The Genetics of Immunity

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In this commentary, Brian P. Lazzaro and David S. Schneider examine the topic of the Genetics of Immunity as explored in this month's issues of *GENETICS* and *G3: Genes|Genomes|Genetics*. These inaugural articles are part of a joint Genetics of Immunity collection (ongoing) in the GSA journals.

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Defense against infection is increasingly recognized to have a complex determination, shaped by the contributions of multiple genes and a multitude of environmental factors. While we may conventionally imagine defense against infection to be determined primarily by the activity of the host immune system, recent studies have established diverse biological mechanisms for regulating defense. The genetics of immunity is being studied using a wide variety of approaches and organisms from agriculturally relevant plants to genetic models such as *Drosophila* to humans. In this spirit of greater discourse among researchers across disciplines, the journals of the Genetics Society of America—*GENETICS* and *G3: Genes|Genomes|Genetics*—invite submissions that address the broad reach and complexity of the genetics of immunity. Several such articles are presented in the June issues of both journals. The call for papers is ongoing, and future articles will be highlighted in the Genetics of Immunity collection.

COMPLEX REGULATION OF IMMUNITY

Several of the highlighted articles report studies of immune regulation in novel contexts. Anjum *et al.* (2013) examined modulation of the Toll pathway, best known in *Drosophila* for its role in activating synthesis of antimicrobial peptides in response to bacterial infection and in vertebrates for inducing inflammatory responses to microbial and viral elicitors. Anjum *et al.* (2013) focused on Toll pathway activity in larval *Drosophila* and conclude that misactivation results in inflammation-like phenotypes of increased lamellocyte (immune cell) proliferation, appearance of melanotic masses, and induction of antimicrobial peptides. They found that Toll activity in *Drosophila* larvae is negatively regulated by sumoylation controlled by the β-arrestin gene *Kurtz*. Loss of *Kurtz* or the SUMO protease Ulp1 results in ectopic immune activity and inappropriate inflammation-like responses. Notably, however, the distinct immune reactions vary in their relative magnitudes in the mutants, indicating that sumoylation probably interacts with other elements of the cellular machinery to balance the multiple activities of the highly pleiotropic Toll pathway. Additionally, because *Kurtz* and *Ulp1* mutants result in global disruption of SUMO activity, there is probably dysregulation of other pathways that contribute to control of inflammation and immunity.

De Arras *et al.* (2014) employed a clever cross-species mutant screen to identify a regulator that controls splicing of messenger RNA (mRNA) encoding the Toll pathway adapter MyD88, and hence immune activity. They took advantage of high-throughput RNA interference (RNAi) screening in *Caenorhabditis elegans* to scan the entire genome for genes whose inhibition blocks immune induction. They found 32 well-supported candidates, 20 of which have clear orthologs in the mouse. Disruption of 8 of these genes in mice also yields clear immune deficiency, and one of them, *Eftud2*, gives the reciprocal phenotype of enhanced IL-6 expression in response to lipopolysaccharide when overexpressed. Further investigation revealed that Eftud2 protein mediates the relative balance between a long (activating) and a short (inhibiting) spliced form of MyD88. Loss of *Eftud2* function results in a proportionally much larger decrease in the short form relative to the long form, thus blocking Toll-pathway activity and immune defense. This article nicely illustrates the power of comparative genomics and immunology to uncover conserved biological functions.

In another dissection of pathway regulation, Stronach *et al.* (2014) tackled the role of mitogen-activated protein kinases (MAPKs) in developmental *vs.* immunological cellular contexts. MAPKs activate the Jun Kinase (JNK) pathway in response to infection and stress and are themselves regulated by upstream kinases (MAPKKs and MAPKKKs, or

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MAP3Ks). Stronach *et al.* (2014) posited that MAP3Ks are broken into a functional domains—some that receive stimulus or determine subcellular localization, plus a distinct protein kinase domain. Under this hypothesis, it should be possible to swap the kinase domains on MAP3Ks that phosphorylate the same substrate and recover full function in the chimeric proteins. The authors tested this idea with the MAP3K Slpr, which is required for developmental signaling, and Tak1, which contributes to immune activation via the JNK and Imd pathways. Swapping the kinase domains between these two proteins results in partial rescue of the respective mutant phenotypes, but in neither reciprocal direction does the chimeric protein fully compensate for loss of the native protein. Thus, it seems clear that the kinase domains are not simply phosphorylating targets but are also potentially involved in interactions with other protein partners and certainly contribute to the inherent specificity of the proteins.

Two articles in the June issue of *GENETICS* address the role of reactive oxygen species (ROS) as signaling and defense molecules. Oxidative radicals are highly reactive, and their cytotoxicity can be harnessed in antipathogen defense. Tiller and Garsin (2014) identified a novel peroxidase, *skpo1*, that controls ROS production in the *C. elegans* hypodermal epithelia and correspondingly determines defense against infection by the bacterium *Enterococcus faecalis*. Small *et al.* (2014) found an unexpected pleiotropy between ROS production and immune cell differentiation mediated by Notch signaling in *Drosophila*. Notch signaling promotes the embryonic differentiation of crystal cells, which are responsible for ROS production in larvae attacked by parasitoid wasps. Infection by parasitoid wasps also results in proliferation of lamellocytes in the larval lymph gland. Unexpectedly, Small *et al.* (2013) found that Notch signaling regulates this lamellocyte differentiation. It appears that Notch signaling acts in a non-cell-autonomous manner in the lymphatic organ to hold lamelloctye precursor cells in quiescence, but inhibition of Notch by RNAi or parasitoid infection allows lamellocyte differentiation to proceed. This surprising pleiotropy establishes Notch as a key regulator of alternative immune cellular lineages in *Drosophila*, with important consequence for the effectiveness of defense against infection.

AGRICULTURALLY AND ECOLOGICALLY RELEVANT SYSTEMS

Infection and immunity are critical in agriculture. For example, bovine mastitis (infection of the udders) costs the U. S. dairy industry >$1.7 billion per year (Jones and Baily 2009). In this issue of *G3*, Lawless *et al.* (2014) correlate the inflammatory response to mastitis caused by *Streptococcus uberis* with microRNAs (miRNAs) that are anticipated to alter mRNA expression profiles. Monocytes released from bone marrow are recruited to the site of infection by chemokine-mediated attraction, where they switch from oxidative phosphorylation to glycolysis and effect an inflammatory response. Lawless *et al.* (2014) hypothesize that this switch is mediated by a suite of miRNAs, with upregulated miRNAs tending to target metabolic transcript RNAs, presumably driving the switch to glycolysis. In a twist, however, downregulated miRNAs are highly enriched for targeting immune genes, indicating that these miRNAs probably function as inhibitors of inflammation in the absence of infection.

In another example of agricultural disease, Connell *et al.* (2013) conducted a case-control study to determine why some birds in chicken flocks are genetically resistant to *Camplyobacter jejuni* infection. Genome-wide mapping reveals significant associations between resistance and polymorphism in the T-cadherin and calmodulin genes. These associations make sense, as previous work has revealed infectious interaction between *C. jejuni* and a related E-cadherin, and calmodulin is a well-known modulator of cadherin function. The phenotypic distributions of the *C. jejuni* load in birds carrying either variant are striking, with some chickens absolutely resistant to infection and others carrying loads of up to 10^{10} bacteria after inoculation. The mapped variants appear to be regulatory, and importantly, the resistant phenotype is determined by the relative—not absolute—expression levels of T-cadherin and calmodulin, with susceptible chickens expressing an approximate 25% increase in the ratio of T-cadherin to calmodulin. With these resistance factors identified, the genetic markers can be employed for selective breeding of resistant flocks.

In contrast, agricultural systems can sometimes be engineered for disease resistance, as Subbaiah *et al.* (2013) have shown by establishing stably transformed silkworms that are resistant to *Bombyx mori* nucleopolyhedrovirus (BmNPV). The transformed silkworms carry RNAi constructs that target four essential viral genes, generating >75% survival of experimental viral infection relative to <15% survival in the parental strain. Even more promising, the few viral occlusion bodies derived from the transformed *B. mori* are impaired in their ability to infect naive silkworms, further limiting disease spread. As BmNPV can result in loss of >50% of commercial silk cocoon yield, the transgenic lines have great potential economic impact.

Natural systems do not lend themselves to easy manipulation to prevent or limit disease establishment and spread, and we often struggle to understand what determines relative susceptibility and resistance in natural settings. *Batrachochytrium dendrobatidis* (Bd) is a fungal pathogen that is devastating amphibian populations globally, and we have a poor understanding of why some species are more susceptible than others. Ellison *et al.* (2014) examined gene expression profiles in the highly susceptible Panama Golden Frog after exposure to attenuated fungus. They found strong immune activation, but that does not translate into effective vaccination or protection from secondary infection. Instead, the data suggest that inflammation of the skin might be pathological in the presence of infection and that reducing inflammation could be protective. The transcriptional data also reveal upregulation of antibacterial immune defenses in frogs infected with Bd, possibly indicating secondary infection facilitated by the primary fungal infection. Intriguingly, Ellison *et al.* (2014) hypothesize that Bd may suppress immune reactions in susceptible frog species based on their observation that expression of genes diagnostic for B cells and T cells is reduced at late stages of infection with fully active Bd, suggesting that the fungus might kill the progenitors of these cells or block their differentiation. This interpretation is bolstered by the observation of reduced spleen size in infected frogs. The work of Ellison *et al.* (2014) suggests that the Panama Golden Frog attempts to resist Bd infection, but that the immune defense is compromised and perhaps itself pathological, resulting in reduced tolerance of infection. The effective defense observed in some other amphibian species may then be due to more managed immune activation and avoidance of pathogen mechanisms for immune suppression.

COMPLEXITY IN IMMUNITY

Perhaps the most interesting frontier in the genetics of immunity arises in the interaction between immune activity and other physiological or developmental processes. Such interactions must shape the balance of traits in an organism, determining overall health in the context of infection. Evolutionary and functional studies in this domain are of particular interest in the *GENETICS/G3* emphasis on the genetics of immunity, and a few such articles are highlighted below.

Johnston *et al.* (2014) applied transcriptional profiling in a time series after immune challenge of the mealworm beetle *Tenebrio molitor*. They found that different components of the immune response have different timings of expression, which indicates complex and specific regulatory control. The authors emphasize that expression of genes

encoding the Toll pathway, antimicrobial peptide genes, and iron sequestration processes remain upregulated a week after infection, although their experiments do not exclude the possibility that residual bacteria may continue to stimulate immune activity. Most notably, however, infectious challenge in *Tenebrio* results in repression of genes involved in glucose metabolism and biosynthesis of lipids, triglycerides, and vitamins. These data parallel an early *Drosophila* microarray study in which benign immune challenge was also found to suppress transcription of basal metabolic genes (De Gregorio *et al.* 2001) and support the hypothesis that immunological activity is energetically costly. At the same time, insects have been reported to reduce feeding in response to infection (*e.g.*, Adamo *et al.* 2007; Ayres and Schneider 2009), and an alternative, and not mutually exclusive, interpretation is that hosts alter metabolism to sequester nutrients as part of a nutrient restriction strategy to manage pathogen growth.

Reciprocally, immunity may be constrained by competing demands on the host. Short *et al.* (2012) have shown that mating and reproduction limit immune competence in *Drosophila*, but that the effect is dependent on an intact female germline. In an article published in *G3*, Short and Lazzaro (2013) report a transcriptional analysis of the response to infection in virgin and mated female *Drosophila melanogaster* with and without germlines. Confirming their previous study, they find that virgin females induce immune genes more strongly than reproductively active females do, although the difference in expression patterns between females with and without germlines is surprisingly small. A novel but logical finding is that egg production genes show reduced expression after infection in virgins but not in mated females, suggesting that females prioritize egg laying over immune defense when given the choice. The transcriptional data also reveal other interesting patterns that provide fodder for follow-up study, such as signatures of egg maturation in response to mating and altered feeding behavior in response to mating and infection.

Although there is a tendency to treat immune capability as a static property of the host, immune competence is a dynamic trait that changes over the host life span. Intuitively, we might expect that immune capacity would decline with host age, a process known as immunosenescence, and indeed this is observed at advanced ages. Felix *et al.* (2012), however, have shown that capacity to clear nonpathogenic *Escherichia coli* infection may increase or decrease between young and middle ages (1 and 4 weeks post-eclosion adults), although the magnitude of variation among genetic lines increases at the older age. They also evaluated genome-wide gene expression in a panel of inbred *D. melanogaster* lines at 1 and 4 weeks of adult age. Consistent with previously published articles, Felix *et al.* (2012) noted a tendency for immune gene expression to increase with age, even in the absence of infection, and again found repressed expression of genes involved in lipid metabolism after infection of flies of both ages. Unexpectedly, however, they found that the genetic architecture of immune capacity (the correlation structure between clearance of infection and genome-wide transcription) differed between the two ages, with a stronger relationship between energy metabolism and immune capacity in older flies.

CONTINUED EMPHASIS ON THE GENETICS OF IMMUNITY

GENETICS and *G3* continue to seek submissions of manuscripts on the genetics of immunity, and both journals will continue to highlight articles published in this area. Topics of particular interest for future publication include:

Elucidation of genetic mechanisms leading to tolerance of, or resistance to, infection

Genetic or signaling networks that affect immune system function

Genetic responses to environmental factors that modulate resistance and tolerance

Genetic interactions among hosts, pathogens, and symbionts that shape infection outcomes

Defense responses stimulated by host tissue damage

Neuroimmunology and behavioral immunity

Mechanisms for specific recognition or memory in invertebrates

The genetics of immunity is an area of considerable research opportunity, particularly where conventional immune pathways intersect with other aspects of host physiology and function. We look forward to reading about the next set of advances in the field.

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