Drosophila melanogaster as an organism model for studying cystic fibrosis and its major associated microbial infections

Hamadoun Touré, Jean-Louis Herrmann, Sébastien Szuplewski, Fabienne Girard-Misguich

AMERICAN SOCIETY FOR MICROBIOLOGY | Infection and Immunity

Host-Microbial Interactions | Minireview

Drosophila melanogaster as an organism model for studying cystic fibrosis and its major associated microbial infections

Hamadoun Touré,[1] Jean-Louis Herrmann,[1,2] Sébastien Szuplewski,[3] Fabienne Girard-Misguich[1]

AUTHOR AFFILIATIONS See affiliation list on p. 9.

ABSTRACT Cystic fibrosis (CF) is a human genetic disease caused by mutations in the *cystic fibrosis transmembrane conductance regulator* gene that encodes a chloride channel. The most severe clinical manifestation is associated with chronic pulmonary infections by pathogenic and opportunistic microbes. *Drosophila melanogaster* has become the invertebrate model of choice for modeling microbial infections and studying the induced innate immune response. Here, we review its contribution to the understanding of infections with six major pathogens associated with CF (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Burkholderia cepacia*, *Mycobacterium abscessus*, *Streptococcus pneumoniae,* and *Aspergillus fumigatus*) together with the perspectives opened by the recent availability of two CF models in this model organism.

KEYWORDS *Drosophila*, cystic fibrosis, CFTR, ENaC, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Mycobacterium abscessus*

BACTERIAL INFECTIONS IN CYSTIC FIBROSIS

Cystic fibrosis (CF) is a human genetic disease with a recessive autosomal transmission. It is the most common genetic disease among Caucasians and affects approximately 7.97/100,000 persons in the USA and 7.37/100,000 in the European Union (1). Although the pulmonary form is the most severe clinical manifestation, other exocrine organs may also be affected (e.g., the pancreas and intestine). The disease is caused by loss-of-function mutations in the *cystic fibrosis transmembrane conductance regulator* (*cftr*) gene (2–4), which encodes a member of the adenosine triphosphate (ATP)-binding cassette (ABC) protein superfamily (3). CFTR is an ATP-gated ion channel that conducts chloride ions across epithelial cell membranes (5, 6), as well as glutathione thiocyanates and bicarbonates.

In addition to modulating the chloride transport, it regulates the activity of other ion channels such as the trimeric epithelial sodium channel (ENaC), which consists of the subunits α, β, and γ. How CFTR negatively regulates ENaC is still controversial. According to König and collaborators, this regulation occurs indirectly through the accumulation of intracellular chlorine (7). However, conflicting results have shown that inhibition of ENaC by CFTR is independent of the direction and extent of chloride transport (8). Studies have shown that CFTR inhibits ENaC through a direct physical interaction (9) or by regulating ENaC subunit quantities (10).

In any case, CFTR dysfunction leads to an excessive activity of the trimeric ENaC channel, causing uncontrolled sodium and excessive water entry into the epithelial cells following the osmotic gradient. This leads to dehydration of the intraluminal surface and an increase in the thickness of the mucus bordering the epithelium (11). In the lungs, the accumulation of thick viscous secretions causes obstruction and inflammation of the airways. These prevent the proper functioning of the mucociliary barrier, which is the primary protective barrier against many pathogens (12). In addition, this mucus has poor antibacterial activity owing to its reduction in acidity. Indeed, CFTR dysfunction prevents

Editor Anthony R. Richardson, University of Pittsburgh, Pittsburgh, Pennsylvania, USA

Address correspondence to Hamadoun Touré, hamadountoure56@gmail.com.

The authors declare no conflict of interest.

See the funding table on p. 9.

Published 17 October 2023

the exit of bicarbonate ions. This modified mucus constitutes the ideal environment for the accumulation, proliferation, and persistence of pathogenic and/or opportunistic microorganisms.

Chronic and recurrent infections and persistent inflammation cause airway damage that can lead to bronchiectasis and thus, a decline in respiratory function (13). The ensuing respiratory failure is the cause of more than 90% of the recorded deaths (14). According to the 2021 report of the French Cystic Fibrosis Registry, these infections are mainly due to *Staphylococcus aureus* (60.6%), *Pseudomonas aeruginosa* (34%), *Aspergillus fumigatus* (21.6%), *Haemophilus influenzae* (10.1%), *Stenotrophomonas maltophilia* (9.3%), *Achromobacter xylosoxidans* (6.4%), *Streptococcus pneumoniae* (1.7%), non-tuberculous mycobacteria (NTM; 3.7%), and *Burkholderia cepacia* (2%). The prevalence of these pathogens varies according to the geography. For comparison, in the USA, *S. aureus, P. aeruginosa,* and NTM have approximately 63%, 24%, and 9.6%, respectively, of the overall prevalence according to the 2021 report of the Cystic Fibrosis Foundation (CFF). The dynamics of the prevalence of the isolated pathogens also changes with time. For example, the respective trends in the prevalence of *S. aureus* and *P. aeruginosa* have taken opposite trajectories over time in the USA. While the former is becoming increasingly prevalent (29% in 1991 vs. 63% in 2021), the latter is less prevalent over time (61% in 1991 vs. 24% in 2021). The same trend is observed in Europe (15).

DROSOPHILA, AN ESTABLISHED ORGANISM MODEL FOR THE STUDY OF PATHOGENS

Drosophila melanogaster is a century-old organism model that is used in various aspects of life sciences such as genetics, developmental biology, cellular biology, neurobiology, and immunity. The constant development and availability of different genetic tools have facilitated its genetic manipulation, making *Drosophila* central to the study of responses to infection and host-pathogen interactions in the last three decades. In their own natural environment, fruit flies face a panel of viruses, bacteria, fungi, and parasites [e.g., wasp (16)]. In the laboratory, *Drosophila* is used as an experimental host to study infection with its natural pathogens as well as human ones. Indeed, *Drosophila* has become an attractive and emergent model for studying host response, virulence factors, and pathophysiology of pathogens associated with human infectious diseases, such as those caused by Zika Virus, *Mycobacterium marinum*, *Listeria monocytogenes*, and *Candida albicans* (17–21).

Drosophila is a dipteran with three larval stages and a complete metamorphosis. In laboratory, third instar larvae and adults are usually infected either orally, locally by wounding or systemically by injecting the microorganism.

Drosophila lacks an adaptive immune response but has innate immunity involving conserved signaling pathways. In both mammals and flies, the JNK, JAK-STAT, and NFκB signaling pathways are critical for immune response regulation (22). To note, the response mediated by Toll-like receptors was discovered in this organism (23). *Drosophila* pattern recognition receptors (PRRs) recognize the pathogen-associated molecular patterns (PAMPs) of microbes, such as peptidoglycan (PGN) or lipoteichoic acid (LTA) (24). They induce an adequate immune response involving both cellular and humoral response (25).

The cellular response is based on blood cells (hemocytes) which are equivalent to mammalian monocytes and macrophages. Until recently, three morphologically distinct types of hemocytes have been identified: plasmatocytes, crystal cells [involved in wound healing, reactive oxygen species (ROS) production, and hypoxic response], and lamellocytes (involved in response to wasp parasitization) (26). The most abundant hemocytes are plasmatocytes that respond to wound signals and control the coagulation response. They also phagocytose and encapsulate invading pathogens and clear apoptotic bodies (27). However, this simplistic classification of hemocytes into three subtypes has been reviewed thanks to recent studies based on single-cell sequencing on either larval (28–31), adult (32), or pupal (33) hemocytes. Collectively, these studies

have identified at least eight distinct specialized hemocyte subpopulations waiting to be functionally characterized.

The existence of an inducible humoral response in fruit flies was first reported in 1972 (34). This response is mainly based on the production of antimicrobial peptides (AMPs), primarily by the fat body, which is functionally homologous to mammalian adipocytes and liver. AMPs can also be produced locally by epithelial cells or hemocytes. Two conserved NFκB signaling pathways, Toll and Immune deficiency (Imd), mediate AMP production. The former is implicated in response to both bacteria with Lys-type PGN (mainly Gram-positive) and fungal infections, while the second is involved in responses to infection by bacteria with DAP-type PGN (mainly Gram-negative) (22, 23, 35, 36). In addition to AMPs production, humoral response also includes the generation of ROS by DUOX proteins locally at the epithelial level (37, 38).

LESSONS FROM *DROSOPHILA* INFECTIONS WITH SOME CF MAJOR PATHOGENS

Drosophila is commonly used to study infections with a single pathogen associated with CF or co-infection. Here, we review the contributions of this model organism to the identification of host receptors, *in vivo* validation of virulence factors, and to the screening of effective drugs. We will follow the prevalence of these pathogens as reported by the French Cystic Fibrosis Registry in 2021, and the number of relevant publications. An overview is provided by Table 1.

Stenotrophomonas maltophilia and *Achromobacter xylosoxidans* were excluded for the following reasons. *Stenotrophomonas maltophilia* has been isolated at the surface and in the gut of wild female *Drosophila* captured in Puerto Rico (39). Its intestinal presence was confirmed in laboratory strains (40). *Achromobacter xylosoxidans*, has been reported to be pathogenic for *Drosophila*, as its injection in adult males leads to rapid dose-dependent death (41).

Staphylococcus aureus

Infections with the Gram-positive bacterium *Staphylococcus aureus* (*S. aureus*) are among the most prevalent in CF patients. Injection of live *S. aureus* into *Drosophila* leads to an important transcriptional response and a systemic infection resulting in a reduction in fly life expectancy (42, 43). Phagocytosis plays a major role in the response to *S. aureus* infection as flies devoid of plasmatocytes succumb more rapidly (44, 45). *Drosophila* Schneider 2 (S2) cells were used as a surrogate for hemocytes. Indeed, this widely used cell line, derived from late embryos, is phagocytic. Genetic screening of S2 cells identified Eater and Croquemort, as *S. aureus* scavenger receptors (44). This was confirmed in adult hemocytes (44) and mammalian macrophages (46). Croquemort is the first CD36 family member to be described as being involved in bacterial recognition. Eater does not recognize LTA, a cell wall polymer found in Gram-positive bacteria. Indeed, the *ltaS* mutant strain (deficient in LTA synthesis) was phagocytosed less by wild-type hemocytes than the wild-type *S. aureus* strain. Moreover, the *ltaS* mutant strain was equally phagocytosed by wild-type and Eater-lacking larval hemocytes (47). However, this was not the case for hemocytes lacking the receptor Draper, whose extracellular region binds LTA, strongly suggesting that this cell wall component is its ligand, contrary to Eater (47).

The integrin βυ is also involved in *S. aureus* recognition by the hemocytes but through peptidoglycan. Indeed, a mutant bacterial strain that produces reduced levels of PGN, due to defective UDP-N-acetylenolpyruvylglucosamine reductase, was less efficiently phagocytosed by integrin βυ-deficient hemocytes (48).

PGN recognition proteins (PGRPs), such as PGRP-SA and PGRP-SC1a, are also important for the recognition and phagocytosis of *S. aureus* (49). However, wall teichoic acids (WTAs), which are covalently linked to PGN, mitigate *S. aureus* recognition by these *Drosophila* immune receptors. Indeed, infection with strains with defective WTA production led to a reduction of *S. aureus* virulence. This loss of pathogenicity is due to increased PGN binding and detection by PGRP-A (50). Complementary to inducing a

TABLE 1 Modeling the infections by major CF pathogens in *Drosophila*

Pathogen	Host immune response					Host manipulation by the pathogen	*In vivo* validated antimicrobial
	Cellular response		Humoral response		Other response(s)		
	Phagocytosis	ROS production	Toll	IMD			
S. aureus	✓ *Receptors*: Croquemort, Draper, Eater	✓	✓	✗	?	• Neutralization of host oxidative and antimicrobial responses by catalase • Reduction of PGN detection and humoral response by producing D-alanylated teichoic acid	• Nisin • NAI-107 • Plumbagin
P. aeruginosa	✓ *Receptors*: ?	?	✓	✓	• Activation of JNK pathway in enterocytes during oral infection • Nutritional immunity: iron sequestration from the hemolymph and relocation to the fat body	• Prevention of phagocytosis by hemocytes by RhIR and the exotoxin ExoS • Induction of apoptosis of S2 cells by ExoS and Exotoxin A • Suppression of AMP production	• Lytic phage MPK1 • Lytic phage MPK6 • Baicalin
B. cepacia	? *Receptors*: ?	?	✓	✓	Activation of TOR pathway for tolerance and resistance	?	?
M. abscessus	*Receptors*: ?	?	✓	✓	Granzyme-mediated cytotoxic response by thanacytes	?	• Tigecycline • Linezolid
S. pneumoniae	✓ ✓ *Receptors*: ?	?	✓	✓	Activation of adenosine signaling for metabolic switch	Loss of circadian regulation of locomotor activity	?
A. fumigatus	? *Receptors*: ?	?	✓	✗	?	?	• Voriconazole • Posaconazole • Terbinafine

cellular response, *S. aureus* PGRP-SA-mediated recognition systemically activates the Toll pathway leading to AMPs production (e.g., Drosomycin, Defensin, and Metchnikowin) (51). Although Imd-related AMPs are not induced, this pathway is required for effective clearance of the infection (52).

Moreover, fly infections have been used to validate known virulence factors, such as hemolysin α (53), as well as to identify new ones. An example is the production of D-alanylated teichoic acid, which reduces PGN recognition by host receptors and thus interferes with the host humoral response to *S. aureus* infection (51).

Drosophila infections have confirmed that methicillin-resistant *S. aureus* (MRSA) isolates, notably the USA300 and PFGE strains, were less virulent than non-MRSA isolates (54). Correlations with clinical observations were found for the community-associated MRSA strains USA300, USA400, and CMRSA2. Indeed, the latter are more virulent than the hospital-associated strain CMRSA6 (53). Recently, a model of oral USA300 infection in *Drosophila* larvae showed that bacterial catalase neutralizes a DUOX-mediated oxidative response that promotes AMPs production through Toll pathway activation (55).

To identify drugs effective against these MRSAs, a panel of antibacterial peptides was screened *in vivo*. Two antibiotics, nisin and NAI-107, have been shown to have the ability to rescue adult flies from fatal infections with the USA300 strain. NAI-107 presented an efficacy equivalent to that of vancomycin, a widely applied antibiotic for the treatment of serious MRSA infections (56).

The antimicrobial activity of plumbagin, a phytochemical, was also validated with the *Drosophila* systemic infection model, whether with *S. aureus* alone or in co-infection with *C. albicans*, as is often observed in the urinary tract in humans. Plumbagin (5-hydroxy-2-methyl-1,4-naphthoquinone) has been identified *in vitro* as a potent antimicrobial agent against *S. aureus* and *C. albicans* (57).

Pseudomonas aeruginosa

Drosophila is susceptible to both oral and systemic infections by the Gram-negative bacterium *P. aeruginosa*. This leads to the invasion of host tissues, then their degradation and ultimately death through the bacterial spread in the hemolymph (34, 58–60).

P. aeruginosa infections induce systemic AMP production mediated by both the Toll and Imd pathways (60, 61), a local epithelial Imd-dependent one and a cellular response (59). More recently, a novel and evolutionarily conserved defense mechanism has been reported (62). *P. aeruginosa* infection induces the overexpression of the iron transferrin 1-encoding gene in the fat body. The consequence is sequestration of iron from the hemolymph and its relocation to the fat body. The importance of the competition for iron between *P. aeruginosa* and its host is further supported by the reduced pathogenicity of a siderophore-defective strain of *P. aeruginosa* in *Drosophila* (62).

Fruit flies have been used to screen *P. aeruginosa* mutants and thus to validate (63) and identify new virulence factors (e.g., relA) (64). Similarly, the contribution of certain virulence factors has been characterized *in vivo* in fruit flies. Examples include the oxylipins involved in biofilm formation and virulence (65), glutathione biosynthesis genes *gshA* and *gshB* (66), transcriptional regulators PA1226 and PA1413, which modulate the virulence (67), reactive chlorine species resistance factor RcsA (68), glucose transport regulator GltB (69), and the nitrite reductase NirA (70). The essential role of the *P. aeruginosa* respiratory chain in virulence and pathogenicity has also been demonstrated in *Drosophila*. Indeed, a PA4427-PA4431 operon mutant strain, defective for respiratory chain complex III (*cytobc1*), induces less mortality in *Drosophila* than the PAO1 reference strain (71).

Many pathogenic Gram-negative bacteria, including *P. aeruginosa*, possess a type III secretion system (T3SS), which injects virulence factors into their host (72). The presence and activation of T3SS are required in *P. aeruginosa* to induce fly death (73). The exotoxin ExoS, whose injection into the host cell cytoplasm is mediated by T3SS, interferes with bacterial phagocytosis by hemocytes (74). ExoS is known to target host Rho GTPases and the contribution of different fly Rho GTPases to *P. aeruginosa* infection resistance

has been assessed *in vivo*, revealing that Rac2 is the main target of ExoS to prevent engulfment (75). ExoS can also induce apoptosis at least in *Drosophila* S2 cells (76), similar to Exotoxin A (77).

P. aeruginosa uses quorum sensing (QS) to regulate and adapt its gene expression. During infection, the QS signaling molecule N-3-oxododecanoyl homoserine lactone (3OC12-HSL) is essential for the bacterial virulence in flies. *Drosophila* lacks Paraoxonases (PONs) which are able to degrade 3OC12-HSL *in vitro*. Transgenic expression of human PON1 protects flies against *P. aeruginosa* infection lethality by interfering with 3OC12-HSL-dependent QS (78). The QS transcription factor RhlR interferes with the host's cellular immune response during the early stages of infection (59). *P. aeruginosa* can also inhibit the host response by suppressing AMP production (61).

Chronic *P. aeruginosa* infection in patients with CF is associated with the formation of mucoid micro-colonies called biofilms. These are observed in the *Drosophila* crop, the functional equivalent of the mammalian stomach, after oral infection. Bacteria recovered from this *in vivo* biofilm present an increased antibiotic resistance and less virulence than the planktonic bacteria (79). Transcriptional regulator PA3898 controls biofilm formation and virulence in *Drosophila* (80). Furthermore, oral infection with *P. aeruginosa* leads to midgut hyperplasia. This is due to activation of the stress response JNK pathway in enterocytes, leading to their apoptosis and indirectly to the overproliferation of intestinal stem cells (81).

Fruit flies can help to find alternative effective therapeutic strategies against *P. aeruginosa* infections, in addition to antibiotics. Indeed, the *in vivo* antibacterial efficacy of *P. aeruginosa*-targeting lytic phages, such as MPK1 and MPK6, has been assessed and proven in *Drosophila* (82, 83). Moreover, Baicalin, has been validated *in vivo* in *Drosophila* (84). This extract from the Chinese herb *Scutellariae radix* has been proposed as an alternative anti-*P. aeruginosa* compound targeting bacterial T3SS.

Aspergillus fumigatus

Immuno-compromised patients as well as those living with CF are prone to invasive aspergillosis. In order to examine the conserved Toll pathway associated with the response to fungal infection in *Drosophila,* including *A. fumigatus* (85), the virulence of different strains of the cosmopolitan filamentous fungus *A. fumigatus* was assessed using Toll-deficient flies (86). Infections were induced by injecting, feeding, or rolling flies with conidia (87). Concordance with results obtained in mammalian models was observed with either the hypovirulent strain *Δalb1* (88) or other *A. fumigatus* mutant strains defective in siderophore biosynthesis, starvation stress response (89), or Glicotoxin production (90).

Toll-deficient *Drosophila* have also been used to assess the *in vivo* efficacy of orally absorbed antifungal agents such as voriconazole and posaconazole, which are commonly used as prophylaxis and treatment for the fungus (88, 91). An *in vitro* pre-exposure of *A. fumigatus* to these molecules was performed before *Drosophila* infection with *A. fumigatus*. This pre-treatment of *A. fumigatus* did not affect the fungal virulence or the efficacy of the same molecules to clear the infection *in vivo* (88, 91). Synergistic effects have been observed when voriconazole was combined with terbinafine (87, 91, 92).

In vivo toxicity of volatile organic compounds (VOCs) produced by filamentous fungi (e.g., alcohols, aldehydes, thiols, esters) has been explored in flies. Exposition of *Drosophila* larvae to VOCs emitted by living fungi delayed metamorphosis toward the pupae stage and subsequently to the adult stage. In addition, this exposure was detrimental to both larval and adult survival (93–95). This toxigenic effect suggests that VOCs may contribute to the fungal pathogenesis, at least in flies.

Burkholderia cepacia complex

Drosophila is an established model for studying systemic infections caused by species of opportunistic Gram-negative bacteria belonging to the *Burkholderia cepacia* complex

(Bcc). It has been used to characterize the virulence of different strains (96), the phenotype of some mutants (97–99), and also to identify virulence factors of strains isolated from CF patients (100).

In response to *B. cepacia* infection, fruit flies produce AMPs, such as Drosomycin and Diptericin, via both the Toll and Imd pathways (101). We recently demonstrated that the induced AMPs are crucial for *Drosophila* survival against *B. cepacia* infection (102).

Drosophila mutants for the *period* gene, whose circadian rhythm is altered, are more tolerant to Bcc infection (101). This study also revealed that both glucose and amino-acid intake improved host tolerance to infection and that the TOR pathway mediates both resistance and tolerance to Bcc infections (101).

Mycobacterium abscessus and the NTM

Drosophila is also a validated model for studying mycobacterial infections. As recently reviewed, most studies have focused on the pathogenic slow-growing *Mycobacterium marinum* to model tuberculosis (103). The most frequently isolated NTM in patients with CF are species of *M. abscessus* and *M. avium* complexes, *M. fortuitum* being rarely found (104, 105). In a study including French patients, *M. abscessus* accounted for more than half of the NTM isolated (104). This bacterium causes the most deleterious pulmonary infections in patients with CF (106). *M. abscessus* belongs to the group of fast-growing mycobacteria which are predominantly saprophytic. It is considered the most pathogenic species within this group (107).

After systemic injection, *M. abscessus* can proliferate within *Drosophila*, leading to severe tissue damage and, ultimately, death (108). It is recognized by PGRP-SA and activates the production of Drosomycin, a Toll-mediated AMP (108). Recently, we confirmed and extended this observation. Indeed, *M. abscessus* injection induced the expression of AMPs encoding genes, either Toll- or Imd-regulated and showed that these AMPs did not seem to play a major role for *Drosophila* survival during *M. abscessus* infection, as indicated by the similar survivals of wild-type and AMP-deficient flies (102). We therefore hypothesized and demonstrated that the intracellular localization of *M. abscessus* protects it from AMPs, particularly Defensin, which we have shown to have a direct bactericidal action against extracellular *M. abscessus* (109). Indeed, after its injection, *M. abscessus* is rapidly internalized by *Drosophila* plasmatocytes in which it grows (102), as observed during fly infection with *M. marinum* (18).

Fly infections have been used to validate mutants for genes encoding known virulence factors, such as the *Δ0855* and *Δ4532* c strains, both defective for intracellular growth (110, 111), as well as to identify some new genes such as *MAB_0471*, *MAB_0472,* and *MAB_3317*c (112).

Drosophila have also highlighted *M. abscessus* resistance to host innate cytotoxic responses. Indeed, thanacytes, a newly described hemocyte subpopulation identified by single-cell sequencing (29), induce caspase-dependent apoptosis in *M. abscessus*-infected plasmatocytes through the action of two serine proteases, encoded by *CG30088* and *CG30090*. However, *M. abscessus* resists this lysis and spreads systemically, leading to bacteremia and subsequent death of infected flies. The resistance of *M. abscessus* to cytotoxic lysis of phagocytes was validated in a mammalian model after contact of infected murine primary macrophages with autologous natural killer cells. This propensity of *M. abscessus* to resist the host cytotoxic innate response, typical of strict pathogenic mycobacteria such as *M. tuberculosis*, could partially explain its superior pathogenicity among fast-growing mycobacteria.

M. abscessus is also multi-resistant to antibiotics, including most of the anti-tuberculosis drugs (113), making it difficult to treat its infections in patients with CF (114). *Drosophila* have been used to test the effectiveness of antibiotics against *M. abscessus in vivo*. Tigecycline treatment was the most efficient and its potency was increased when combined with linezolid (115).

Streptococcus pneumoniae

Injection of the Gram-positive bacterium *S. pneumoniae* in *Drosophila* causes lethal infections. Fly exposure to sublethal doses primes resistance to subsequent infections by *S. pneumoniae* (116). Phagocytosis by plasmatocytes is crucial for resistance to streptococcal infections (116–118). It is activated by Eiger, a *Drosophila* homolog of humans TNFα (119). Hemocyte activation requires increased consumption of energy, which is obtained by a systemic metabolic switch involving the release of glucose from glycogen. This is mediated by adenosine signaling and is modulated by adenosine deaminase ADGF-A to prevent the loss of energy reserves during chronic infection (118). Interestingly, this effect of adenosine has also been observed in a mice lung streptococcal infection model in which it regulates pulmonary neutrophil recruitment (120).

The *Drosophila* response to a systemic infection with *S. pneumoniae* is not limited to the immune cellular response because it also includes the production of AMPs, mediated by both Toll and Imd pathways (118).

S. pneumoniae infections have been used to assess whether interactions between circadian rhythm and immunity exist in flies, as observed in mammals (121). Infected wild-type flies lose circadian regulation of locomotor activity, whereas mutant flies for *timeless* or *period*, which encode components of the central circadian clock, were more sensitive than wild-type flies to *S. pneumoniae* infection (122).

CO-INFECTION MODELS

Most patients with CF are prone to polymicrobial infections. *Drosophila* has been used to study such interactions between pathogens as well as those with the host microbiota. Indeed, flies were orally infected with a combination of *P. aeruginosa* and strains isolated from the oral flora of patients with CF to compare bacterial virulence genes and host AMP gene expression with mono-infections. Thus, it was observed that co-infection with *Streptococcus* sp. and *P. aeruginosa* increased the production of the flagellar filament protein fliC in *P. aeruginosa*, most likely to increase its motility (123). Upon co-infection with Gram-positive bacteria, *P. aeruginosa* also presents an increased virulence, due to the production of antimicrobials and toxins that kill the other bacteria as well as the host cells. The latter is induced by the detection by *P. aeruginosa* of Gram-positive bacteria PGN (124).

Conversely, *Streptococcus parasanguinis*, a Gram-positive colonizer of the airway of patient with CF, hijacks *P. aeruginosa* exopolysaccharide alginate production to form a biofilm that limits *P. aeruginosa* growth. This biofilm contains streptococcal adhesins, which are also key factors for fly colonization and mortality (125). Nitrite reductase production is crucial for *P. aeruginosa* virulence (126).

A more recent model of co-infection with two common pathogens found in patients with CF was based on the co-injection with *S. aureus* and *P. aeruginosa* in adult *Drosophila* (127).

MODELING CF IN *DROSOPHILA*

Two CF-like models have been proposed in *Drosophila*. The first consists of mutant flies for the *bereft* gene which encodes *miR-263a*, a microRNA negatively regulating the quantity of transcripts encoding the α and β subunits of ENaC (*ppk4* and *ppk28,* respectively). Thus, these flies are a model of ENaC hyperactivity. Indeed, phenotypes in their midgut are similar to those observed in epithelia of patients with CF. It was observed that there was excessive sodium entry within enterocytes, the most abundant intestinal cells, leading to an incoming flow of water following the osmotic gradient and to a dehydration of the intraluminal area bordering the epithelium (128).

These phenotypes are also observed in the second gastro-intestinal *Drosophila* CF, which has been more recently reported (129). It is a CFTR mutant model obtained by depleting in enterocytes the transcripts of *CG5789/Cftr*. This gene encodes the *Drosophila* structural and functional equivalent of human CTFR. Indeed, the expression of human

CFTR in this CF model rescued gastro-intestinal phenotypes. Partial suppression of these phenotypes was also observed upon overexpression of *miR-263a*, suggesting that ENaC may act downstream of CFTR, as in humans (129).

Both models exhibit increased levels of antimicrobial peptides due to the activation of the Imd pathway in response to increased bacterial accumulation in the midgut. Moreover, they are more susceptible to oral infections with *Pseudomonas aeruginosa* (128, 129). Here again, *human CFTR* expression rescued this phenotype in flies depleted of *Cftr* transcripts, establishing a new model to study CF pathophysiology, particularly in respect to the susceptibility to pathogen infections (129).

It would be interesting to determine the susceptibility of both models to other major pathogens found in CF. To note, ENaC has been proposed to be involved in airway liquid clearance (130). One may wonder whether the CF phenotypes observed in *miR-263a* mutant flies are only restricted to the midgut and whether this model is more susceptible to systemic infections.

CONCLUDING REMARKS

The recent use of certain CFTR modulators has brought relief to many CF patients; but unfortunately, not to all. The development of relevant models is crucial for understanding CF pathophysiology and consequently for searching for effective molecules that can be beneficial in all kinds of *cftr* mutations leading to CF. *Drosophila* can meet this need, all the more so as CFTR and ENaC channels are present and their deregulation leads to a CF phenotype. As we have shown in this review, fruit flies have already allowed the identification of many virulence factors of the most common pathogens in patients with CF, as well as numerous host factors required to counter these infections. *Drosophila* use should make it possible to study and understand host resistance factors that are modulated in the context of CF. In the long term, treatments based on the modulation of the evolutionarily conserved susceptibility and predisposition factors could reduce CF-associated infections.

AUTHOR AFFILIATIONS

[1]Université Paris-Saclay, UVSQ, INSERM, Infection et Inflammation, Montigny-le-Bretonneux, France
[2]Assistance Publique-Hôpitaux de Paris, Hôpitaux Universitaires Ile-de-France Ouest, GHU Paris-Saclay, Hôpital Raymond Poincaré, Garches, France
[3]Université Paris-Saclay, UVSQ, Laboratoire de Génétique et Biologie Cellulaire, Montigny-le-Bretonneux, France

AUTHOR ORCIDs

Hamadoun Touré http://orcid.org/0000-0003-2131-3703
Sébastien Szuplewski http://orcid.org/0000-0003-1722-0707
Fabienne Girard-Misguich http://orcid.org/0000-0002-7571-6519

FUNDING

Funder	Grant(s)	Author(s)
Association Vaincre la Mucoviscidose (French CF Association)	RIF20190502522	Jean-Louis Herrmann
Institut National de la Santé et de la Recherche Médicale (Inserm)		Jean-Louis Herrmann
Université de Versailles Saint-Quentin-en-Yvelines (UVSQ)		Jean-Louis Herrmann

AUTHOR CONTRIBUTIONS

Hamadoun Touré, Conceptualization, Methodology, Writing – original draft, Writing – review and editing | Jean-Louis Herrmann, Conceptualization, Funding acquisition, Supervision, Validation, Writing – review and editing | Sébastien Szuplewski, Conceptualization, Supervision, Writing – review and editing | Fabienne Girard-Misguich, Conceptualization, Supervision, Writing – review and editing

REFERENCES

1. Farrell PM. 2008. The prevalence of cystic fibrosis in the European Union. J Cyst Fibros 7:450–453. https://doi.org/10.1016/j.jcf.2008.03.007
2. Kerem B, Rommens JM, Buchanan JA, Markiewicz D, Cox TK, Chakravarti A, Buchwald M, Tsui LC. 1989. Identification of the cystic fibrosis gene: genetic analysis. Science 245:1073–1080. https://doi.org/10.1126/science.2570460
3. Riordan JR, Rommens JM, Kerem B, Alon N, Rozmahel R, Grzelczak Z, Zielenski J, Lok S, Plavsic N, Chou JL. 1989. Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. Science 245:1066–1073. https://doi.org/10.1126/science.2475911
4. Rommens JM, Iannuzzi MC, Kerem B, Drumm ML, Melmer G, Dean M, Rozmahel R, Cole JL, Kennedy D, Hidaka N. 1989. Identification of the cystic fibrosis gene: chromosome walking and jumping. Science 245:1059–1065. https://doi.org/10.1126/science.2772657
5. Anderson MP, Gregory RJ, Thompson S, Souza DW, Paul S, Mulligan RC, Smith AE, Welsh MJ. 1991. Demonstration that CFTR is a chloride channel by alteration of its anion selectivity. Science 253:202–205. https://doi.org/10.1126/science.1712984
6. Nagel G, Hwang TC, Nastiuk KL, Nairn AC, Gadsby DC. 1992. The protein kinase A-regulated cardiac Cl- channel resembles the cystic fibrosis transmembrane conductance regulator. Nature 360:81–84. https://doi.org/10.1038/360081a0
7. König J, Schreiber R, Voelcker T, Mall M, Kunzelmann K. 2001. The cystic fibrosis transmembrane conductance regulator (CFTR) inhibits ENaC through an increase in the intracellular Cl- concentration. EMBO Rep 2:1047–1051. https://doi.org/10.1093/embo-reports/kve232
8. Chabot H, Vives MF, Dagenais A, Grygorczyk C, Berthiaume Y, Grygorczyk R. 1999. Downregulation of epithelial sodium channel (ENaC) by CFTR co-expressed in *Xenopus oocytes* is independent of Cl- conductance. J Membr Biol 169:175–188. https://doi.org/10.1007/s002329900529
9. Yan W, Samaha FF, Ramkumar M, Kleyman TR, Rubenstein RC. 2004. Cystic fibrosis transmembrane conductance regulator differentially regulates human and mouse epithelial sodium channels in *Xenopus oocytes*. J Biol Chem 279:23183–23192. https://doi.org/10.1074/jbc.M402373200
10. Rubenstein RC, Lockwood SR, Lide E, Bauer R, Suaud L, Grumbach Y. 2011. Regulation of endogenous ENaC functional expression by CFTR and Δf508-CFTR in airway epithelial cells. Am J Physiol Lung Cell Mol Physiol 300:L88–L101. https://doi.org/10.1152/ajplung.00142.2010
11. Boucher RC. 2007. Cystic fibrosis: a disease of vulnerability to airway surface dehydration. Trends Mol Med 13:231–240. https://doi.org/10.1016/j.molmed.2007.05.001
12. Hoegger MJ, Fischer AJ, McMenimen JD, Ostedgaard LS, Tucker AJ, Awadalla MA, Moninger TO, Michalski AS, Hoffman EA, Zabner J, Stoltz DA, Welsh MJ. 2014. Impaired mucus detachment disrupts mucociliary transport in a piglet model of cystic fibrosis. Science 345:818–822. https://doi.org/10.1126/science.1255825
13. Ratjen F, Döring G. 2003. Cystic fibrosis. Lancet 361:681–689. https://doi.org/10.1016/S0140-6736(03)12567-6
14. O'Sullivan BP, Freedman SD. 2009. Cystic fibrosis. The Lancet 373:1891–1904. https://doi.org/10.1016/S0140-6736(09)60327-5
15. Hatziagorou E, Orenti A, Drevinek P, Kashirskaya N, Mei-Zahav M, De Boeck K, ECFSPR. Electronic address: ECFS-Patient.Registry@uz.kuleuven.ac.be, ECFSPR. 2020. Changing epidemiology of the respiratory bacteriology of patients with cystic fibrosis-data from the European cystic fibrosis society patient Registry. J Cyst Fibros 19:376–383. https://doi.org/10.1016/j.jcf.2019.08.006
16. Kim-Jo C, Gatti J-L, Poirié M. 2019. *Drosophila* cellular immunity against parasitoid wasps: a complex and time-dependent process. Front Physiol 10:603. https://doi.org/10.3389/fphys.2019.00603
17. Liu Y, Gordesky-Gold B, Leney-Greene M, Weinbren NL, Tudor M, Cherry S. 2018. Inflammation-induced STING-dependent autophagy restricts Zika virus infection in the *Drosophila* brain. Cell Host Microbe 24:57–68. https://doi.org/10.1016/j.chom.2018.05.022
18. Dionne MS, Ghori N, Schneider DS. 2003. *Drosophila melanogaster* is a genetically tractable model host for *Mycobacterium marinum*. Infect Immun 71:3540–3550. https://doi.org/10.1128/IAI.71.6.3540-3550.2003
19. Mansfield BE, Dionne MS, Schneider DS, Freitag NE. 2003. Exploration of host-pathogen interactions using *Listeria monocytogenes* and *Drosophila melanogaster*. Cell Microbiol 5:901–911. https://doi.org/10.1046/j.1462-5822.2003.00329.x
20. Davis MM, Alvarez FJ, Ryman K, Holm ÅA, Ljungdahl PO, Engström Y, Skoulakis EMC. 2011. Wild-type *Drosophila melanogaster* as a model host to analyze nitrogen source dependent virulence of *Candida albicans*. PLoS One 6:e27434. https://doi.org/10.1371/journal.pone.0027434
21. Yang H, Hultmark D. 2017. *Drosophila* muscles regulate the immune response against wasp infection via carbohydrate metabolism. Sci Rep 7:15713. https://doi.org/10.1038/s41598-017-15940-2
22. Lemaitre B, Hoffmann J. 2007. The host defense of *Drosophila melanogaster*. Annu Rev Immunol 25:697–743. https://doi.org/10.1146/annurev.immunol.25.022106.141615
23. Lemaitre B, Nicolas E, Michaut L, Reichhart JM, Hoffmann JA. 1996. The dorsoventral regulatory gene cassette Spätzle/toll/cactus controls the potent antifungal response in *Drosophila* adults. Cell 86:973–983. https://doi.org/10.1016/s0092-8674(00)80172-5
24. Bergman P, Seyedoleslami Esfahani S, Engström Y. 2017. *Drosophila* as a model for human diseases-focus on innate immunity in barrier epithelia. Curr Top Dev Biol 121:29–81. https://doi.org/10.1016/bs.ctdb.2016.07.002
25. Lu Y, Su F, Li Q, Zhang J, Li Y, Tang T, Hu Q, Yu X-Q. 2020. Pattern recognition receptors in *Drosophila* immune responses. Dev Comp Immunol 102:103468. https://doi.org/10.1016/j.dci.2019.103468
26. Banerjee U, Girard JR. 2019. *Drosophila* as a genetic model for hematopoiesis. Genetics 211:367–417.
27. Vlisidou I, Wood W. 2015. *Drosophila* blood cells and their role in immune responses. FEBS J. 282:1368–1382. https://doi.org/10.1111/febs.13235
28. Cho B, Yoon S-H, Lee D, Koranteng F, Tattikota SG, Cha N, Shin M, Do H, Hu Y, Oh SY, Lee D, Vipin Menon A, Moon SJ, Perrimon N, Nam J-W, Shim J. 2020. Single-cell Transcriptome maps of myeloid blood cell lineages in *Drosophila*. Nat Commun 11:4483. https://doi.org/10.1038/s41467-020-18135-y
29. Fu Y, Huang X, Zhang P, van de Leemput J, Han Z. 2020. Single-cell RNA sequencing identifies novel cell types in *Drosophila* blood. J Genet Genomics 47:175–186. https://doi.org/10.1016/j.jgg.2020.02.004
30. Tattikota SG, Cho B, Liu Y, Hu Y, Barrera V, Steinbaugh MJ, Yoon S-H, Comjean A, Li F, Dervis F, Hung R-J, Nam J-W, Ho Sui S, Shim J, Perrimon N. 2020. A single-cell survey of *Drosophila* blood. eLife 9. https://doi.org/10.7554/eLife.54818
31. Cattenoz PB, Sakr R, Pavlidaki A, Delaporte C, Riba A, Molina N, Hariharan N, Mukherjee T, Giangrande A. 2020. Temporal specificity and heterogeneity of *Drosophila* immune cells. EMBO J 39:e104486. https://doi.org/10.15252/embj.2020104486
32. Boulet M, Renaud Y, Lapraz F, Benmimoun B, Vandel L, Waltzer L. 2021. Characterization of the *Drosophila* adult hematopoietic system reveals

a rare cell population with differentiation and proliferation potential. Front Cell Dev Biol 9:739357. https://doi.org/10.3389/fcell.2021.739357
33. Hirschhäuser A, Molitor D, Salinas G, Großhans J, Rust K, Bogdan S. 2023. Single-cell Transcriptomics identifies new blood cell populations in Drosophila released at the onset of metamorphosis. Development 150:dev201767. https://doi.org/10.1242/dev.201767
34. Boman HG, Nilsson I, Rasmuson B. 1972. Inducible antibacterial defence system in *Drosophila*. Nature 237:232–235. https://doi.org/10.1038/237232a0
35. Lemaitre B, Kromer-Metzger E, Michaut L, Nicolas E, Meister M, Georgel P, Reichhart JM, Hoffmann JA. 1995. A Recessive Mutation, immune deficiency (IMD), defines two distinct control pathways in the *Drosophila* host defense. Proc Natl Acad Sci U S A 92:9465–9469. https://doi.org/10.1073/pnas.92.21.9465
36. Ferrandon D, Imler J-L, Hetru C, Hoffmann JA. 2007. The *Drosophila* systemic immune response: sensing and signalling during bacterial and fungal infections. Nat Rev Immunol 7:862–874. https://doi.org/10.1038/nri2194
37. Ritsick DR, Edens WA, McCoy JW, Lambeth JD. 2004. The use of model systems to study biological functions of NOx/Duox enzymes. Biochem Soc Symp, no. 71:85–96. https://doi.org/10.1042/bss0710085
38. Ha E-M, Oh C-T, Bae YS, Lee W-J. 2005. A direct role for dual oxidase in *Drosophila* gut immunity. Science 310:847–850. https://doi.org/10.1126/science.1117311
39. Ramírez-Camejo LA, Maldonado-Morales G, Bayman P. 2017. Differential microbial diversity in *Drosophila melanogaster*: are fruit flies potential vectors of opportunistic pathogens Int J Microbiol 2017:8526385. https://doi.org/10.1155/2017/8526385
40. Tan FHP, Liu G, Lau S-Y, Jaafar MH, Park Y-H, Azzam G, Li Y, Liong M-T. 2020. *Lactobacillus* probiotics improved the gut microbiota profile of a *Drosophila melanogaster* Alzheimer's disease model and alleviated neurodegeneration in the eye. Benef Microbes 11:79–89. https://doi.org/10.3920/BM2019.0086
41. Aryal SK, Carter-House D, Stajich JE, Dillman AR. 2017. Microbial associates of the Southern mole cricket (*Scapteriscus borellii*) are highly pathogenic. J Invertebr Pathol 150:54–62. https://doi.org/10.1016/j.jip.2017.09.008
42. Needham AJ, Kibart M, Crossley H, Ingham PW, Foster SJ. 2004. *Drosophila melanogaster* as a model host for *Staphylococcus aureus* infection. Microbiology (Reading) 150:2347–2355. https://doi.org/10.1099/mic.0.27116-0
43. Troha K, Im JH, Revah J, Lazzaro BP, Buchon N. 2018. Comparative transcriptomics reveals CrebA as a novel regulator of infection tolerance in *D. melanogaster*. PLoS Pathog 14:e1006847. https://doi.org/10.1371/journal.ppat.1006847
44. Nehme NT, Quintin J, Cho JH, Lee J, Lafarge M-C, Kocks C, Ferrandon D. 2011. Relative roles of the cellular and humoral responses in the *Drosophila* host defense against three gram-positive bacterial infections. PLoS One 6:e14743. https://doi.org/10.1371/journal.pone.0014743
45. Defaye A, Evans I, Crozatier M, Wood W, Lemaitre B, Leulier F. 2009. Genetic ablation of *Drosophila* phagocytes reveals their contribution to both development and resistance to bacterial infection. J Innate Immun 1:322–334. https://doi.org/10.1159/000210264
46. Stuart LM, Deng J, Silver JM, Takahashi K, Tseng AA, Hennessy EJ, Ezekowitz RAB, Moore KJ. 2005. Response to *Staphylococcus aureus* requires CD36-mediated phagocytosis triggered by the COOH-terminal cytoplasmic domain. J Cell Biol 170:477–485. https://doi.org/10.1083/jcb.200501113
47. Hashimoto Y, Tabuchi Y, Sakurai K, Kutsuna M, Kurokawa K, Awasaki T, Sekimizu K, Nakanishi Y, Shiratsuchi A. 2009. Identification of Lipoteichoic acid as a ligand for Draper in the Phagocytosis of *Staphylococcus aureus* by *Drosophila hemocytes*. J Immunol 183:7451–7460. https://doi.org/10.4049/jimmunol.0901032
48. Shiratsuchi A, Mori T, Sakurai K, Nagaosa K, Sekimizu K, Lee BL, Nakanishi Y. 2012. Independent recognition of *Staphylococcus aureus* by two receptors for phagocytosis in *Drosophila*. J Biol Chem 287:21663–21672. https://doi.org/10.1074/jbc.M111.333807
49. Garver LS, Wu J, Wu LP. 2006. The Peptidoglycan recognition protein PGRP-Sc1A is essential for toll signaling and phagocytosis of *Staphylococcus aureus* in *Drosophila*. Proc Natl Acad Sci U S A 103:660–665. https://doi.org/10.1073/pnas.0506182103
50. Atilano ML, Yates J, Glittenberg M, Filipe SR, Ligoxygakis P, Schneider DS. 2011. Wall teichoic acids of *Staphylococcus aureus* limit recognition by the *Drosophila* peptidoglycan recognition protein-SA to promote pathogenicity. PLoS Pathog 7:e1002421. https://doi.org/10.1371/journal.ppat.1002421
51. Tabuchi Y, Shiratsuchi A, Kurokawa K, Gong JH, Sekimizu K, Lee BL, Nakanishi Y. 2010. Inhibitory role for D-alanylation of wall teichoic acid in activation of insect toll pathway by peptidoglycan of *Staphylococcus aureus*. J Immunol 185:2424–2431. https://doi.org/10.4049/jimmunol.1000625
52. Hori A, Kurata S, Kuraishi T. 2018. Unexpected role of the IMD pathway in *Drosophila* gut defense against *Staphylococcus aureus*. Biochem Biophys Res Commun 495:395–400. https://doi.org/10.1016/j.bbrc.2017.11.004
53. Wu K, Conly J, Surette M, Sibley C, Elsayed S, Zhang K. 2012. Assessment of virulence diversity of methicillin-resistant *Staphylococcus aureus* strains with a *Drosophila melanogaster* infection model. BMC Microbiol 12:274. https://doi.org/10.1186/1471-2180-12-274
54. Ben-Ami R, Watson CC, Lewis RE, Albert ND, Arias CA, Raad II, Kontoyiannis DP. 2013. *Drosophila melanogaster* as a model to explore the effects of methicillin-resistant *Staphylococcus aureus* strain type on virulence and response to linezolid treatment. Microbial Pathogenesis 55:16–20. https://doi.org/10.1016/j.micpath.2012.11.012
55. Ramond E, Jamet A, Ding X, Euphrasie D, Bouvier C, Lallemant L, He X, Arbibe L, Coureuil M, Charbit A. 2021. Reactive oxygen species-dependent innate immune mechanisms control methicillin-resistant *Staphylococcus aureus* virulence in the *Drosophila* larval model. mBio 12:e0027621. https://doi.org/10.1128/mBio.00276-21
56. Thomsen TT, Mojsoska B, Cruz JCS, Donadio S, Jenssen H, Løbner-Olesen A, Rewitz K. 2016. The lantibiotic nai-107 efficiently rescues *Drosophila melanogaster* from infection with methicillin-resistant *Staphylococcus aureus* USA300. Antimicrob Agents Chemother 60:5427–5436. https://doi.org/10.1128/AAC.02965-15
57. Nair SV, Baranwal G, Chatterjee M, Sachu A, Vasudevan AK, Bose C, Banerji A, Biswas R. 2016. Antimicrobial activity of plumbagin, a naturally occurring naphthoquinone from plumbago rosea, against *Staphylococcus aureus* and *Candida albicans*. Int J Med Microbiol 306:237–248. https://doi.org/10.1016/j.ijmm.2016.05.004
58. Apidianakis Y, Rahme LG. 2009. *Drosophila melanogaster* as a model host for studying *Pseudomonas aeruginosa* infection. Nat Protoc 4:1285–1294. https://doi.org/10.1038/nprot.2009.124
59. Limmer S, Haller S, Drenkard E, Lee J, Yu S, Kocks C, Ausubel FM, Ferrandon D. 2011. *Pseudomonas aeruginosa* RhlR is required to neutralize the cellular immune response in a *Drosophila melanogaster* oral infection model. Proc Natl Acad Sci U S A 108:17378–17383. https://doi.org/10.1073/pnas.1114907108
60. Lau GW, Goumnerov BC, Walendziewicz CL, Hewitson J, Xiao W, Mahajan-Miklos S, Tompkins RG, Perkins LA, Rahme LG. 2003. The *Drosophila melanogaster* toll pathway participates in resistance to infection by the gram-negative human pathogen *Pseudomonas aeruginosa*. Infect Immun 71:4059–4066. https://doi.org/10.1128/IAI.71.7.4059-4066.2003
61. Apidianakis Y, Mindrinos MN, Xiao W, Lau GW, Baldini RL, Davis RW, Rahme LG. 2005. Profiling early infection responses: *Pseudomonas aeruginosa* eludes host defenses by suppressing antimicrobial peptide gene expression. Proc Natl Acad Sci U S A 102:2573–2578. https://doi.org/10.1073/pnas.0409588102
62. Iatsenko I, Marra A, Boquete J-P, Peña J, Lemaitre B. 2020. Iron sequestration by transferrin 1 mediates nutritional immunity in *Drosophila melanogaster*. Proc Natl Acad Sci U S A 117:7317–7325. https://doi.org/10.1073/pnas.1914830117
63. Kim S-H, Park S-Y, Heo Y-J, Cho Y-H. 2008. *Drosophila melanogaster*-based screening for multihost virulence factors of *Pseudomonas aeruginosa* PA14 and identification of a virulence-attenuating factor, HudaA. Infect Immun 76:4152–4162. https://doi.org/10.1128/IAI.01637-07
64. Erickson DL, Lines JL, Pesci EC, Venturi V, Storey DG. 2004. *Pseudomonas aeruginosa* relA contributes to virulence in *Drosophila melanogaster*.

Infect Immun 72:5638–5645. https://doi.org/10.1128/IAI.72.10.5638-5645.2004
65. Martínez E, Campos-Gómez J. 2016. Oxylipins produced by *Pseudomonas aeruginosa* promote biofilm formation and virulence. Nat Commun 7:13823. https://doi.org/10.1038/ncomms13823
66. Wongsaroj L, Saninjuk K, Romsang A, Duang-Nkern J, Trinachartvanit W, Vattanaviboon P, Mongkolsuk S. 2018. *Pseudomonas aeruginosa* glutathione biosynthesis genes play multiple roles in stress protection, bacterial virulence and biofilm formation. PLoS One 13:e0205815. https://doi.org/10.1371/journal.pone.0205815
67. Heacock-Kang Y, Zarzycki-Siek J, Sun Z, Poonsuk K, Bluhm AP, Cabanas D, Fogen D, McMillan IA, Chuanchuen R, Hoang TT. 2018. Novel dual regulators of *Pseudomonas aeruginosa* essential for productive biofilms and virulence. Mol Microbiol 109:401–414. https://doi.org/10.1111/mmi.14063
68. Nontaleerak B, Duang-Nkern J, Wongsaroj L, Trinachartvanit W, Romsang A, Mongkolsuk S. 2020. Roles of RcsA, an AhpD family protein, in reactive chlorine stress resistance and virulence in *Pseudomonas aeruginosa*. Appl Environ Microbiol 86:e01480-20. https://doi.org/10.1128/AEM.01480-20
69. Xu C, Cao Q, Lan L. 2021. Glucose-binding of periplasmic protein GltB activates GtrS-GltR two-component system in *Pseudomonas aeruginosa*. Microorganisms 9:447. https://doi.org/10.3390/microorganisms9020447
70. Armstrong T, Fenn SJ, Hardie KR. 2021. JMM profile: carbapenems: a broad-spectrum antibiotic. J Med Microbiol 70:001462. https://doi.org/10.1099/jmm.0.001462
71. Shen L, Gao L, Yang M, Zhang J, Wang Y, Feng Y, Wang L, Wang S. 2021. Deletion of the PA4427-PA4431 operon of *Pseudomonas aeruginosa* Pao1 increased antibiotics resistance and reduced virulence and pathogenicity by affecting quorum sensing and iron uptake. Microorganisms 9:1065. https://doi.org/10.3390/microorganisms9051065
72. Dey S, Chakravarty A, Guha Biswas P, De Guzman RN. 2019. The type III secretion system needle, tip, and translocon. Protein Sci 28:1582–1593. https://doi.org/10.1002/pro.3682
73. Fauvarque M-O, Bergeret E, Chabert J, Dacheux D, Satre M, Attree I. 2002. Role and activation of type III secretion system genes in *Pseudomonas aeruginosa*-induced *Drosophila* killing. Microb Pathog 32:287–295. https://doi.org/10.1006/mpat.2002.0504
74. Avet-Rochex A, Bergeret E, Attree I, Meister M, Fauvarque M-O. 2005. Suppression of *Drosophila* cellular immunity by directed expression of the ExoS toxin GAP domain of *Pseudomonas aeruginosa*. Cell Microbiol 7:799–810. https://doi.org/10.1111/j.1462-5822.2005.00512.x
75. Avet-Rochex A, Perrin J, Bergeret E, Fauvarque M-O. 2007. Rac2 is a major actor of *Drosophila* resistance to *Pseudomonas aeruginosa* acting in phagocytic cells. Genes Cells 12:1193–1204. https://doi.org/10.1111/j.1365-2443.2007.01121.x
76. Sharma AK, FitzGerald D. 2010. *Pseudomonas exotoxin* kills *Drosophila* S2 cells via apoptosis. Toxicon 56:1025–1034. https://doi.org/10.1016/j.toxicon.2010.07.007
77. Jia J, Wang Y, Zhou L, Jin S. 2006. Expression of *Pseudomonas aeruginosa* toxin ExoS effectively induces apoptosis in host cells. Infect Immun 74:6557–6570. https://doi.org/10.1128/IAI.00591-06
78. Stoltz DA, Ozer EA, Taft PJ, Barry M, Liu L, Kiss PJ, Moninger TO, Parsek MR, Zabner J. 2008. *Drosophila* are protected from *Pseudomonas aeruginosa* lethality by transgenic expression of paraoxonase-1. J Clin Invest 118:3123–3131. https://doi.org/10.1172/JCI35147
79. Mulcahy H, Sibley CD, Surette MG, Lewenza S. 2011. *Drosophila melanogaster* as an animal model for the study of *Pseudomonas aeruginosa* biofilm infections in vivo. PLoS Pathog 7:e1002299. https://doi.org/10.1371/journal.ppat.1002299
80. Heacock-Kang Y, Sun Z, Zarzycki-Siek J, Poonsuk K, McMillan IA, Chuanchuen R, Hoang TT. 2018. Two regulators, PA3898 and PA2100, modulate the *Pseudomonas aeruginosa* multidrug resistance MexAB-OprM and EmrAB efflux pumps and biofilm formation. Antimicrob Agents Chemother 62:e01459-18. https://doi.org/10.1128/AAC.01459-18
81. Apidianakis Y, Pitsouli C, Perrimon N, Rahme L. 2009. Synergy between bacterial infection and genetic predisposition in intestinal dysplasia. Proc Natl Acad Sci U S A 106:20883–20888. https://doi.org/10.1073/pnas.0911797106
82. Heo Y-J, Lee Y-R, Jung H-H, Lee J, Ko G, Cho Y-H. 2009. Antibacterial efficacy of phages against *Pseudomonas aeruginosa* infections in mice and *Drosophila melanogaster*. Antimicrob Agents Chemother 53:2469–2474. https://doi.org/10.1128/AAC.01646-08
83. Lindberg HM, McKean KA, Wang I-N. 2014. Phage fitness may help predict phage therapy efficacy. Bacteriophage 4:e964081. https://doi.org/10.4161/21597073.2014.964081
84. Zhang P, Guo Q, Wei Z, Yang Q, Guo Z, Shen L, Duan K, Chen L. 2021. Baicalin represses type three secretion system of *Pseudomonas aeruginosa* through PQS system. Molecules 26:1497. https://doi.org/10.3390/molecules26061497
85. Huang C, Xu R, Liégeois S, Chen D, Li Z, Ferrandon D. 2020. Differential requirements for mediator complex subunits in *Drosophila melanogaster* host defense against fungal and bacterial pathogens. Front Immunol 11:478958. https://doi.org/10.3389/fimmu.2020.478958
86. Ben-Ami R, Lamaris GA, Lewis RE, Kontoyiannis DP. 2010. Interstrain variability in the virulence of *Aspergillus fumigatus* and *Aspergillus terreus* in a toll-deficient *Drosophila* fly model of invasive aspergillosis. Med Mycol 48:310–317. https://doi.org/10.1080/13693780903148346
87. Lionakis MS, Kontoyiannis DP. 2012. *Drosophila melanogaster* as a model organism for invasive aspergillosis. Methods Mol Biol 845:455–468. https://doi.org/10.1007/978-1-61779-539-8_32
88. Lionakis MS, Lewis RE, May GS, Wiederhold NP, Albert ND, Halder G, Kontoyiannis DP. 2005. Toll-deficient *Drosophila* flies as a fast, high-throughput model for the study of antifungal drug efficacy against invasive aspergillosis and aspergillus virulence. J Infect Dis 191:1188–1195. https://doi.org/10.1086/428587
89. Chamilos G, Bignell EM, Schrettl M, Lewis RE, Leventakos K, May GS, Haas H, Kontoyiannis DP. 2010. Exploring the concordance of *Aspergillus fumigatus* pathogenicity in mice and toll-deficient flies. Med Mycol 48:506–510. https://doi.org/10.3109/13693780903225813
90. Spikes S, Xu R, Nguyen CK, Chamilos G, Kontoyiannis DP, Jacobson RH, Ejzykowicz DE, Chiang LY, Filler SG, May GS. 2008. Gliotoxin production in *Aspergillus fumigatus* contributes to host-specific differences in virulence . J Infect Dis 197:479–486. https://doi.org/10.1086/525044
91. Lionakis MS, Kontoyiannis DP. 2010. The growing promise of toll-deficient *Drosophila melanogaster* as a model for studying *Aspergillus* pathogenesis and treatment. Virulence 1:488–499. https://doi.org/10.4161/viru.1.6.13311
92. Lamaris GA, Ben-Ami R, Lewis RE, Kontoyiannis DP. 2008. Does pre-exposure of *Aspergillus fumigatus* to voriconazole or posaconazole *in vitro* affect its virulence and the *in vivo* activity of subsequent posaconazole or voriconazole, respectively? A study in a fly model of aspergillosis. J Antimicrob Chemother 62:539–542. https://doi.org/10.1093/jac/dkn224
93. Inamdar AA, Zaman T, Morath SU, Pu DC, Bennett JW. 2014. *Drosophila melanogaster* as a model to characterize fungal volatile organic compounds. Environ Toxicol 29:829–836.
94. AL-Maliki HS, Martinez S, Piszczatowski P, Bennett JW. 2017. *Drosophila melanogaster* as a model for studying *Aspergillus fumigatus*. Mycobiology 45:233–239.
95. Zhao G, Yin G, Inamdar AA, Luo J, Zhang N, Yang I, Buckley B, Bennett JW. 2017. Volatile organic compounds emitted by filamentous fungi isolated from flooded homes after Hurricane sandy show toxicity in a *Drosophila* bioassay. Indoor Air 27:518–528. https://doi.org/10.1111/ina.12350
96. Castonguay-Vanier J, Vial L, Tremblay J, Déziel E, Leulier F. 2010. *Drosophila melanogaster* as a model host for the *Burkholderia cepacia* complex. PLoS One 5:e11467. https://doi.org/10.1371/journal.pone.0011467
97. Vial L, Groleau M-C, Lamarche MG, Filion G, Castonguay-Vanier J, Dekimpe V, Daigle F, Charette SJ, Déziel E. 2010. Phase variation has a role in *Burkholderia ambifaria* niche adaptation. ISME J 4:49–60. https://doi.org/10.1038/ismej.2009.95
98. Agnoli K, Schwager S, Uehlinger S, Vergunst A, Viteri DF, Nguyen DT, Sokol PA, Carlier A, Eberl L. 2012. Exposing the third chromosome of *Burkholderia cepacia* complex strains as a virulence plasmid. Mol Microbiol 83:362–378. https://doi.org/10.1111/j.1365-2958.2011.07937.x

99. Chapalain A, Vial L, Laprade N, Dekimpe V, Perreault J, Déziel E. 2013. Identification of quorum sensing-controlled genes in *Burkholderia ambifaria*. Microbiologyopen 2:226–242. https://doi.org/10.1002/mbo3.67
100. Schwager S, Agnoli K, Köthe M, Feldmann F, Givskov M, Carlier A, Eberl L. 2013. Identification of *Burkholderia cenocepacia* strain H111 virulence factors using nonmammalian infection hosts. Infect Immun 81:143–153. https://doi.org/10.1128/IAI.00768-12
101. Allen VW, O'Connor RM, Ulgherait M, Zhou CG, Stone EF, Hill VM, Murphy KR, Canman JC, Ja WW, Shirasu-Hiza MM. 2016. Period-regulated feeding behavior and TOR signaling modulate survival of infection. Curr Biol 26:184–194. https://doi.org/10.1016/j.cub.2015.11.051
102. Touré H, Galindo LA, Lagune M, Glatigny S, Waterhouse RM, Guénal I, Herrmann J-L, Girard-Misguich F, Szuplewski S. 2023. *Mycobacterium abscessus* resists the innate cellular response by surviving cell lysis of infected phagocytes. PLoS Pathog 19:e1011257. https://doi.org/10.1371/journal.ppat.1011257
103. Marshall EKP, Dionne MS. 2022. Drosophila versus mycobacteria: a model for mycobacterial host-pathogen interactions. Mol Microbiol 117:600–609. https://doi.org/10.1111/mmi.14819
104. Roux A-L, Catherinot E, Ripoll F, Soismier N, Macheras E, Ravilly S, Bellis G, Vibet M-A, Le Roux E, Lemonnier L, Gutierrez C, Vincent V, Fauroux B, Rottman M, Guillemot D, Gaillard J-L, Jean-Louis Herrmann for the OMA Group. 2009. Multicenter study of prevalence of nontuberculous mycobacteria in patients with cystic fibrosis in France. J Clin Microbiol 47:4124–4128. https://doi.org/10.1128/JCM.01257-09
105. Griffith DE, Aksamit T, Brown-Elliott BA, Catanzaro A, Daley C, Gordin F, Holland SM, Horsburgh R, Huitt G, Iademarco MF, Iseman M, Olivier K, Ruoss S, von Reyn CF, Wallace RJ, Winthrop K, ATS Mycobacterial Diseases Subcommittee, American Thoracic Society, Infectious Disease Society of America. 2007. An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. Am J Respir Crit Care Med 175:367–416. https://doi.org/10.1164/rccm.200604-571ST
106. Qvist T, Taylor-Robinson D, Waldmann E, Olesen HV, Hansen CR, Mathiesen IH, Høiby N, Katzenstein TL, Smyth RL, Diggle PJ, Pressler T. 2016. Comparing the harmful effects of nontuberculous mycobacteria and Gram negative bacteria on lung function in patients with cystic fibrosis. J Cyst Fibros 15:380–385. https://doi.org/10.1016/j.jcf.2015.09.007
107. Johansen MD, Herrmann J-L, Kremer L. 2020. Non-tuberculous mycobacteria and the rise of *Mycobacterium abscessus*. Nat Rev Microbiol 18:392–407. https://doi.org/10.1038/s41579-020-0331-1
108. Oh C-T, Moon C, Jeong MS, Kwon S-H, Jang J. 2013. *Drosophila melanogaster* model for *Mycobacterium abscessus* infection. Microbes and Infection 15:788–795. https://doi.org/10.1016/j.micinf.2013.06.011
109. Touré H, Durand N, Guénal I, Herrmann J-L, Girard-Misguich F, Szuplewski S. 2023. *Mycobacterium abscessus* opsonization allows an escape from the defensin bactericidal action in *Drosophila*. Microbiol Spectr 11:e0077723. https://doi.org/10.1128/spectrum.00777-23
110. Dubois V, Viljoen A, Laencina L, Le Moigne V, Bernut A, Dubar F, Blaise M, Gaillard J-L, Guérardel Y, Kremer L, Herrmann J-L, Girard-Misguich F. 2018. MmpL8MAB controls *Mycobacterium abscessus* virulence and production of a previously unknown glycolipid family. Proc Natl Acad Sci USA 115:E10147–E10156. https://doi.org/10.1073/pnas.1812984115
111. Dubois V, Pawlik A, Bories A, Le Moigne V, Sismeiro O, Legendre R, Varet H, Rodríguez-Ordóñez MDP, Gaillard J-L, Coppée J-Y, Brosch R, Herrmann J-L, Girard-Misguich F. 2019. *Mycobacterium abscessus* virulence traits unraveled by transcriptomic profiling in amoeba and macrophages. PLoS Pathog 15:e1008069. https://doi.org/10.1371/journal.ppat.1008069
112. Boeck L, Burbaud S, Skwark M, Pearson WH, Sangen J, Wuest AW, Marshall EKP, Weimann A, Everall I, Bryant JM, Malhotra S, Bannerman BP, Kierdorf K, Blundell TL, Dionne MS, Parkhill J, Andres Floto R. 2022. *Mycobacterium abscessus* pathogenesis identified by phenogenomic analyses. Nat Microbiol 7:1431–1441. https://doi.org/10.1038/s41564-022-01204-x
113. Medjahed H, Gaillard J-L, Reyrat J-M. 2010. *Mycobacterium abscessus*: a new player in the mycobacterial field. Trends Microbiol. 18:117–123. https://doi.org/10.1016/j.tim.2009.12.007
114. Floto RA, Olivier KN, Saiman L, Daley CL, Herrmann J-L, Nick JA, Noone PG, Bilton D, Corris P, Gibson RL, Hempstead SE, Koetz K, Sabadosa KA, Sermet-Gaudelus I, Smyth AR, van Ingen J, Wallace RJ, Winthrop KL, Marshall BC, Haworth CS. 2016. US cystic fibrosis foundation and European cystic fibrosis society consensus recommendations for the management of non-tuberculous mycobacteria in individuals with cystic fibrosis. Thorax 71:i1–i22. https://doi.org/10.1136/thoraxjnl-2015-207360
115. Oh C-T, Moon C, Park OK, Kwon S-H, Jang J. 2014. Novel drug combination for *Mycobacterium abscessus* disease therapy identified in a *Drosophila* infection model. J Antimicrob Chemother 69:1599–1607. https://doi.org/10.1093/jac/dku024
116. Pham LN, Dionne MS, Shirasu-Hiza M, Schneider DS. 2007. A specific primed immune response in *Drosophila* is dependent on phagocytes. PLoS Pathog 3:e26. https://doi.org/10.1371/journal.ppat.0030026
117. Chambers MC, Lightfield KL, Schneider DS. 2012. How the fly balances its ability to combat different pathogens. PLoS Pathog 8:e1002970. https://doi.org/10.1371/journal.ppat.1002970
118. Bajgar A, Dolezal T. 2018. Extracellular adenosine modulates host-pathogen interactions through regulation of systemic metabolism during immune response in *Drosophila*. PLoS Pathog 14:e1007022. https://doi.org/10.1371/journal.ppat.1007022
119. Schneider DS, Ayres JS, Brandt SM, Costa A, Dionne MS, Gordon MD, Mabery EM, Moule MG, Pham LN, Shirasu-Hiza MM. 2007. *Drosophila* eiger mutants are sensitive to extracellular pathogens. PLoS Pathog 3:e41. https://doi.org/10.1371/journal.ppat.0030041
120. Bou Ghanem EN, Clark S, Roggensack SE, McIver SR, Alcaide P, Haydon PG, Leong JM, Mitchell TJ. 2015. Extracellular adenosine protects against *Streptococcus pneumoniae* lung infection by regulating pulmonary neutrophil recruitment. PLoS Pathog 11:e1005126. https://doi.org/10.1371/journal.ppat.1005126
121. Majde JA, Krueger JM. 2005. Links between the innate immune system and sleep. J Allergy Clin Immunol 116:1188–1198. https://doi.org/10.1016/j.jaci.2005.08.005
122. Shirasu-Hiza MM, Dionne MS, Pham LN, Ayres JS, Schneider DS. 2007. Interactions between circadian rhythm and immunity in *Drosophila melanogaster*. Curr Biol 17:R353–R355. https://doi.org/10.1016/j.cub.2007.03.049
123. Sibley CD, Duan K, Fischer C, Parkins MD, Storey DG, Rabin HR, Surette MG. 2008. Discerning the complexity of community interactions using a *Drosophila* model of polymicrobial infections. PLoS Pathog 4:e1000184. https://doi.org/10.1371/journal.ppat.1000184
124. Korgaonkar A, Trivedi U, Rumbaugh KP, Whiteley M. 2013. Community surveillance enhances *Pseudomonas aeruginosa* virulence during polymicrobial infection. Proc Natl Acad Sci U S A 110:1059–1064. https://doi.org/10.1073/pnas.1214550110
125. Scoffield JA, Duan D, Zhu F, Wu H. 2017. A commensal streptococcus hijacks a *Pseudomonas aeruginosa* exopolysaccharide to promote biofilm formation. PLoS Pathog 13:e1006300. https://doi.org/10.1371/journal.ppat.1006300
126. Scoffield JA, Wu H. 2016. Nitrite reductase is critical for *Pseudomonas aeruginosa* survival during co-infection with the oral commensal *Streptococcus parasanguinis*. Microbiology (Reading) 162:376–383. https://doi.org/10.1099/mic.0.000226
127. Lee Y-J, Jang H-J, Chung I-Y, Cho Y-H. 2018. *Drosophila melanogaster* as a polymicrobial infection model for *Pseudomonas aeruginosa* and *Staphylococcus aureus*. J Microbiol 56:534–541. https://doi.org/10.1007/s12275-018-8331-9
128. Kim K, Hung R-J, Perrimon N. 2017. miR-263a regulates ENaC to maintain osmotic and intestinal stem cell homeostasis in *Drosophila*. Dev Cell 40:23–36. https://doi.org/10.1016/j.devcel.2016.11.023
129. Kim K, Lane EA, Saftien A, Wang H, Xu Y, Wirtz-Peitz F, Perrimon N. 2020. *Drosophila* as a model for studying cystic fibrosis pathophysiology of the gastrointestinal system. Proc Natl Acad Sci U S A 117:10357–10367. https://doi.org/10.1073/pnas.1913127117
130. Liu L, Johnson WA, Welsh MJ. 2003. *Drosophila* DEG/ENaC pickpocket genes are expressed in the tracheal system, where they may be involved in liquid clearance. Proc Natl Acad Sci U S A 100:2128–2133. https://doi.org/10.1073/pnas.252785099

AUTHOR BIOS

Hamadoun Touré is holder of a master's degree in parasitology followed by a master's degree in host-pathogen interactions in 2019. Having always been interested in the latter field, he completed his PhD on the mechanisms of resistance of Mycobacterium abscessus to the host innate immune response using Drosophila as a model in Prof. Jean-Louis Herrmann's laboratory at Université Paris-Saclay. After defending his thesis in May 2023, he will join Dr. Meghan Koch's laboratory at the Fred Hutchinson Cancer Center in October 2023 as a postdoctoral fellow to study how the acquisition of maternal factors through breast milk contributes to shaping offspring immunity.

Jean-Louis Herrmann is University Professor and Hospital Practitioner, Head of the Bacteriology-Hygiene Laboratories at the R. Poincaré and A Paré hospitals, University Hospital Group of Paris-Saclay and Director of the Mixed Research Unit UMR1173 (Université de Versailles St Quentin en Yvelines and INSERM). His field of research concerns host-pathogen interactions, with a particular focus on mycobacteria, and currently Mycobacterium abscessus. He is currently involved in the development of diagnostic and therapeutic tests to tackle infectious diseases on the basis of this research.

Sébastien Szuplewski is Assistant Professor at the University of Versailles-Saint-Quentin-en-Yvelines (UVSQ). He received his Ph.D. in Developmental Biology from University of Paris XI, known nowadays as University Paris-Saclay, under the supervision of Dr. Régine Terracol at Institut Jacques Monod (IJM). Following his postdoctoral research in the laboratories of Pr. Stephen M. Cohen at EMBL, Temasek Life Sciences Laboratory (TLL) and Institute of Molecular and Cell Biology (IMCB), he joined the UVSQ-Laboratory of Genetics and Biology of the Cell (LGBC) working in part on host/pathogen interactions between Drosophila and *Mycobacterium abscessus*.