Disease Pathology: Wasting Energy Fighting Infection

Drosophila melanogaster **infected with** ***Mycobacterium marinum*** **suffer metabolic wasting similar to that seen in humans suffering from tuberculosis. This wasting is linked to insulin signaling and hastens host death.**

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It is not the infection that matters, it is the sickness. In both clinical and ecological settings, it is the symptoms of disease that draw our attention to infection and immunity and the severity of symptoms that dictates the impact of being infected. Some disease symptoms, such as coughing, can be promoted by and benefit the infectious agent. Others, like fever, are integral to host defenses. In many instances, however, the proximate causes of, and even which party benefits from, observed disease symptoms are unknown. Understanding the bases for these pathologies is central to understanding and treating disease.

Tuberculosis is a disease with a particularly interesting pathology. It begins with pulmonary infection by the bacterium *Mycobacterium tuberculosis* [1] and was almost invariably fatal prior to the widespread availability of antibiotics, though the period between contraction of the infection and ultimate death might span years. One symptom of advanced tuberculosis is profound wasting, a loss of body mass and energy stores so severe that tuberculosis was widely referred to as "consumption" in the 1800s. It is as though the disease consumes the patient from the inside out. Tuberculosis is now largely controlled in the industrialized world, but the mechanism of consumptive wasting continues to hold considerable interest for pathologists.

Mycobacterium marinum is a close relative of *M. tuberculosis*. With an optimal growth temperature of around 30°C, *M. marinum* does not typically cause human disease. *M. marinum* does, however, cause epidemic disease outbreaks in poikilotherms such as amphibians and fish [2]. *M. marinum* can also establish lethal infection when injected into the fruit fly *Drosophila melanogaster* [3], raising the possibility that the *M. marinum–D. melanogaster* system could be used as an inexpensive and genetically tractable model to study the progression of a tuberculosis-like infection. Parallel efforts have been initiated to study *M. marinum* infection in zebrafish (*Danio rerio*) [4]. It is not known whether zebrafish infected with *M. marinum* experience wasting similar to that observed in advanced tuberculosis, although microarray analysis of zebrafish in the late stages of *M. marinum* infection reveals substantial disregulation of metabolic genes [5]. In a recent report in *Current Biology*, Dionne *et al.* [6] have now shown that *Drosophila* infected with *M. marinum* do exhibit tuberculosis-like energetic wasting.

During intermediate and late stages of *M. marinum* infection, *Drosophila* lose glycogen and fat stores, become hyperglycemic and exhibit depressed expression of genes involved in triglyceride and glycogen metabolism [6]. The elevated levels of free glucose observed in the hemolymph of infected *Drosophila* are reminiscent of type-2 diabetes in humans. This is compelling because, in humans, tuberculosis and diabetes are often clinically linked [7]. It further appears that, as in type-2 diabetes, *M. marinum* induced wasting can be linked to a disruption in insulin signaling. Under normal circumstances, insulin triggers the phosphorylation of the kinase Akt, causing Akt to negatively regulate the transcription factor Foxo. Foxo activity suppresses the storage of energy in the forms of fat and glycogen, so when insulin signaling deactivates Foxo, it promotes long-term energy storage.

Dionne *et al.* [6] found that *M. marinum*-infected *Drosophila* do not properly phosphorylate Akt, but that Akt phosphorylation can be restored by injection of human insulin. Furthermore, flies mutant for the *foxo* gene, when infected with *M. marinum*, die more slowly, express energy metabolism genes at closer-to-normal levels, and waste slightly less than their wild-type counterparts. Neither injection of insulin nor mutation of the *foxo* gene makes flies fully resistant to *M. marinum*; these treatments only dampen the symptoms of infection.

The metabolic alterations observed in *Drosophila* infected with *M. marinum* could stem from manipulation of host physiology by the bacterium, or they could reflect host reapportionment of energy budgets during the transition from normal homeostasis to active immune defense. These two possibilities obviously are not exclusive. There are many documented examples of pathogens that manipulate the behavior or physiology of their hosts to the apparent benefit of the pathogen and detriment of the host [8]. There is also ample evidence that immune defense is energetically costly [9], and that a robust immune response must necessarily draw finite energetic resources away from other physiological processes. In the case of *Drosophila* infected by *M. marinum*, wasting may occur because the bacterium is actively manipulating the metabolism of the fly in order to

create a favorable environment for infection, or because the fly is expending a tremendous amount of energy in an ultimately futile battle against a lethal pathogen.

Dionne *et al.* [6] favor the interpretation that the metabolic wasting observed in *Drosophila* infected by *M. marinum*, and perhaps in humans infected with *M. tuberculosis*, is a consequence of sustained energy reallocation toward immune function. As in excessive fever or allergic responses, wasting induced by infection may be a consequence of normal immune activity gone awry. There remain, however, some experimental ambiguities that must be resolved before this hypothesis can be fully embraced. Energetic wasting is reduced in *foxo* mutant *Drosophila*, which increases the longevity of the flies but does not appear to reduce *M. marinum* proliferation. It thus appears that wasting accelerates death in infected flies, but confers no direct benefit to the infecting bacteria, logically supporting the hypothesis that wasting may be a side effect of immunity. However, even though the *foxo* mutants retain glycogen significantly better than do wild-type flies, they still become hyperglycemic. Because infection causes equivalent increases in free glucose levels in the hemolymph of both *Drosophila* genotypes, the current data cannot address whether *M. marinum* profit from higher glucose availability. The possibility that *M. marinum* actively stimulates hyperglycemia has yet to be explored experimentally.

More broadly, the hypothesis that wasting is the result of sustained, but otherwise normal, energetic reallocation to defense suggests that the transcriptional and metabolic signatures of this reallocation should be generic across infections. Unfortunately, little data exist to test this prediction. Previous whole genome transcriptional analyses of *Drosophila* infected with other bacteria have not revealed the metabolic alterations observed during *M. marinum* infection. These experiments have typically been conducted using non-pathogenic bacteria that are cleared by the immune response within hours [10,11] or highly virulent bacteria that cause death within hours [12]. In either case, the measurable course of infection is so short that it may not be possible to capture substantial energetic reallocation to defense. Among the mycobacteria, infection of *Drosophila* with *M. smegmatis* elicits neither the same wasting [6] nor the mortality [3] observed after infection with *M. marinum*, but *M. smegmatis* causes a lower-intensity infection [3], perhaps obviating the need for substantial energetic reallocation. Whether wasting is a general property of sustained severe infections or is specific to certain infectious agents remains to be conclusively determined.

The study by Dionne *et al.* [6] nicely demonstrates that model systems can be infected with human pathogens or their close relatives to explore general properties of human disease, an approach that these researchers have previously applied with success in *Drosophila* [13,14]. This work reflects an important shift in perspective from an emphasis on mechanisms of immunity to a more holistic analysis of pathology in studies utilizing model systems. A focus on the broader physiological consequences of infection can also be extended to the study of pathogens that afflict model organisms in nature, especially since these are likely to possess interesting pathologies that can be interpreted in appropriate evolutionary and ecological context. Studies of infection pathology that better connect mechanistic laboratory experiments with the philosophies of clinical medicine will open new routes for translational research and will result in a more complete understanding of disease.

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