Minireview

Screening the fruitfly immune system

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Abstract

The anti-microbial defense system of *Drosophila* shows functional similarities with the vertebrate innate immune system. Two recent gene-expression profiling studies of fruitflies challenged with infectious agents have identified key molecular players in the fruitfly's response to bacterial and fungal infection, as well as a large number of immune-regulated genes with unknown immunological function.

In recent years, *Drosophila* has emerged as a popular model system for the study of immunity to infection. The reasons for this are two-fold. First, the fly immune system is mechanistically similar to the mammalian innate immune system and thus the fly is a model for vertebrate innate immunity without having the distraction of the more complex adaptive immune system of vertebrates, which employs huge repertoires of antigen-specific lymphocytes. Second, the fly is a model for arthropods (such as mosquitoes, fleas and ticks) that carry human diseases; any knowledge of *Drosophila* immunity is likely to be pertinent in the study of how these animals spread diseases to people.

It is in this context that De Gregorio *et al.* [1] and Irving *et al.* [2] have carried out a genome-wide analysis of the *Drosophila* response to challenges to the immune system from infectious agents. These studies have intrinsic differences from previous expression-profile studies of immunity. Whereas previous studies typically focused on one or a few purified cell types, De Gregorio *et al.* [1] and Irving *et al.* [2] were able to analyze gene expression in the whole animal; this has the advantage of allowing detection of responses that require interactions between tissues. Because data were collected at several time points after infection, these experiments may also allow the chronological ordering of complex multi-tissue responses. Moreover, the whole-animal experiment has the potential to find responses in tissues in which one would not ordinarily look; it remains to be seen whether or not the present array data will be informative in these regards. There is a potential disadvantage to the whole-animal approach, however: it is possible that immune responses that take place only in specific tissues are undetectable in a whole-animal experiment because they are drowned out by baseline expression in non-responsive tissues. By this reasoning, the whole-animal approach will probably work well in measuring the systemic response to infections of the fat body, a relatively large tissue responsible for anti-microbial peptide production in flies, but might miss expression changes at the infection site and in the cellular immune system, each of which might reflect only a few cells within an otherwise unaffected tissue.

De Gregorio *et al.* [1] and Irving *et al.* [2] both used the Affymetrix *Drosophila* oligonucleotide microarray, or chip, for their analyses, and both used the same immune challenges: the Gram-negative bacterium *Escherichia coli*, the Gram-positive bacterium *Micrococcus luteus* and the fungus *Beauvaria bassiana*. Bacterial infections were induced by pricking the flies with a septic needle, whereas fungal infections were induced simply by coating the flies with spores. (*Beauvaria* is an insect pathogen that has evolved techniques for penetrating the cuticle.) This raises the question of how wounding alone affects gene regulation. This may be a difficult question to answer, as flies in culture are far from axenic but instead grow in cultures that contain other organisms. Any wound could therefore introduce a broad range of microorganisms that would set off all the alarms in the immune system of the infected fly.

The genetics of the *Drosophila* immune response have been analyzed in a fair degree of detail [3]. This enables us to ask whether genes isolated in genetic screens for immune-response mutants have been found in the microarray analyses by De Gregorio *et al.* [1] and Irving *et al.* [2]. The answer is mixed. For example, some of the genes of the *Toll* pathway, which is critical for anti-fungal defense in adult flies and whose homologs are involved in innate immunity against bacterial pathogens in mammals, were detectably upregulated by immune challenge in both studies. In contrast, the genes of the parallel *imd* pathway, which is critical for defense against Gram-negative bacteria in *Drosophila*, were mostly not found to be upregulated in these analyses. (The two studies did not identify identical sets of differentially regulated genes; this is partly the result of different experimental conditions but may also suggest that a cut-off of two-fold expression difference, which is roughly what was used in both studies, is not large enough to lend absolute reproducibility to the conclusions.) One category of genes that did appear to be reliably upregulated in both analyses are immune effectors, as opposed to signaling components. For example, the attacin and cecropin genes, which encode secreted anti-microbial peptides, were induced several-fold in these experiments. Effectors have been elusive genetic targets, presumably because of either redundancy or the immune challenges typically used in genetic screens, which suggests that one of the virtues of expression profiling will be that it finds a different spectrum of genes from those found using traditional genetic approaches.

Other conclusions can be drawn by comparing the genes found in the two recent chip analyses with those already established in the literature as being responsive to immune challenge. Because De Gregorio *et al.* [1] defined their cut-off for immune-responsive genes according to previous findings, a qualitative comparison between their findings and previous work is not meaningful. Quantitatively, however, the extent to which genes are upregulated in this experiment is much smaller than that seen using northern blots or quantitative reverse transcriptase (RT)-coupled PCR. It is unclear whether this is the result of some difference in infection protocols or a difference that is broadly true of chip experiments. Generally, though, both the chip data sets [1,2] are broadly consistent with previous findings. We can also compare the chip data with those from studies of immune-responsive genes in mosquito cells, which give many similar results, including differential regulation of large numbers of serpins (serine-protease inhibitors), pattern-recognition proteins, such as the peptidoglycan recognition protein (PGRP) family, and effectors involved in melanization and coagulation, such as prophenol oxidase [4].

Of course, one common characteristic of microarray experiments is that they tend to suggest more microarray experiments. Will the comparison of flies infected with the same pathogen by injection and natural infection begin to pick apart which portions of the response are simply the result of wounding? How different are the transcriptional profiles induced by different pathogens, and, more specifically, will pathogenic bacteria suppress specific subsets of responses, as is suggested by recent experiments with both flies and vertebrates [5-7]? Will examination of responses in mutant flies uncover subtleties not obvious from wild-type studies? The studies by De Gregorio *et al.* [1] and Irving *et al.* [2] also suggest further genetic experiments: of the 400 genes isolated as immune-regulated by De Gregorio and colleagues [1], only 32 have been previously studied as mediators of fly immunity; the immunological functions of the other 368 remain to be defined. Irving *et al.* [2] also found many genes with unknown functions. More traditional 'forward' and 'reverse' genetic techniques will help the characterization of these genes. The combination of further large-scale genomic analyses and traditional screens has great potential for giving us insights into the fly's anti-microbial defense system and ultimately into the more complex innate immune system of vertebrates.

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