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Balancing sensitivity, risk, and immunopathology in immune regulation

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Activation of an immune response is energetically costly and excessive immune system activity can result in immunopathology, yet a slow or insufficient immune response carries the risk of pathogen establishment with consequent pathology arising from the infection. Mathematical theory and empirical data demonstrate that hosts balance the costs of immunity against the risk of infection by closely regulating immunological dynamics. An optimal immune system is rapidly and robustly deployed against a true infectious threat and rapidly deactivated once the threat has been controlled. Genetic variation in the sensitivity of an immune system, as well as in the activation and shutdown kinetics of host immune responses, can contribute to the evolution of pathogen virulence and host tolerance of infection. Improved understanding of the adaptive forces that operate on immune regulatory dynamics will clarify fundamental principles governing the evolution and maintenance of innate immune systems.

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Intuition suggests that pathogenic infection should be met immediately with an overwhelming immune defense that smothers the infection before it can become established. In fact, one might envision an ever-active immune system poised to suppress any pathogen that breaches epithelial barriers. Yet most immune systems remain fairly inactive under normal circumstances and are only induced to high levels of activity after infection occurs. The ubiquity of inducible immune reactions implies that the fully active state is costly, and that the cost is not worth bearing in the absence of dangerous infection. The costs are real and manifest in terms of energetic demands and risk of autoimmune damage. Yet the alternative strategy of deactivating the immune system in the absence of infection carries a risk of its own, providing the pathogen with time to become established while immune defense is being ramped up. The lag time to full activation of an inducible defense therefore sets up a high-stakes race between the host and pathogen, and the host must balance risk against costs in assessing what threats warrant a response and how strongly to react.

Insects and other non-vertebrates rely on innate immune systems, while vertebrates supplement their innate immunity with highly specific, antibody-mediated acquired immune systems. In innate antimicrobial immune responses, a eukaryotic host recognizes conserved molecular structures characteristic of microbes but absent from eukaryotes, including β-glucans (fungal cell walls), peptidoglycan and lipopolysaccharides (bacterial cell walls and membranes), and components of flagella (bacteria). These stimulatory molecules are sometimes called Microbe-Associated Molecular Patterns, or MAMPs [1•]. Recognition of parasitic infection stimulates an induced response, which in insects can be composed of phagocytosis of small pathogens by macrophage-like cells, encapsulation of large pathogens within multilayered sheaths of host cells, and production of cytotoxic oxidative free radicals and antimicrobial peptides to kill pathogens [2,3•]. Each of these defense mechanisms may come at considerable cost. Phagocytes are finite in number and cannot always be quickly recycled [3•]. Forming a multicellular capsule around a pathogen requires substantial investment into hematopoiesis [4]. Reactive oxygen species are undiscriminating and can do damage to host tissues as well as to pathogens [5,6,7••]. Antimicrobial peptides may be produced at levels as high as 100 μmol in insect hemolymph [8], posing a substantial transcriptional and translational burden on host cells. The costs of an immune response in terms of energetic expenditure and self-damage can be so great that, in some cases, the pathology associated with an infection is due as much to the host response as it is to direct virulence of the pathogen [9].

How, then, should a host optimize the immune response? Rapid activation is critical and small differences in the speed or intensity of immune induction over the first few hours of infection can make a life-or-death difference in

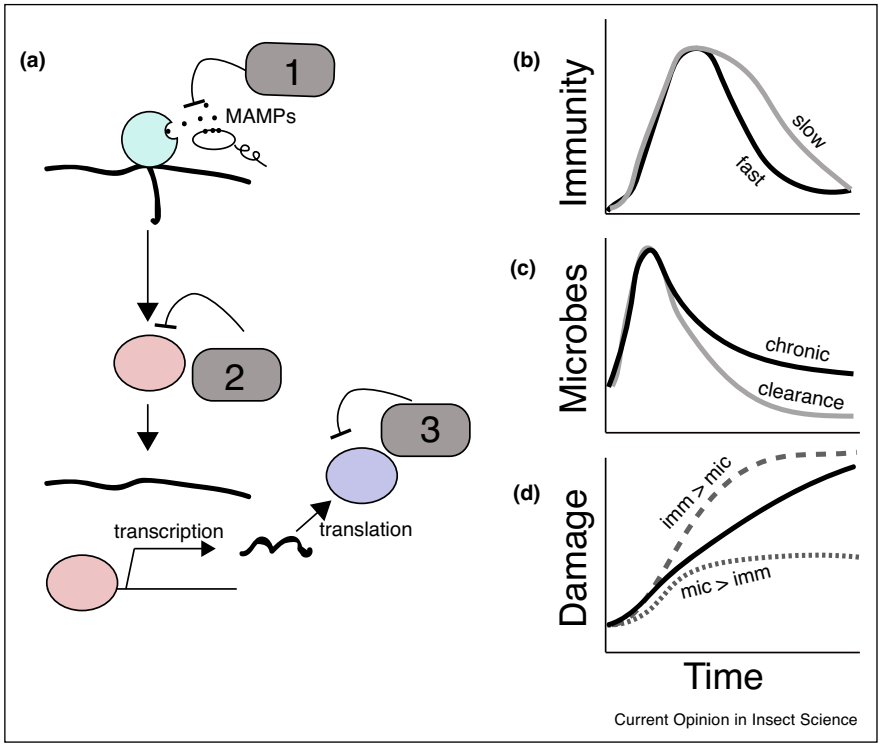
infection outcome [10,11••, Lafont *et al. bioRxiv* doi: https://doi.org/10.1101/2021.10.19.464998], but the costs of an overactive response could be substantial. The host must react quickly and intensely to a true threat without being hypersensitive to non-threats such as beneficial microbes and benign commensals. Since even non-pathogenic microbes present MAMPs, simple recognition that a microbe is present is too low of a threshold for inducing full immune activation. Instead, a more discriminating strategy is to couple surveillance for microbes with surveillance for indicators of cellular damage such as intracellular molecules in the extracellular space (sometimes called Damage Associated Molecular Patterns; DAMPs) [12,13] or for common pathogen virulence mechanisms such as toxin production or protease secretion [14,15,16•]. That a discriminating immune system should mount a full response only in the joint presence of both DAMPs and MAMPs is the central premise of Maztinger's Danger Model [17]. Extending Matzinger's logic and incorporating the costs of immunity, Lazzaro and Rolff subsequently argued that the healthiest host need not completely eradicate a pathogen but need only reduce pathogen burden below a threshold where the cost of damage done by the pathogen is less than would be the cost of an immune reaction to control the infection [18]. The residual pathogen burden would be tolerated by the host. Those authors drew a parallel to the economic injury level as applied in agriculture [19], which is the threshold at which herbicide or insecticide application is warranted because the cost of damage done by an agricultural pest exceeds the cost of implementing control. Similarly, a well-tuned immune system might balance risks and costs by activating a response only when both MAMPs and danger signals are jointly present, and only when the potential damage from the pathogen is greater than the energetic and immunopathological cost of defense.

Of course, it is quite a lot to ask that any system should be able to anticipate future costs of unchecked infection and titrate exactly the correct amount of immunity for control. In practice, infection presents a combination of stimuli, some of which may be only indirectly related to potential virulence or toxicity of the pathogen, and the immune system must respond to those stimuli as they exist in the moment as opposed to what they might represent for the future. While activating immunity certainly comes with costs, failing to adequately suppress a pathogenic infection would cost much more. In an early theoretical model, Frank showed that hosts achieve highest fitness when pathogenic infection is rapidly detected and an intense response is mounted to guarantee control of infection, provided the response is quickly shut down after the infection has been cleared to limit immunopathology [20]. Subsequent work by Urban *et al.* [21] demonstrated that the optimal host strategy is to err in the direction of excess immune activation to ensure suppression of the pathogen, but again emphasized the importance of rapid shutdown after the infection has been cleared. Interestingly, however, the fitness surface in the Frank [20] model was fairly flat and the Urban *et al.* model [21] yielded a flat shoulder to the fitness surface beyond the point where immune activity was adequate for control of infection. These theoretical findings suggested that natural populations might harbor substantial genetic variability for immune regulatory kinetics as long as activation exceeds some minimum threshold. Consistent with these models, natural insect populations harbor considerable genetic variation for the quantitative degree of immune gene expression in response to infection challenge [e.g. Refs. 22,23••,24•]. However, more empirical work is necessary to determine the degree to which that variability stems specifically from polymorphism in surveillance sensitivity, intensity of activation, and rate of shutdown, as well as how such variability relates to pathogen control and host fitness.

The theoretical prediction that potential threats should elicit a rapid and overwhelming initial response is well supported by data. In insects, a sterile injury is sufficient to induce a transient prophylactic antimicrobial response even in the absence of microbes or MAMPs [e.g. Ref. 25]. However, the presence of microbes is required to sustain the response beyond a few hours. Insect immune reactions to sterile injury or legitimate bacterial infection include several-hundred-fold increase in expression of genes encoding a broad suite of antimicrobial peptides [e.g. Refs. 25–28,29•]. Surprisingly, however, most of the antimicrobial peptides produced seem to have no effect in controlling any given infection [30••]. Thus, it would appear that instead of attempting to restrict expression to the specific peptide(s) that are appropriate for controlling a given infection, insects abundantly express an entire catalog of peptides so that a few functionally effective peptides will be included among them. This may be due to lack of host ability to rapidly and accurately discriminate among infections, but the consequence could be that an unnecessarily high cost of immune activation is borne in order to ensure that infection is controlled.

The prediction of robust negative regulation is also well supported empirically. A ubiquitous insect defense mechanism is production of cytotoxic reactive oxygen species (ROS) from quinone and semiquinone intermediates via phenoloxidase. This is an extremely rapid response, activated in minutes by protease cascades that are much faster than responses that depend on transcription and translation. However, simultaneously, ROS detoxifiers like superoxide dismutase and catalase are produced to defuse the ROS even as they are being generated [3•; Figure 1]. Similarly, the expression of genes encoding antimicrobial peptides is triggered by activation of two signaling pathways, the Toll and Imd pathways, which are almost completely conserved across insects. These two pathways activate expression of proteins that dampen flux

Figure 1



Regulation of immune signaling during infection. Negative regulators of immune responses **(a)** can degrade MAMPs and DAMPs to prevent signal transduction from immune receptors (1, for example, peptidoglycan-recognition proteins with amidase activity), they can inhibit or degrade signaling proteins and transcription factors that propagate those signals (2, for example, the proteins Cactus and PIRK), or they can modify the activity of the resulting effector response (3, for example, antioxidants that detoxify immune-generated ROS). The net effect of these regulators is to slow the induction of an immune response and to accelerate its decay. Lower activity of a negative regulator can result in a prolonged immune response (**(b)**, gray line) relative to a response that returns more quickly to baseline (black line). While reducing negative regulation on the system could potentially result in faster activation and/or more complete clearance of microbes compared to a dampened reaction that might allow chronic persistence of an infection **(c)**, a host with more aggressive immunity could suffer a net higher damage (**(d)**, gray dashed line, where immunopathology exceeds the damaged done by infection) compared to a host whose immune system is under moderate negative regulation (d, black line, where immunopathology is reduced but damage due to chronic infection continues to accumulate) or one whose immune system is strongly regulated so that immunopathological damage is lower than direct damage from the infection (d, dotted gray line).

through the pathways [1•; Figure 1], resulting in a negative feedback loop that shuts down the immune system once the activating stimulus is gone. Furthermore, the Toll and Imd pathways upregulate production of proteins that degrade immunostimulatory MAMPs [31,32; Figure 1], actively removing the stimulus to prevent sustained signaling in response to microbes that have already been killed or in response to benign commensals [33]. Theory predicts [34] that shutdown kinetics should be especially rapid when the cost of immune deployment is high, as in the case of antimicrobial peptide production, or when there is a high risk of immunopathology, as is the case with ROS production. High levels of immunopathology can even shift the fitness balance toward sustaining chronic infections, provided the pathology of the chronic infection is less than the pathology associated with a level of immunity required to eradicate it [34].

Genetic variation in sensitivity to immune stimulation and consequent immunopathology could contribute to among-individual variation in tolerance of infection, where tolerance is defined as the ability to sustain health and fitness despite a pathogen burden [35]. Tolerance can be empirically measured as the relationship between health and parasite number, and variation in tolerance can be determined at the population level by measuring both host health and pathogen burden in a sample of individuals or genotypes drawn from the population. Immune sensitivity is determined by several distinct variables, including the number of microbes needed to cross activation thresholds [36], the strength of induction per microbe [23••], and the kinetics of negative regulators relative to positive ones [37]. Genetic variation in any of these traits could lead to variability in the capacity to control infection, as well as to variation in

immunopathology and tolerance of infection. Insects are especially amenable to studying variation in tolerance because they often can be maintained as genetically homogeneous strains, which can be used for replicated measure of host health over an experimental range of pathogen burdens. Such studies have revealed considerable naturally occurring genetic variation in tolerance of microbial infection [e.g. Refs. 38–41,42•]. This observation is qualitatively consistent with the theoretical predictions of Frank [20] and Urban *et al.* [21] that populations may be genetically variable for immune activation and shutdown kinetics, and indeed a pair of recent articles recently showed that negative regulators of the *Drosophila* immune system promote tolerance of infection [Prakash *et al. bioRxiv* doi: 10.1101/2021.09.23.461574, Prakash *et al. bioRxiv* doi: 10.1101/2021.09.23.461578]. Future research should additionally test the relationship between tolerance and sensitivity to immune activation, while recognizing that a vast number of physiological processes could contribute to tolerance and that the determinants of genetic variation in tolerance are unlikely to lie solely in the immune system [28,40,43].

Optimal immune reactivity may vary demographically. Metcalf *et al.* [44] noted that long-lived hosts might be expected to evolve reduced sensitivity to infection in order to minimize immunopathology accumulated over a lifetime. This is superficially contrary to an intuitive expectation that long-lived hosts should be more immunologically vigilant because of cumulative risk of exposure, but is supported by widespread observation that overactivation of immune systems early in life results in decreased longevity [6,45,46]. Instead, long-lived hosts could be predicted to evolve forms of acquired immunological memory and immune plasticity to deal with rapidly evolving pathogens [47–49]. This hypothesis could be tested by contrasting the immune systems of long-lived and short-lived invertebrates.

When immunopathology is a major determinant of overall pathology (in contrast to when virtually all pathology arises from parasite exploitation of the host), immunopathology can become an indirect driver of pathogen virulence evolution [50]. For the host, the cost of infection is the cumulative combination of pathology arising from both pathogen virulence and the host's own immune reactions (Figure 1). Direct selection on the pathogen is always for increased transmission, but host immunopathology can become a factor in transmission-virulence tradeoffs for the pathogen. When pathogen virulence mechanisms stimulate increased immunopathology to the point of decreasing transmission (e.g. because of early host death), there can be selective pressure on pathogens to become both less virulent and less immunostimulatory, effectively increasing host tolerance of infection [51,52]. Reduced immunostimulation may be less likely to evolve when transmission and immunopathology are positively correlated (e.g. when host symptoms mediate transmission). Similarly, host evolution of tolerance mechanisms that mitigate pathogen-induced damage without directly attacking the pathogen itself (such as wound repair and neutralization of virulence factors) can facilitate pathogen evolution of escalated virulence if the virulence mechanisms directly promote transmission [53].

While both theoretical and empirical studies have begun to explore the challenge of optimizing immune reactivity to sufficiently control infection while minimizing immunopathology, much work remains to be done. How the host might achieve this optimization given limited and imperfect information about potential virulence in the early stages of an infection is a question ripe for theoretical exploration. Mechanistically disentangling the counterbalancing forces of immune induction versus active signal decay and system shutdown is a top priority, including comparative evaluation of how direct versus indirect negative regulation impacts infection dynamics. So, too, is determining the health and fitness consequences of drifting away from immunological optimality. Because of their experimental tractability, insect systems are ideal for performing this work. Insects are regularly observed to tolerate low-level chronic infections instead of immunologically eradicating pathogens [e.g. Refs. 38,41,54,55], and the importance of this for the evolution of pathogen virulence and transmission should be explored theoretically. Achieving better and more complete understanding of the proximate and evolutionary consequences of variation in the regulation of inducible immune responses will clarify fundamental principles driving the evolution and maintenance of innate immune systems.

Conflict of interest statement

Nothing declared.

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