triggers inflammasome signaling, a process that has been coined "functional degradation"<sup>47,48</sup>. Detection by Pypin of the glucosylation of Rho induced by bacterial toxins represents another example of mammalian ETI<sup>49</sup>.

Although PTI and ETI are distinct modes of recognition, they significantly overlap. The direct sensing by a receptor of a microbial effector (as opposed to the sensing of its activity), is very similar to pattern recognition but is generally considered part of ETI. In PTI, MAMPs are viewed as 'structural' and therefore passive, while ETI refers to the sensing of effector activity. However, this may be an artificially binary distinction, as many instances of pathogen detection combine recognition of both patterns and effector activity. In plants, it is recognized that the separation between PTI and ETI is partly artificial, and that the spectrum from MAMPs to effectors constitutes a continuum<sup>50,51</sup>. Future studies are likely to provide further examples of continuity between these two sensing modes.

Instead of recognizing patterns typical of pathogens, some immune receptors can recognize the absence of a molecular pattern that all or virtually all host components express and that is not expressed by 'foreign' entities. This self-signal is sensed by an inhibitory receptor that blocks the immune response; detection of intruders that lack this self-signal removes the inhibition and triggers an immune response. This recognition mode is parsimonious, as it does not require the recognition of a specific pathogen. However, it can be subverted relatively easily by pathogens that succeed in expressing host 'self-markers'.

This mode of recognition was first characterized in 1986 in natural killer (NK) cells by Kärre, as the “missing self” hypothesis<sup>52</sup>. NK cells respond to the absence of expression of major histocompatibility (MHC) class I proteins. MHC-I, which is constitutively expressed on all nucleated cells, is often down-regulated when a cell becomes infected by a virus or undergoes cancerous transformations. Two other examples are the regulation of C3 convertase in the alternative complement pathway and the regulation of phagocytosis via CD47. Host cells are spared because they inhibit the formation of active C3 convertase, while pathogens do not and are eliminated<sup>53</sup>. CD47 functions as a self-recognition molecule, sending a negative “don’t eat me” signal to macrophages and dendritic cells, while cells that do not express CD47 or downregulate its expression are eliminated<sup>54</sup>. Self-tissues of *Drosophila* are also encapsulated when the basement membrane is degraded, which is sufficient to recruit hemocytes and produce melanization<sup>55,56 55,57–61</sup>. This suggests that patrolling hemocytes identify intact basement membrane as self, while parasitoid eggs are encapsulated because they lack a self-signal. Another fascinating example of recognition of ‘absence of self’ is the restriction-modification system of bacteria that allows destruction of unmethylated DNA from invading bacteriophages, where methylation DNA acts as a ‘self’ mark<sup>62</sup>. Interestingly, missing self recognition has also been invoked in allorecognition in Urochordates<sup>63,64</sup>.

The missing self concept has been considered by some as a part of ETI<sup>45</sup>, particularly when the detected ‘missing self’ is a host modification induced by a pathogen (typically, MHC-I downregulation in a host cell infected by a virus), rather than a spontaneous host modification (e.g., a cell that becomes cancerous).

This mode of recognition is based on the detection of altered or abnormal self-patterns, i.e., what is immunologically recognized is the modification of host components. This often manifests in the detection of a host component in a place where it is normally absent. This may be referred to as ‘recognition of the abnormal location of host components’. For instance, in the mechanism of efferocytosis, apoptotic cells expose an ‘eat me’ signal, inducing their elimination by phagocytes. Phosphatidylserine, a phospholipid usually confined to the inner layer of the cellular membrane, is exposed on the outer membrane of cells undergoing apoptosis<sup>65</sup>. Recognition of phosphatidylserine by conserved receptors of the Draper-CED1 family triggers engulfment of the dying cell<sup>66,67</sup>. Recognition of modified host components can also be involved in host defense. An example is “assisted apoptosis”, whereby virally infected or transformed cells (typically cancerous cells) express molecular markers recognized by other cells, and these cells non-autonomously induce the apoptosis of the virally infected or transformed cells<sup>68</sup>. Another important example is the detection by NK cells of stressed host cells that express proteins such as MICA and MICB, which are induced upon viral infection<sup>69</sup>.

The guard hypothesis in plants and studies of ETI has more generally strengthened the view that innate immune responses can be mediated by the recognition of altered self. Accordingly, the recognition of modified self has often also been considered part of ETI<sup>45,70</sup>. Nevertheless, the two ideas are conceptually distinct: ETI is about detecting activity of pathogen effectors, whereas the ‘altered self’ proposes that immune systems can recognize modifications of the self even in a non-infectious context (such as cancer). Thus, a more rigorous framework would distinguish two modes of recognition of modified self: an *extrinsic* one, where host modifications are due to the activities of a pathogen within the body, and an *intrinsic* one, where host modifications are internally produced, without the intervention of a pathogen (e.g., most cases of cancer-related ‘altered self’). Only the extrinsic mode can be considered part of ETI.

The ‘altered self’ also raises the question of whether the immune system truly detects alterations of components originating from the self only or instead detects all alterations of the host, both ‘self’ and ‘nonself’ in origins. The discontinuity theory of immunity, which proposes that immune systems have been selected through evolution for their capacity to detect significant structural and activity-based changes in the host<sup>71</sup>, considers the ‘altered self’ and the ‘missing self’ as subcategories of such changes<sup>71</sup>.

According to the ‘danger theory’<sup>72,73</sup>, effector immune responses are due not to ‘nonself’ or ‘infectious nonself’ but to the detection by the host of ‘danger signals’. The broad and sometimes imprecise term ‘danger signal’ was later replaced by the more specific ‘damage-associated molecular patterns’ (DAMPs)<sup>74</sup>, in analogy to the ‘PAMP’ concept<sup>75</sup>. The main advantage is that ‘DAMP’ focuses on the central idea that immune responses are induced by damage to host tissue and encompasses sterile inflammation, the activation of the immune system in absence of infection. One early and important objection has been that DAMPs trigger a repair response rather than an anti-pathogenic immunological response, but the increasing recognition that repair responses are part of the overall immunological response (**Box 1**), have perhaps attenuated this objection<sup>76</sup>.

Although the ‘danger signal’ theory was originally intended to explain adaptive immune responses<sup>77</sup>, it has proved useful in understanding innate immune responses by offering an additional layer of complexity to the PRR-PAMP framework. It is now clear that many DAMPs (including heat-shock proteins, cell death-

associated signals, uric acid, F-actin<sup>78</sup>, and high-mobility-group box 1 protein), together with inflammasomes, play a very important role in mammalian innate immune responses<sup>75,79</sup>. An example in *Drosophila* is the sensing of actinin that triggers the JAK-STAT pathway to orchestrate a systemic wound response<sup>80</sup>. As actinin is an intracellular cytoskeletal component, its release into the extracellular compartment signals cell damage.

DAMPs exist also in plants<sup>81,82</sup>. Plant cell wall-degrading enzymes are secreted by many plant pathogens to gain entrance into plant hosts, several of which contribute to the release of oligogalacturonide fragments from the carbohydrate plant cell walls that act as danger signals<sup>83</sup>. However, DAMPs not only include cell wall fragments, but also peptides, nucleotides and amino acids that are able to activate both local and systemic responses. An example of these are phyto cytokines, plant endogenous peptides that are released from damaged cells and of which Systemin, a peptide induced by wounding and herbivore attack in solanaceous plants to activate long-distance defense responses, is best characterized<sup>84</sup>.

DAMP sensing and ETI have interesting overlaps but are conceptually distinct<sup>45</sup>. ETI is a response to a pathogenic activity or effector whereas DAMPs are endogenous host molecules released in response to stress or damage. The potential overlap comes from the fact that some receptors recognize both pathogen effectors and DAMPs and many pathogens induce cellular stress, generating DAMPs. Yet, if the cellular stress in such cases can be explained by pathogen activity, it seems reasonable to consider the situation as an instance of ETI.

The concept of 'surveillance' was elaborated in the study of *C. elegans* host defense. This nematode lacks many of the usual families of PRRs found in vertebrates or insects. However, infection elicits immune responses<sup>85</sup>, and is triggered not by PRRs or specific virulence factors, but instead by generalist stress pathways<sup>86</sup>. A major example of surveillance in *C. elegans* and other animals is the detection of pathogen-induced translational inhibition. In *C. elegans*, the immunological response to *P. aeruginosa* is triggered in response to exotoxin ToxA, which can prevent the translation of crucial host defense molecules. What is sensed is not ToxA, but rather ToxA activity in inhibiting protein synthesis by blocking EF-2 function<sup>86,87</sup>. Thus, reduced translation, which occurs when cells are stressed, is coupled to induction of protective immune responses. In metazoans, pore-forming toxins damage host cell membranes and trigger a specific repair program (MAPK, p38), including inhibition of translation, aimed at restoring plasma membrane integrity and ion homeostasis<sup>88,89</sup>.

The surveillance concept significantly overlaps with ETI and DAMPs but insists that detection of infection is mediated downstream of generalist stress pathways, which can be activated by a greater range of events beyond infection. Similar to DAMPs, the concept of surveillance is more closely related to activation of stress and repair pathways as a result of host-pathogen interactions, rather than an immune response *sensu stricto*. It however reminds us that, in addition to detecting infections caused by evolving biotic agents, organisms display stress responses in presence of physical stress, some of which contribute to host defense. The concept of surveillance could also be applied to many mechanisms of resilience that contribute to host survival without directly targeting pathogens (**Box 1**). In mammalian cells, host membrane damage induced by bacterial pore-forming toxins or insertion of the bacterial type three secretion system triggers an amino acid starvation response that results in mTOR-regulated induction of defensive stress responses, including anti-bacterial autophagy<sup>90-92</sup>. While ETI suggests a specific interaction between virulence factors of pathogens and host sensors, surveillance points to the sensing of broad perturbations (membrane pores, translation inhibition).

## Signaling pathways downstream of recognition leading to specificity

Recognition mechanisms activate signaling pathways, leading to the production of effectors. A key feature of any immune system is target-specificity – the ability to produce effectors tailored to the encountered agents. In contrast to adaptive immunity and its 'antigen-specific' responses, innate immunity has long been considered to lack specificity. Yet many innate responses are tailored to the invading pathogen<sup>1,93</sup>.

Pattern recognition and other modes of innate sensing trigger immune responses by recruiting adaptors that activate downstream signal transduction pathways. Studies in vertebrates, insects, and plants show that pathogen recognition converges on a limited number of signaling pathways (e.g., NF- $\kappa$ B, JAK-STAT). Some specificity is achieved when distinct innate immune sensors activate different signaling cascades, leading to the production of distinct sets of effectors<sup>93,94</sup>. Innate recognition carries information about the class of pathogens along with some contextual information (e.g., tissue and subcellular localization and associated damages) that are integrated by recruiting specific signaling pathways. This is best exemplified by the selective activation of the Toll and Imd pathways in *Drosophila* by, respectively, fungi and Gram-negative bacteria<sup>17,93</sup>, triggering specific antifungal or antibacterial effectors. Similarly, nucleic acid ligands—the only PAMPs often available to

detect viruses—tend to elicit type I IFN production in mammals, a specialized anti-viral cytokine. The same PAMPs can generate different responses depending on the subcellular location in which they are detected. For example, extracellular LPS, flagellin, and DNA all engage TLRs and produce a transcriptional response, whereas cytosolic detection of these same PAMPs results in inflammasome activation and cell death. This differential response makes sense: if the cell is infected with an intracellular pathogen, cell death will limit the ability of the pathogen to exploit the intracellular niche, whereas if the cell is uninfected and merely in the vicinity of an infection, then a transcriptional response to produce or recruit antimicrobial effectors or cells is appropriate<sup>34,95</sup>. A similar phenomenon has been described in plants where signalling pathways regulated by both the plant hormone salicylic acid and the hormones jasmonate and ethylene, govern defense responses against biotrophic plant pathogens that need living host tissue to thrive, versus necrotrophic pathogens that induce host cell death to retrieve nutrients, respectively<sup>96,97</sup>.

Moreover, various immune cells express different repertoires of innate receptors and are therefore mobilized during an infection only when they possess the appropriate receptors<sup>98</sup>. The infection route also makes considerable difference, as a key feature of any immune system is compartmentalization, with specialized adaptations according to tissue. The immune response to a pulmonary infection by a virus is distinct from the immune response to gut infection by a worm. In mammals, such distinct immune programs are achieved at both local and systemic levels by producing different sets of cytokines. The concept of immune polarization (type 1 vs. type 2 response), initially developed in the context of T cells, has been extended to innate immunity (e.g., M1-like vs. M2-like macrophages<sup>99</sup>). In summary, the coupling of different sensors to distinct effectors via signaling pathways, combined with the recruitment of subsets of immune cells according to the repertoire of sensors they express, make innate immune responses to a pathogen quite specific<sup>94</sup>.

In most cases, innate responses are far less specific than the antigen specificity of vertebrate adaptive responses. Yet some innate responses are as specific as adaptive responses, as exemplified by RNA interference (RNAi)<sup>100,101</sup>, which displays both innate and adaptive properties. RNAi is triggered in response to a MAMP, namely, double stranded RNA by the RNase III enzyme Dicer. It leads to the production of siRNA and induces sequence-specific destruction of distinct viruses through the RNA-induced silencing complex (RISC). This is a key antiviral defense mechanism in plants, insects, and nematodes, and also plays a role in mammals<sup>102,103</sup>.

## Innate immune effector mechanisms

The recognition of pathogens and activation of signaling pathways trigger effector mechanisms. Although effector mechanisms have received less attention conceptually than recognition and signaling, they are crucial: recognition is about what the immune system *sees*; effector mechanisms are about what the immune system *does*. Eliminating threats without causing excessive host damage (immunopathology) is a delicate challenge.

Innate immune effectors, despite their diversity across plants and animals, ultimately rely on a limited number of mechanisms revolving around a few principles. (**Figure 2**). A first category, which we can call '*destruction of the radically different*', are effectors that specifically target certain classes of pathogens due to specific characteristics they have that are absent in host cells. This is best exemplified by cationic antimicrobial peptides which disrupt bacterial and fungal membranes because they are more negatively charged than eukaryotic membranes<sup>104–106</sup>. This category also includes some virus-blocking restriction factors, anti-parasitic compounds, and peptides that target specific bacterial molecules (e.g., lysozymes that digest peptidoglycan). A second class of effectors, here called '*anti-virulence effectors*', are host defense molecules that do not target the pathogen itself but instead its virulence factors, inhibiting its infective ability. For example, in response to fungal proteases that target the host, animals and plants produce protease inhibitors (e.g., serpins)<sup>107,108</sup>. Furthermore, some immune peptides can block the action of bacterial toxins<sup>109</sup>. A third group, referred to as '*innate sensor-assisted elimination*', are effector mechanisms coupled to an innate sensor that restricts its impact to pathogens and not to the host. Such effector mechanisms can only be guided by innate sensors that *directly* recognize infectious agents, typically via MAMPs or via the recognition of absence of self signal. This includes the Membrane Attack Complex that kills pathogens through the control of complement, and the neutralization of pathogens by opsonins that recruit clotting factors and blood cells. When immune effectors are deleterious to both the pathogen and the host, they can nonetheless be used by confining the pathogen to a specialized destructive compartment isolated from host tissues. This is a variant of innate sensor-assisted elimination, as it is guided by innate sensors. Phagocytosis eliminates small pathogens in phagosomes that subject the pathogen to high amounts of reactive oxygen species (ROS), lysosomal enzymes, and acidity that might be otherwise toxic to the host. Encapsulation of parasitoid wasps by hemocytes in *Drosophila* or granuloma formation around tuberculosis limits the infectious site and diffusion of potentially toxic immune reactions. A fourth type of effector mechanism observed in plants and animals is '*suicide of infected cells*'

through the activation of special cell death pathways, limiting the spread of infectious agents to neighboring host tissues<sup>110</sup>. In plants, this is exemplified by the hypersensitive response that leads to necrosis of host tissues, preventing dissemination<sup>111</sup>, which echoes pyroptotic cell death of infected cells in mammals, that can be activated by NLRs<sup>112</sup>. Death of infected cells is sometimes induced by specialized patrolling immune cells (e.g., NK cells) that can recognize aberrant cells ('assisted cell death'). A fifth type of effector mechanism, called 'nutritional immunity' is the sequestration of metabolites critical for pathogen growth. For instance, in insects and vertebrates, but also in plants<sup>113</sup>, sequestration of iron by transferrin or lactoferrin limits the availability of this element, which is indispensable to bacterial growth<sup>114</sup>. RNAi, which triggers the destruction of viral RNA in a sequence-specific manner, could be considered a sixth distinct mechanism, albeit related to innate sensor assisted elimination. CRISPR might also belong to this category, or may instead be viewed as a component of a bacterial adaptive immune system<sup>115</sup>.

Although the above classification of innate immune effector mechanisms remains preliminary and non-exhaustive, it is noteworthy that similar mechanisms of pathogen elimination have emerged across plants and metazoans and are either evolutionarily conserved (homology) or convergent (analogy). Future comparisons of these innate immune effector mechanisms across various taxa are likely to identify 'weak points' of pathogens commonly targeted by immune systems, which may ultimately inspire therapeutic strategies<sup>116</sup>.

Importantly, although we focus here on mechanisms of pathogen elimination, it is increasingly appreciated that a host can survive an infection through other ways, including disease tolerance, symbiont-mediated immunity, and behavioral immunity (**Box 1**).

It was long assumed that the effector response was not specific and that multiple innate immune effectors acted redundantly to combat an infection. Yet recent studies have challenged this 'cocktail hypothesis' with examples where a single effector among many induced downstream of an immune pathway, can be critical to survive an infection. This is exemplified by Diptericin A, one of the forty peptides induced by the Imd pathway during *Drosophila* immune response, which is critical in combatting infection with *P. rettgeri* while its paralog Diptericin B is critical against *Acetobacter pomorum*<sup>117,118</sup>. Similarly, in mammals, defects in innate immune effectors (complement, NK cells) often lead to acute susceptibility to a narrow set of pathogens. In humans, an illustration of this is provided by patients carrying rare mutations in genes critical for innate immune defense. For instance, mutations of genes associated with the interferon gamma pathway predispose to infection with *Mycobacteria*<sup>119</sup>. Similarly, rare mutations in the gene encoding IRAK-4, a key mediator of TLR signaling, are selectively associated with pyogenic bacterial infections<sup>120</sup>. Full redundancy would not make sense evolutionarily as the maintenance of immune effectors is very costly<sup>116,121</sup>. This further emphasizes the importance of the specificity of innate immune responses, at the effector as well as recognition level.

Another conceptual change in the innate immunity field is a refined appreciation of the importance of physical and chemical barriers as well as constitutive immune mechanisms. When entering a host, most pathogens first face effective physical, chemical, and physiological barriers. These include the mucus or simple and stratified epithelium of mammals, the chitin-based cuticle or peritrophic membrane of insects, and the waxy cuticle and cell wall of plants<sup>122</sup>. Barriers work differently in different tissues. Barriers are dynamic and fully integrated into the whole immune response. Innate recognition often leads to barrier reinforcement by increasing mucus secretion, increased peristalsis in intestinal infection<sup>123,124</sup>, or modification of the cuticle in *C. elegans*<sup>125</sup>. In mammals, a specific subset of colonic goblet cells, known as sentinel goblet cells<sup>126</sup>, detect MAMPs through a TLR-NLRP6 signaling axis, resulting in the rapid release of mucus. These cells seemingly work as a "rheostat" system to reinforce the colonic mucus barrier when microbes (and their ligands) get too close to the epithelial barrier. Analogously, plant cells respond with cell wall fortifications following attempted pathogen ingress, which includes the oxidative cross-linking of carbohydrates and the deposition of callose<sup>127</sup>.



In addition to barriers, organisms display diverse constitutive effector mechanisms<sup>128</sup>. Examples include the constitutive expression of antimicrobial peptides or lactoferrin in various epithelia<sup>3</sup>, and of antiviral restriction factors in stem cells<sup>129</sup>. Besides constitutively expressed antimicrobial peptides, plants also pre-produce low-molecular-weight secondary metabolite compounds with antimicrobial properties known as phytoanticipins<sup>130</sup>. Tissue architecture is also an important factor, as local cellular organization can favor the emergence of functional gradients of antimicrobial factors to prevent microbe infiltration<sup>131</sup>. This occurs in the intestinal epithelium, where the Paneth cells, the major producers of anti-microbial molecules, are found at the bottom of intestinal crypts. This topology generates an antimicrobial concentration gradient that protects the bottom of the crypt from intruders, thereby shielding the vital intestinal stem cells, also found at the crypt bottom<sup>132</sup>. Constitutive defenses can be regulated by tissue-specific transcription factors independent of infection or can result from low amounts of immune stimulation by the microbiota (sometimes called 'physiological inflammation'). Indeed, genes encoding antimicrobial proteins produced by Paneth cells are among the most differentially expressed genes when comparing germ-free versus conventionally-raised mice<sup>133</sup>. Constitutive innate defenses provide a basal level of protection in the absence of infection. They prevent most infections by environmental microbes without triggering costly and sometimes deleterious immune responses. In some cases they prevent PRR activation, thus diminishing risks of unwanted damage<sup>128</sup>. Thus, in addition to traditional inducible immune responses, barriers and constitutive defenses should be taken into consideration when conceptualizing host immunity.

Although immunity has long been thought to be conveyed by specialized immune cells, recently it has been increasingly appreciated that all cells can mount 'cell-intrinsic' or 'cell-autonomous' responses against certain pathogens, typically viruses and intracellular bacteria and that, in metazoans, cellular self-defense synergizes with whole-body protection provided by traditional immunity to eliminate pathogens<sup>134-136</sup>. Within-cell sensing of viral and bacterial nucleic acids is an essential aspect of cell-intrinsic immunity, leading to the production of type I interferons. Cell-intrinsic immunity operates across all three domains of life, and some specific mechanisms such as cGAS-STING<sup>137</sup> seem to be evolutionarily conserved<sup>138</sup>. The cell, with its organelles, represents a miniaturized immune system able to sense and activate host defenses, such as production of intracellular antibacterial peptides, autophagy, and metabolic competition between organelles and pathogens<sup>139,140</sup>. Much remains to be discovered about cell-intrinsic immunity, which will constitute a major topic in future studies.

### Innate immune memory

Immune memory refers to the observation that a host, once infected, becomes more resistant to a secondary infection by the same pathogen<sup>141</sup>. Memory is a central feature of the adaptive immunity, where it relies on clonal selection and somatic recombination, and constitutes the basis of vaccination. Traditionally, immune memory has four key properties: i) high specificity, ii) return of the immune response to basal level after primary infection, iii) a quicker and more efficient response upon secondary infection, and iv) long-term protection (years or decades).

Despite the long-held dogma that immune memory is limited to lymphocyte-based immunity and vertebrates, the idea that innate immunity can mediate long-term protection is not new<sup>142,143</sup>. However, studies have often been unsatisfactory for several reasons. First, when considering the innate immune system, the term 'memory' is used to describe various processes, some of which would be better described as *persistent* immune responses. Second, many early studies have lacked conceptual precision and appropriate controls, and have not been reproducible<sup>144</sup>. Third, many experiments used very artificial settings, with unclear *in natura* relevance.

Regardless, recent work has revealed a collection of innate immune mechanisms that can provide long-term protection. To clarify and organize existing observations, we have defined four different categories of memory-like processes exhibited by the innate and adaptive immune systems (**Figure 3**). In the first category, a primary infection leads to a *low persistent state of infection*, due to the persistence of the infectious agent in the host, which increases the amount of basal immunity, improving survival to a secondary infection. In the second case, a primo infection leads to a short-term immune response, and protection against a second challenge results from *long-lasting effects of innate immune effectors* in a context where, contrary to the first category, the infectious agent is eliminated. This protection may be due to the perdurance of humoral factors (e.g., antimicrobial peptides) or the differentiation and/or increased numbers of effector immune cells, and can last for days up to several months. NK cell-based memory<sup>145</sup> may also be considered part of Category II.

In the third case, a primo infection triggers a *sustained innate immune response that may provide lifelong protection* against secondary infection. Category III relates to Category II, but differs in that the immune response does not decline. A first case is the induction of broad-spectrum innate immunity that remains

sustained. An illustration could be an innate immune response in an organism devoid of negative regulation. A second case refers to highly pathogen-specific responses that remains sustained. One example is RNAi, found in plants, *C. elegans* and *Drosophila*. Crucially, RNAi is highly specific and can generate transgenerational effects. siRNA can be amplified by RNA-dependent polymerase which generates secondary siRNA, propagating the protection<sup>100,146</sup>. A second example of sustained highly specific protection is the CRISPR-Cas system<sup>147–149</sup> in bacteria and archaea. As new spacers derived from the genome of the invader (typically a phage) are incorporated into the CRISPR array along with a new repeat unit, information about this invader is stored, and can be used to combat subsequent invasions<sup>148,150</sup>. CRISPR-Cas-mediated immune memory displays high specificity and involves modification of the genome like vertebrate adaptive immunity, but moreover it is transmitted to the progeny<sup>115</sup>.

The fourth category, which we propose to call '*reactivation-based memory*', consists of a first immune response that is fully resolved by a return to a basal activation level, followed by a reactivation leading to a second, much stronger, immune response. The protection against secondary challenge is due to a shift in regulatory mechanisms that leaves the immune system in an 'anticipatory' state of immune readiness, enabling stronger or quicker responses upon secondary challenge<sup>151–154</sup>. Naturally, the paradigmatic example of '*reactivation-based memory*' is lymphocyte-mediated immunity in vertebrates, which relies on the persistence of long-lived memory cells. However, the innate immune system can also mediate '*reactivation-based memory*'. Some examples of these are i) increased expression of signaling molecules that allow faster induction of effectors, ii) epigenetic modifications rendering immune genes more accessible to transcription factors, or iii) differentiation of immune cells to a 'pre-activated state' that allows them to react faster upon secondary infection. Such '*reactivation-based memory*' also occurs in plants, where the phenomenon is generally known as 'systemic acquired resistance' (SAR), which is induced by many pathogens that cause tissue necrosis, either as a part of a hypersensitive response or as a symptom of disease. SAR not only provides enhanced resistance in distal plant organs and acts against a returning infection by the initial pathogen, but generally acts more broadly against a distinctive spectrum of pathogens and is long-lasting<sup>155–157</sup>.

With this categorization in mind, further terminology can be introduced. First, immunologists working on innate immunity have often avoided the term 'memory' due to its strong connotations, and instead used the term *priming*, which simply describes higher host resistance to a pathogen upon secondary infection. This concept, which has a long history, has the advantage of applying to all four categories defined above and does not make any assumptions about underlying mechanisms. It implicitly refers to a methodology involving successive challenges. The term '*innate immune memory*' may similarly be used to describe long-term protection provided by innate immunity, but some authors might use it exclusively to describe '*reactivation-based memory*'. Another concept, 'trained immunity', has been proposed in 2011 by Netea et al., based on the observation that, in animal models, a prior infection or vaccination with bacteria, fungi or helminth parasites protects against heterologous infections independent of adaptive immunity<sup>158</sup>. Trained immunity accounts for immunological memory mediated by macrophages and neutrophils<sup>158</sup>, results in an increased nonspecific response to subsequent infections and is based on epigenetic reprogramming<sup>159</sup>. Yet epigenetic modifications, at least in the case of histone marks, are not always easy to monitor and might not be specific to '*reactivation-based memory*'. An additional source of confusion is that many authors use 'trained immunity' and 'priming' interchangeably. While the introduction of the concept of trained immunity has the potential to stimulate research on innate immune memory, it should be restricted to innate immunity-based '*reactivation-based memory*' to avoid further confusions<sup>160</sup>.

### **Innate immune tolerance**

A key feature of immune systems is their ability to maintain host tissues and provide permissive niches to resident microbes without eliminating them. Immune tolerance (which is distinct from 'disease tolerance' (discussed in Box 1) relates to an active immunological process in which inhibitory pathways overcome excitatory pathways, leading to the maintenance (as opposed to the elimination) of the target (which can be exogenous or endogenous). Contrary to the long-held view that autoimmune diseases are concerned exclusively with the adaptive immunity, some autoimmune-like diseases involve only innate immunity. One of the largest phenotypic classes of mutations in *Drosophila* are melanotic tumor mutants, which present with encapsulation of self-tissues<sup>161</sup>. In mammals, mutations in genes encoding innate immune receptors, such as NLR4 or NLRP3, cause auto-inflammatory syndromes<sup>162</sup>. Similarly, mutations in genes (e.g., TREX1) critical for the clearance of cytosolic nucleic acids can trigger constitutive activation of the cGAS-STING innate immune pathway, resulting in diseases such as Aicardi-Goutieres syndrome<sup>163</sup>, or systemic lupus erythematosus<sup>164</sup>. In plants, some mutations in 'accelerated cell death 6' (ACD6) genes reduce plant growth due to the constitutive activation of the immune system<sup>165</sup>. Autoimmune responses have also been reported to occur in plant

hybrids<sup>166,167</sup>. Thus, disruption of innate immunity can lead to a rupture of immune tolerance to self-tissues, causing immunopathology.

Another important challenge is how the innate immune system deals with the microbiome, notably in the gut. We have seen that pattern recognition does not discriminate between pathogens and symbionts. Several common innate immune tolerance principles have recently been uncovered that help maintain the microbiome while preventing excessive innate immune responses. A first principle is *compartmentalization* (physical separation) of symbionts in specialized niches, hiding symbionts from the highly destructive components of the host immune system. The gut microbiome of mammals is lined by mucus that prevents contact with the epithelia. Moreover, as mentioned, a gradient of antimicrobial peptides in the crypt establishes a 'no entry' zone to microbes. Last, pattern-recognition receptors are often disposed in the lamina propria underneath the epithelial layer and react only when microbes breach this barrier<sup>131</sup>, a phenomenon also occurring in plant roots<sup>168</sup>. Similarly, insect endosymbionts remain relatively invisible to the immune system, either because they lack MAMPs or because they reside in so-called 'immune privileged' compartments (cytoplasm, bacteriome).

The second principle is the existence of *negative regulators* that prevent immune activation. They can be either constitutive or inducible, establishing a negative feedback loop. A striking example is the expression of amidase PGRPs in *Drosophila* that degrade peptidoglycan, the MAMP recognized by the PRRs PGRP-LC and PGRP-LE upstream of the Imd pathway<sup>169,170,171</sup>. The expression of these negative regulators determines the degree of immune reactivity to MAMPs in the tissue. Moreover, the existence of negative feedback makes the system more reactive to variations in MAMP concentration (caused for example by pathogen entry) rather than MAMPs *per se*. The principle of compartmentalization and negative regulation has been especially well characterized in the case of the endosymbiotic bacteria *Sodalis pierantonius* and its host, the cereal weevil *Sitophilus oryzae*<sup>172</sup>. First, this endosymbiont, although it exposes MAMPs known to activate the immune response, resides in a specialized organ called the bacteriome. Second, bacterial replication is kept in check by the constitutive expression of an antibacterial peptide in the bacteriome<sup>173</sup>. Third, the immune response triggered by MAMPs released by the endosymbiont is suppressed by the expression of a negative regulator<sup>172</sup>. In plants, various mutualistic interactions occur with microbes, of which the interaction of legume plants with Rhizobium bacteria is probably the best characterized. Inside plant roots, the bacteria occur intracellularly in nodules, a protected environment where the plant provides the bacteria with nutrients, while the bacteria obtain nitrogen from the atmosphere by fixing it into a form that the plant can use. To initiate this symbiosis, and also suppress host immunity, the bacteria secrete lipochitooligosaccharide nodulation (NOD) factors that are perceived by host cell surface receptors that mediate the accommodation of the Rhizobium bacteria<sup>174</sup>.

We conclude that specific features of symbionts (low division rate, modified or absent MAMPs) and two sets of factors of the innate immune system, namely physical separation and negative regulation, allow symbionts to be kept in check while activating effective immune responses against pathogens.

## Concluding Remarks

Thirty years of innate immunity research have deeply transformed the conceptual foundations of immunology. Immunity is now recognized as a universal phenomenon in all living things. Traditionally defined lymphocyte-based adaptive immunity is a fascinating but tiny part of immunity. Although the adaptive immune system is bewitching, it may generate its own necessity by selecting for ever-more-devious parasites<sup>175</sup>. Most organisms rely on innate immunity and, even in those that display lymphocyte-based adaptive immunity, the latter is strongly dependent on foundational innate mechanisms. Innate immunity is diverse and highly complex, with intricate crosstalk between its various levels and mechanisms, as illustrated, for instance, by the multilevel regulation between cell-intrinsic immunity and immunity mediated by specialized cells.

Innate immunity has transformed the core concept of immunity in several ways. First, immunity is both 'systemic' (organized into a complex and integrated system at the whole organism level) and strongly 'local' to the extent that each organ or each tissue, and even each cell type, can be considered to have a unique immune system. Second, although pathogen pressure is clearly one of the most central selective pressures in the evolution of immune systems, immunity goes well beyond just defense against pathogens: innate immune mechanisms are involved in tissue homeostasis, symbiosis, and many other physiological functions<sup>176,177</sup>. Moreover, many other processes cooperate with the immune system in host defense, including disease tolerance, symbiont-mediated immunity, and behavioral immunity.

The study of innate immunity has benefited from various model organisms, for which different methods have been used. How we interrogate the immune system clearly influences how we 'see' it and how we spell out its mechanisms. Habitual methodologies (e.g. mode of infection, dose, temperature, use of opportunistic or co-evolved pathogens) influence the answers we achieve. Despite these limitations, a fruitful dialogue between

scientific communities working with different systems has propelled new generally applicable concepts that transcend the idiosyncrasies of each model. One major example is effector-triggered immunity, a concept that originated in plants, but is now applied across all taxa including mammals. We believe that this comparative approach that has been so critical at the birth of innate immunity studies, with dialogue and mutual enrichment between communities, should be valued and encouraged. While parallels in innate immunity across kingdoms are often appraised against the yardstick of human immunology, deciphering specific mechanisms that are 'not apparently conserved' is critical for a full understanding of immunity.

Perhaps the most radical conceptual transformation in the field has been the questioning of the definition of 'innate immunity' as such. Innate immunity was originally defined by what it was seen to lack: 'memory' and 'specificity'. This vision has perished as these concepts have been found to be generally applicable to innate immunity. Thus, reconceptualizing innate immunity is a necessity. In our view, the two terms 'specific' and 'adaptive', are not adequate to distinguish the innate from the adaptive immune system, except when taken in the implicit sense of the clonal selection of a repertoire of immune genes. In other words, one can still distinguish somatic recombination-based immunity from non-somatic recombination-based immunity, but this distinction does not overlap with the traditional memory-lack of memory distinction nor with the specific-nonspecific distinction.

Finally, the development of innate immunity required the invention of new language, which was facilitated by rich theoretical thinking, from Janeway's infectious nonself hypothesis to Matzinger's danger theory, and guard-hypothesis-inspired ETI to disease tolerance. Although we sometimes feel lost in the multiple, although fascinating, molecular processes underlying host defense, attempts to conceptualize innate immunity within a broader perspective are an important source of inspiration for future experimental work leading to new discoveries.

### **Acknowledgements**

We thank Anna Zaidman-Remy, Mark Hanson, Jonathan Ewbank, Sören Paludan, and Asen Daskalov for helpful comments. We thank Hannah Westlake for comments on the manuscript and for help for the figures. BL was supported by the SNSF grant 310030\_215073. The research of TP is funded by the Gordon and Betty Moore Foundation through grant GBMF9021. B.P.H.J.T. acknowledges funding by the Alexander von Humboldt Foundation and the Deutsche Forschungsgemeinschaft (DFG) under Germany's Excellence Strategy – EXC 2048/1 – Project ID: 390686111.

### **Author contributions**

The paper was written and revised by all the authors.

### **Declaration of interests**

No

### **Figure legends and Box 1**

#### **Figure 1. Six overlapping mechanisms of innate sensing.**

- A.** Pattern-triggered immunity (PTI). Structural molecules specific to a class of microbes (PAMPs/MAMPs), are recognized by host pattern-recognition receptors (PRRs) which trigger the immune response. PRRs can bind directly to microbes (e.g. PGRP-SA to Gram-positive bacteria) or more frequently sense microbe by sensing MAMPs released by microbes (indirect mode). PRRs can be secreted, transmembrane, phagosomal, or intracellular (not shown). They can initiate a transcriptional program or directly trigger effector modules.
- B.** Effector-triggered immunity (ETI). Host receptors directly sense virulence factors, or more frequently 'guard proteins' sense the activity of virulence factors that modify host molecules. There are multiple variations on the mechanisms that allow the sensing of microbial effectors (not shown here, e.g., decoys<sup>178</sup>). Host guard proteins can be extracellular (e.g., detection of microbial protease activity in *Drosophila* by Persephone), transmembrane, or intracellular.
- C.** Recognition of the absence of self. The immune system is activated when a constitutive signal provided by host cells is not detected by host receptors, indicating material of foreign origin.

- D. Recognition of altered self. Some immune receptors can recognize altered or abnormal self-patterns. This is often a host component located at a place where it is normally absent, such as a normally intracellular molecule present in extracellular spaces.
  - E. Recognition of damage-associated molecular patterns (DAMPs). Innate immune responses are triggered by the sensing of host molecules released upon damage to host tissues.
  - F. Surveillance. Innate immune responses are triggered by generalist stress pathways that interpret rupture of cellular homeostasis as an indicator of infection.
- Sensing mechanisms are either direct (pink background) or indirect via the sensing of activities or damages (green background).

### Figure 2: Common principles of innate immune effector mechanisms

Immune effectors involved in innate immunity rely on a limited number of mechanisms that revolve around a few principles. **A** 'destruction of the radically different' (e.g., antimicrobial peptides recognizing negatively charged membrane of bacteria), **B**. 'anti-virulence', **C** 'PRR-assisted elimination' (e.g., complement activation guided by C3b binding to pathogen, or phagocytosis of opsonized microbe), **D**. 'suicide of the infected cells', **E**. 'nutritional immunity'.

### Figure 3 The various forms of immune memory

Survival to infection has long been linked exclusively to the host's ability to combat and eliminate pathogens. Yet recent studies have broadened our perspective by revealing additional mechanisms that contribute to survival during infection, including disease tolerance, symbiont-mediated immunity, and behavioral immunity. These mechanisms are integrated with the rest of the innate immune system and several of them use classical innate signaling pathways, which raises the key conceptual question of how 'inclusive' our understanding of innate immunity should be.

**Disease tolerance versus resistance.** Host defense can be mediated either by *resistance* mechanisms that directly target or limit the growth of pathogens (discussed in the main text) or by *disease tolerance* (or *resilience*) mechanisms that promote host survival without targeting pathogens. The concept of disease tolerance was initially developed by plant ecologists to measure plant survival upon infection by a pathogen<sup>179-184</sup>. Whereas resistance implies that the microbial load is reduced upon infection, tolerance implies that host fitness is less negatively affected by pathogen growth. In the 2000s, the concept was also applied to animals<sup>185-189</sup>. Disease tolerance mechanisms include tissue repair, stress responses, detoxification, and prevention of the deleterious consequences of immunopathology, among others. A commonly used method to distinguish resistance and disease tolerance immune mechanism is to plot survival or disease symptoms against microbial load<sup>186,187</sup>. This approach reasonably assumes that microbe load is more significantly affected by disrupting resistance mechanisms than disease tolerance. However, the complex interplay between survival and pathogen growth makes separating resistance and tolerance difficult<sup>190</sup>. Second, many processes can affect survival that are unrelated to the immune system. Thus, a definition of disease tolerance as "immune related mechanisms that promote host survival without targeting pathogens" (without additionally addressing pathogen load), appears to us more appropriate. Nevertheless, the conceptual change and overall broadening of our understanding of host defense induced by the wide recognition of disease tolerance mechanisms across species constitute some of the most stimulating contributions of recent innate immunity studies.

**Symbiont-mediated immunity.** All animals and plants harbor a network of microorganisms known as the microbiota. This host-microbiota relationship can vary from mutualistic to pathogenic, and is context-dependent<sup>191</sup>. Symbionts can provide their hosts with many advantages, including protection against pathogens, a process called symbiont-mediated immunity<sup>192</sup> or 'co-immunity'<sup>193</sup>. Symbiont-mediated immunity is especially prevalent with facultative endosymbionts (bacteria living inside their host) of insects, which can increase their own transmission by protecting their hosts<sup>194-197</sup>. Symbiont-mediated immunity in insects exists against various pathogens, including parasitic wasps, nematodes, RNA viruses, fungi, and *Plasmodium* parasites. It is also the cornerstone of symbiont-based control strategies, such as the recent field release of *Aedes* mosquitoes transfected with a strain of *Wolbachia* to suppress dengue virus transmission<sup>198</sup>. In plants, many cases of symbiont-mediated protection are documented<sup>199,200</sup>. There are two modes of symbiont-mediated immunity, direct and indirect. A symbiont can protect its host by directly interfering with pathogen growth via either niche competition or by producing an effector that targets the pathogen<sup>197</sup>. In 'colonization resistance', the resident microbiome prevents the arrival of pathogens through direct competition – e.g., by occupying a niche and making it less accessible to intruders<sup>201</sup>, which can be established through collective

consumption of the nutrients that an incoming pathogen species requires to establish itself<sup>202</sup>. This phenomenon, described for the gut microbiota since the 1960s–70s<sup>193,203</sup>, exists in invertebrates<sup>204,205</sup> and plants<sup>206</sup>. The protection can also be indirect, i.e., host-mediated. In this case, the immune system is stimulated by the presence of resident microbes making the host more resistant against potentially invading pathogens<sup>207</sup>. Gut symbionts stimulate the maturation of the mucosal immune system during development and set a basal amount of immune activation<sup>208,209</sup>. Symbionts might also indirectly promote host survival by improving their metabolism, therefore increasing energetic resources that can be used by the immune system to fight infection.

**Behavioral immunity.** The crosstalk between the nervous and the immune system is well established, as exemplified by the production of glucocorticoids by adrenal glands under the control of the Hypothalamic–pituitary–adrenal (HPA) axis. Animals also use their sensory system to detect pathogens and trigger various behaviors that prevent infection. These mechanisms, grouped under the term ‘behavioral immunity’, include avoidance of pathogen-associated odors, hygienic behavior by grooming, food uptake blockage, sickness behaviors required for efficient health recovery, and parental behavior protecting the progeny. In such situations, the immune and nervous systems cooperate to increase host fitness and protect the next generation<sup>210–212</sup>. Vertebrates and invertebrates alike adapt their behaviors in response to volatile olfactory cues related to the microbial environment. In *Drosophila*, olfactory cues shape behavior, and wasp odors can prime progenitor hemocytes to differentiate into lamellocytes, a cell type involved in encapsulation<sup>213</sup>. Strikingly, *Drosophila* can also exhibit parental behaviors to protect their progeny from wasp infection, by laying eggs on ethanol rich substrates that are aversive to wasps<sup>214</sup>. Nematodes also adopt various well-studied behaviors to avoid pathogens<sup>215,216</sup>. Social immunity, observed in ants and bees but also in humans (e.g., grooming by conspecifics) also relates to behavioral immunity<sup>217</sup>. Crucially, the nervous and immune systems not only interact but also overlap. Many neuronal cells express PRRs that allow them to directly sense infection. For instance, reduced egg laying following infection in *Drosophila* is triggered by the sensing of peptidoglycan by PGRP-LE in a subset of octopaminergic neurons in the central brain<sup>218</sup>. Similarly, detection of circulating peptidoglycan fragments by NOD2 in the brain has been recently shown to affect the regulation of body temperature and appetite in mice<sup>219</sup>. Thus, the nervous system can sense infection states using the same mechanisms as the immune system.

## Box 2 Other ways to combat infection

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Table 1

Concept	Definition
<b>Innate immune recognition</b>	
PAMP (pathogen-associated molecular pattern)	Conserved molecular motifs shared across many pathogens and absent from the host, regularly also referred to as microbe-associated molecular pattern (MAMP)
PRR (pattern recognition receptor)	Innate immune receptors that recognize PAMPs/MAMPs.
ETI (effector-triggered immunity)	The ability to detect the presence of pathogens by sensing toxins or 'effectors' they use to infect their host. The sensing of effectors can be direct, or indirect by detecting perturbations these effectors induce.
DAMP (damage-associated molecular pattern)	Host molecules associated with host tissue damage which are immunogenic.
Missing self	Some immune inhibitory receptors can recognize a molecular pattern that all or virtually all host components express intruders that lack this self-signal fail to maintain inhibition, thus triggering an immune response.
Altered self	Some immune receptors can recognize altered or abnormal self-patterns. This often consists in the detection of a host component at a place where it is normally absent.
Surveillance	Disrupted cellular homeostasis upon infection (e.g. inhibition of translation, membrane pores) activates generalist stress pathways that also trigger an innate immune response.
<b>Signaling and effectors</b>	
Target-specificity	The ability to produce effectors tailored to the encountered agents
Destruction of the radically different	Immune effectors that specifically target certain classes of pathogens due to specific characteristics that are absent in host cells
Anti-virulence effectors	Host defense molecules that do not target the pathogen itself but instead its virulence factors.
Innate sensor-assisted elimination	Effector mechanisms coupled to innate sensors that directly recognize infectious agents and restrict effector activity to pathogens and not the host.
Suicide of infected cells	Effector mechanisms involving activation of special cell death pathways that limit the spread of infectious agents to neighboring host tissues.
Nutritional immunity	The sequestration by the host of metabolites critical for pathogen growth.
Barrier-based effector mechanisms	Physical and chemical barriers that separate host internal compartments from the environment.
Constitutive mechanisms	Constitutive expression of immune effectors providing a basal level of protection
Cell-intrinsic immunity	A set of cell-specific ('cell-autonomous') immune defense mechanisms, particularly describing cellular mechanisms that target eukaryotic viruses.
Disease tolerance mechanisms	Mechanisms that promote host survival without targeting pathogens.
Resistance mechanisms	Immune effector mechanisms that directly target or limit the growth of pathogens.
Symbiont-mediated immunity or 'co-immunity'	Host defense protection provided by symbionts carried by the host.
Behavioral immunity	Neuronally mediated behaviors that prevent infection or promote health recovery.
<b>Innate immune memory</b>	
Persistent immune responses	Priming caused by a low chronic infection or by the perdurance of immune effectors
Sustained innate immune response	A lifelong and sustained protection against secondary infection (e.g., RNAi).
Reactivation-based memory	A first immune response is fully resolved by a return to a basal activation level; reactivation produces a much stronger immune response.
Trained immunity	A form of reactivation-based immune memory mediated by innate immune cells and resulting in an increased nonspecific response to subsequent infections.
Innate immune memory	Immune memory not mediated by a lymphocyte-based adaptive immune system. This term should probably be best used exclusively to describe 'reactivation-based memory'.



Immune priming	Improved survival of previously infected individuals compared to naïve controls upon subsequent infection.
Transgenerational immune priming (TGIP)	Improved survival to infection of the progeny of previously infected parents compared to the progeny of naïve control parents.
<b>Immune tolerance</b>	
Innate auto-immunity	Diseases caused by the inappropriate host-targetting activation of the innate immune system.
Microbiota compartmentalization	Physical separation of the microbiota from the host immune system (e.g. in specialized niches such as crypts or by barriers such as mucus).
Negative (feedback) regulation	Mechanisms that prevent excessive activation of the immune system through expression of negative regulators that influence the degree of immune reactivity.

**Table 1. Definition of-concepts of innate immunity discussed in this article**

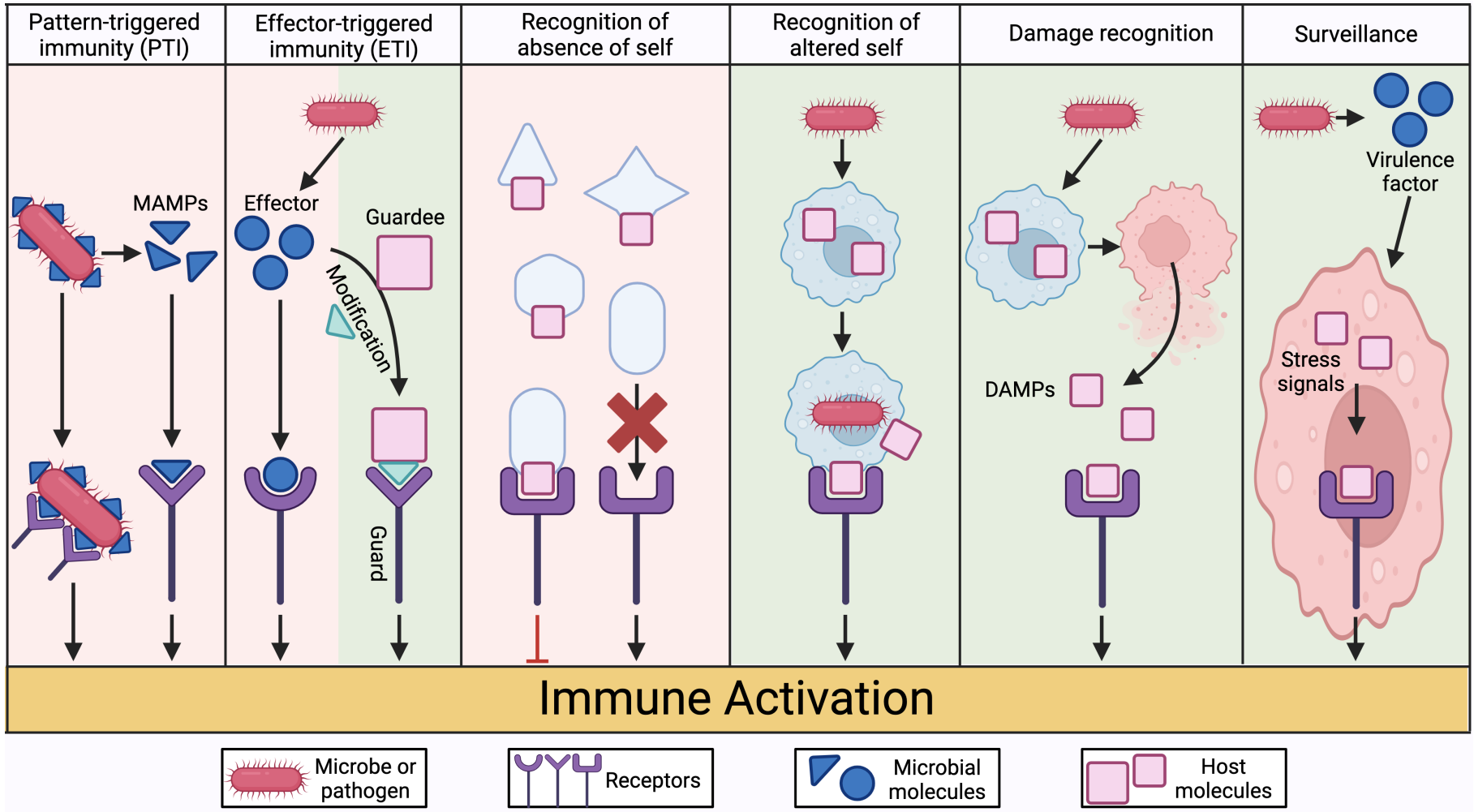


Figure 1

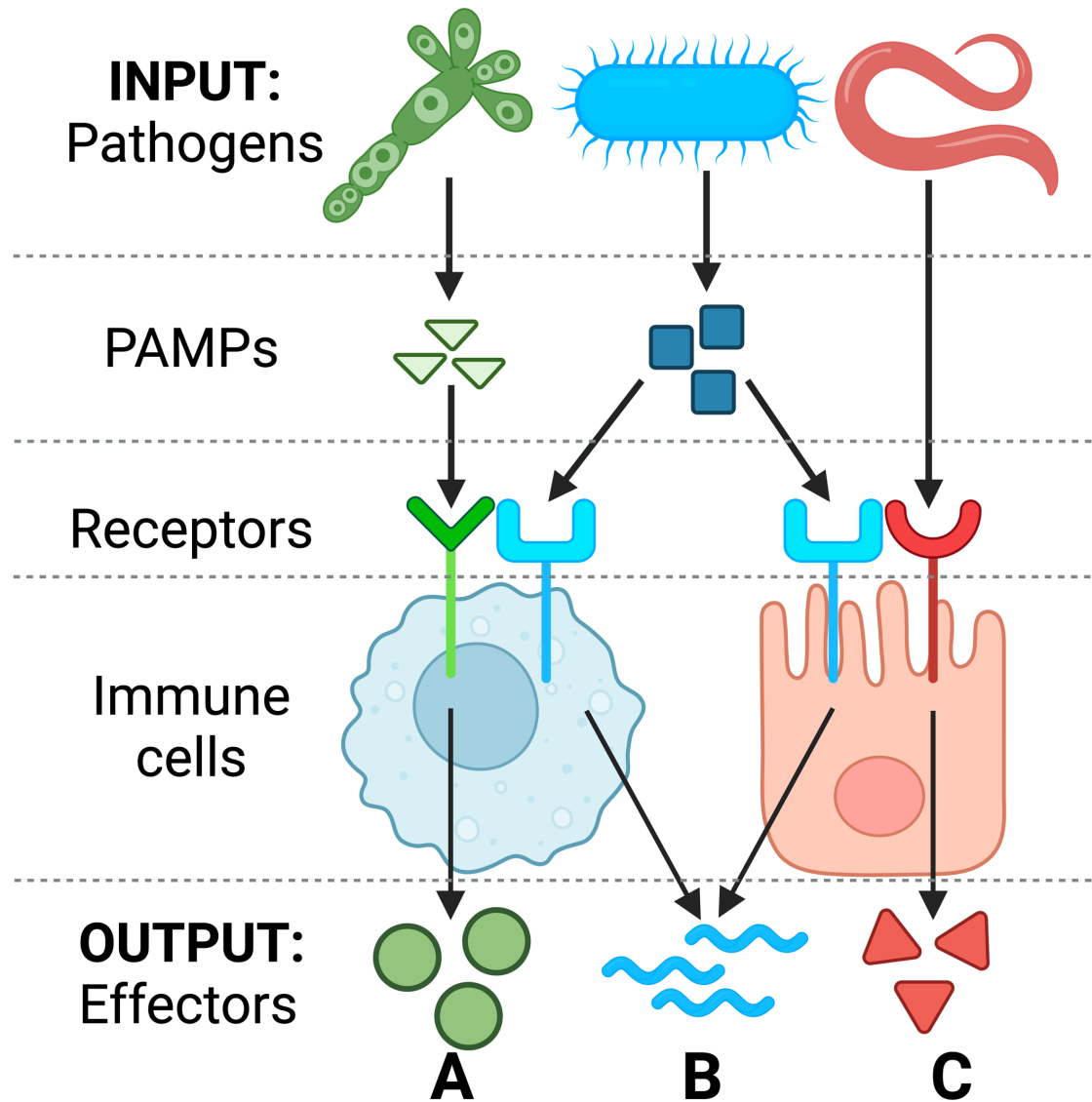


Figure 2

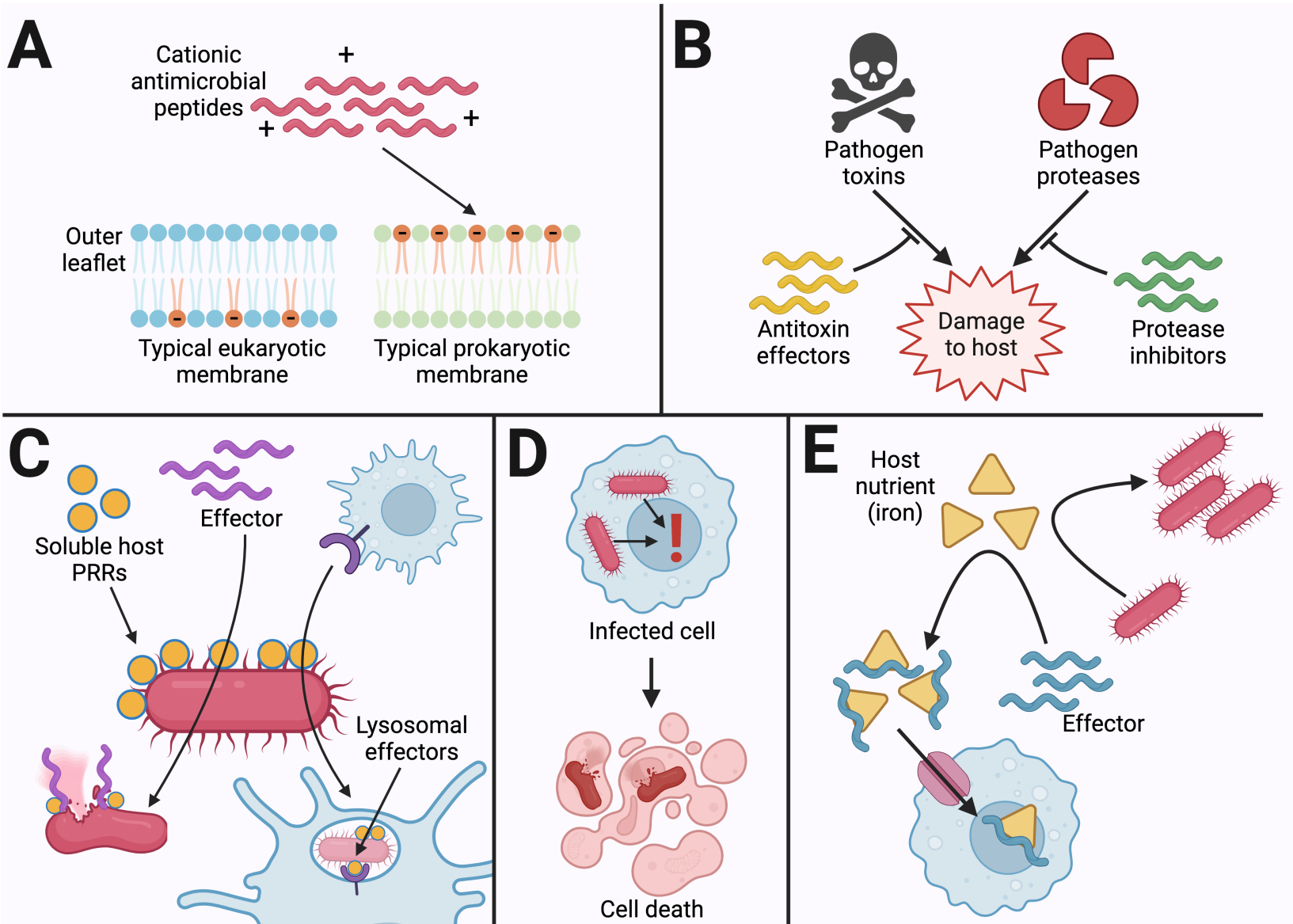


Figure 3

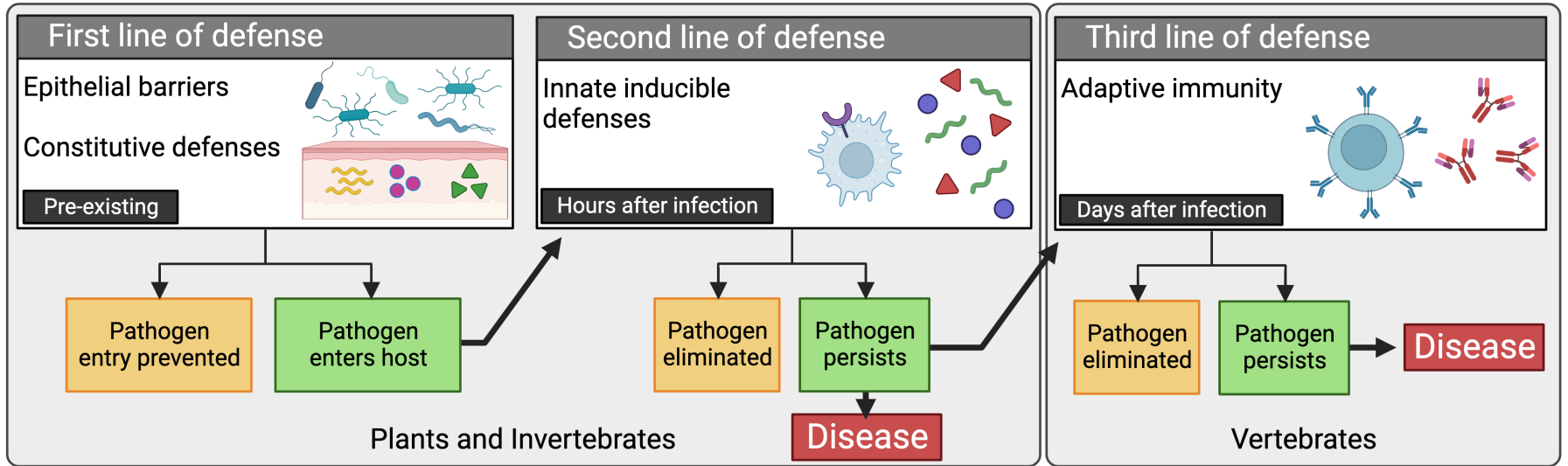


Figure 4

Mechanism	Primo infection	Description	Immune response profile
<b>I. Persistent infection</b> <b>INNATE</b>  Broad protection	Infection, stressors	Low level initial infection increases basal immune activation, increasing resistance against secondary infection.	
<b>II. Persistence of immune effectors</b> <b>INNATE</b>  Broad protection	Infection, stressors	Perdurance of immune effectors (humoral factors, differentiated cells) following an initial immune response provides early and stronger protection against secondary infection.	
<b>III. Sustained immune response,</b> <b>INNATE</b> (RNAi)  Highly specific	Viruses	Continuous amplification of siRNA provides long-lasting protection with high specificity.	
<b>III. Sustained immune response,</b> <b>ADAPTIVE</b> (CRISPR)  Highly specific	Viruses	New spacers derived from the genome of the invader are incorporated into the CRISPR array and used to face subsequent invasions. Transmitted to the next generation.	
<b>IV. Reactivation-based memory</b> <b>INNATE</b> (trained immunity)  Broad protection	Infection, stressors, adjuvants	Basal activation levels return after initial infection, but regulatory changes increase immune readiness, enabling stronger or more rapid responses upon secondary challenge.	
<b>IV. Reactivation-based memory,</b> <b>ADAPTIVE</b> (B and T cell mediated)  Highly specific	Infection, PRR + antigen	Initial infection triggers differentiation of memory T or B cells carrying receptors for specific pathogen markers.	