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Human pathogenic bacteria, fungi, and viruses in *Drosophila*

Disease modeling, lessons, and shortcomings

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Keywords: *Pseudomonas aeruginosa*, *Serratia marcescens*, *Vibrio cholerae*, *Lactobacillus plantarum*, *Francisella tularensis*, *Mycobacterium marinum*, *Salmonella* Typhimurium, *Enterococcus faecalis*, *Listeria monocytogenes*, *Staphylococcus aureus*

Drosophila has been the invertebrate model organism of choice for the study of innate immune responses during the past few decades. Many *Drosophila*–microbe interaction studies have helped to define innate immunity pathways, and significant effort has been made lately to decipher mechanisms of microbial pathogenesis. Here we catalog 68 bacterial, fungal, and viral species studied in flies, 43 of which are relevant to human health. We discuss studies of human pathogens in flies revealing not only the elicitation and avoidance of immune response but also mechanisms of tolerance, host tissue homeostasis, regeneration, and predisposition to cancer. Prominent among those is the emerging pattern of intestinal regeneration as a defense response induced by pathogenic and innocuous bacteria. Immunopathology mechanisms and many microbial virulence factors have been elucidated, but their relevance to human health conventionally necessitates validation in mammalian models of infection.

Introduction

We interact with microorganisms throughout our lives. Some microbes are beneficial for the human body, while others can be pathogenic. The skin and the mucosal surfaces are the primary sites of host–microbe interaction.[1,2] The intestinal mucosa is one of the largest interfaces of the human body and is heavily colonized by numerous bacterial species,[1,3] some of which protect the host by modulating immune responses to fight pathogens, while providing tolerance to non-pathogens.[3] Beneficial bacteria adhesion to the intestinal mucosa may prevent the attachment and compete for space and food with suspected pathogens, thus preventing the colonization and invasion of pathogenic bacteria.[4]

Nevertheless, many bacteria, viruses, and fungi can cause dangerous infections especially under conditions that favor their growth and survival. Pneumonia and diarrhea together are the third cause of death among children under 5 years of age worldwide, accounting for 2 million deaths per year.[5] Food- and waterborne pathogens can cause acute or chronic infections to most individuals, while immunocompromised individuals due to skin burn, cancer treatment, or HIV infection, are highly susceptible to opportunistic pathogens. Also genetically predisposed individuals are more susceptible to infection, because conditions such the inflammatory bowel disease and cystic fibrosis, can alter the microbiota composition and host defense promoting the colonization and invasion of pathogenic bacteria.

Here we list 68 microbial species that have been studied in flies (**Table 1**) and review some of the 43 human microbes that have been modeled in *Drosophila melanogaster* (**Fig. 1**), describing the lessons as well as the shortcomings in studying human microbes in flies. It appears that many human infectious agents can be effectively studied in *Drosophila*, in cases where the pathologies exhibited in flies reflect conserved aspects of human disease or physiology.

Due to space limitations we do not describe the significant work done in *Drosophila* with *Pseudomonas entomophila*, *Erwinia carotovora*, *Beauveria bassiana*, *Drosophila* viruses, and other non-human pathogens (**Table 1**), focusing instead on studies aiming to explore in depth human microbial pathogenesis.

D. melanogaster, a Simple Host for Studying Microbial Diseases

D. melanogaster is a simple model organism for studying diseases caused by a great number of bacteria, fungi, and viruses. It has a short generation time simpler but analogous organ structure compared with mammals, and can be expanded at low cost.[2,134] Despite simplicity, many *Drosophila* defense mechanisms are highly conserved in mammals.[135] NFκB, JNK, and JAK-STAT signaling pathways are critical regulators of the immune responses in both flies and mammals.[135] Similarly to mammals one of the first lines of the *Drosophila* defense against microbes is mediated by barrier epithelia and their responses.[136] Infected tissue homeostasis and regeneration are also part of the defense response.[39,137,138]

Systemically, *Drosophila* fights many microbes primarily via the production of conserved antimicrobial peptides by the fat body (an analog of the mammalian liver), by the deposition of melanin that traps microbes and via phagocytosis by the plasmatocytes, which are analogous to the mammalian macrophages.[135] Bacteria, fungi, and viruses induce Toll and Imd, the two highly conserved

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Table 1. Microbes studied in *Drosophila melanogaster*

Microbes	Human diseases caused	Lessons from *Drosophila melanogaster*
Gram-negative bacteria		
**Burkholderia cepacia* complex (Bcc)	-Respiratory infections in immunocompromised patients[6]	-TNFα pathway might act against Bcc wound infections in humans.[7] -Melanization seem to reduce the ability of bacteria to grow (increase resistance) in flies, but it also reduces the tolerance of flies to Bcc infection, presumably because melanization induces immunopathology.[8]
Burkholderia thailandensis	-A low in virulence relative of *B. pseudomallei*[9]	-Highly pathogenic in wild type flies when injected or orally administered, despite the induction of antimicrobial peptides[9]
**Chlamydophila pneumoniae* **Chlamydia trachomatis*	-Lung carcinoma[10] -Trachoma[11] -Detrimental effects on female reproductive health[11]	-The conserved Tom complex-mediated host defenses show specificity against *C. caviae,* but not against *C. trachomatis*[12]
Enterobacter cloacae		-Infection induces peptide Edin in a Relish-dependent manner in adult flies[13]
Erwinia carotovora		-The Imd and JAK-STAT pathways control the immune responses in the gut. The latter contributes to stem cell proliferation and epithelial renewal.[14] -There is a conserved role of PGRPs in gut homeostasis in both mammals and flies.[15]
Escherichia coli laboratory strains (non-pathogenic, non-commensal)		-Non-pathogenic when injected into wild-type flies.[16]
**Francisella tularensis*	-Tularemia[17]	-*F. tularensis* uses common and host-specific virulence factors to proliferate within *Drosophila* and mammalian phagocytes.[17-21]
**Helicobacter pylori*	-Gastric ulcers and carcinoma[22]	-JNK, RTKs, and MLC are activated in response to CagA in a tissue context-dependent manner.[22-24]
**Legionella pneumophila*	-Legionnaire disease[25]	-Dot/Icm system and the pertinent secreted effectors of *L. pneumophila*, is pivotal in its pathogenicity in flies and humans. -Some bacterial effectors are required for full infectivity of *Drosophila* cells only in specific host genetic backgrounds.
**Mycobacterium abscessus*	-Localized tissue infections -Disseminated infections in immunodeficient patients[26]	-Induction of AMPs production in *Drosophila*[26]
**Mycobacterium fortuitum*	-Skin and soft tissue infections -Postsurgical wound infections -Endocarditis[27]	-CD36 family of proteins is required for mycobacterial infection.[28]
**Mycobacterium marinum*	-Skin infections[29] -Arthritis[29] -Osteomyelitis[29]	-Innate immunity and autophagy stimulants and anabolic and antimycobacterial drugs can be tested in flies against *M. marinum* and other mycobacterial infections.[30-33]
Mycobacterium smegmatis		-Malpighian tubules of *Drosophila* are epithelial tissues that sense microbial invasion[34] -ESCRT machinery may restrict the mycobacterial growth within the host cells[35]
Photorhabdus luminescens		-Induces the Imd pathway[36]
**Providencia species*	-Infect many organisms including humans[37]	-*Providencia* infects *Drosophila;* mechanisms unknown[37]
**Pseudomonas aeruginosa*	-Lethal infections in cystic fibrosis and burn wound patients.[38]	-*P. aeruginosa* modulates the local host defense responses in a tissue-dependent manner and may contribute to epithelial inflammation and cancer in genetically predisposed organisms.[39] -There is an inverse correlation between biofilm formation and acute virulence and the ability of other microbial species to enhance *P. aeruginosa* virulence.[40]
Pseudomonas entomophila		-Causes loss of gut integrity including the loss of stem cells and death.[41] -Induction of systemic expression of antimicrobial peptide genes in flies after oral infection[42]

Asterisk indicates human-related species that have been studied in flies.

Table 1. Microbes studied in *Drosophila melanogaster* (continued)

Microbes	Human diseases caused	Lessons from *Drosophila melanogaster*
Gram-negative bacteria (continued)		
**Salmonella* Typhimurium	-Gastroenteritis[43]	-JNK and p38 MAP kinases may drive the humoral and the cellular innate immune response respectively against *S.* Typhimurium.[44-46] -The secreted effector protein AvrA may inhibit JNK to promote infection.[44]
**Serratia marcescens*	-Pneumonia[47] -Meningitis[47]	-*S. marcescens* may cause intestinal pathologies and concomitant lethality, in accordance to the propensity of bacteria to damage mammalian epithelia.[48-50] -While phagocytosis and NFκB pathway induction promotes host defense, JAK-STAT pathway-induced intestinal regeneration appears to exacerbate infection.[49]
Spiroplasma poulsonii (intracellular symbiont)		-Increases susceptibility of *Drosophila* to certain gram-negative pathogens[51]
**Vibrio cholerae*	-Cholera[52]	-Suppression of intestinal stem cell division is likely a virulence strategy of *V. cholerae* because accelerated epithelial regeneration may protect the host against *V. cholera*.[53] -The barrier-disrupting effects of cholera toxin may act in parallel with Cl^- secretion to drive the pathophysiology of cholera.[54]
Wolbachia (intracellular symbiont)		-Female *Wolbachia*-infected flies are more resistant to *B. bassiana* infection.[55] -Induces resistance to RNA virus infections in flies;[56] not via the siRNA pathway.[57] -The mechanisms of *Wolbachia*-mediated antiviral protection are independent of the mechanisms underlying antibacterial protection[58,59]
Xenorhabdus nematophila		-Induces the Imd pathway[36]
**Yersinia pseudotuberculosis*	-Yersiniosis[60]	-The virulence factor KerV is a possible target for anti-infective drug design.[61]
Gram-positive bacteria		
**Bacillus anthracis*	-Anthrax[62]	-Endocytic recycling and cell membrane cholesterol are targets of *B. anthracis* toxins in flies and probably in humans.[62]
**Bacillus cereus*	-Gastrointestinal and non-gastrointestinal infections[63]	-Host defense mechanisms are not defined[64]
Bacillus thuringiensis		-*M. sexta* larvae Aminopeptidase N is a receptor for the *B. thuringiensis* Cry1Ac1 toxin[65]
**Enterococcus faecalis*	-Nosocomial infections[66]	-*E. faecalis* shows exceptional similarities in natural colonization of *Drosophila* and humans, a property that places *Drosophila* in a suitable position to assess its quorum sensing factors that relate to pathogenicity.[66,67]
**Lactobacillus plantarum*	-Enhancement of the intestinal epithelium barrier function[68]	-Unlike pathogenic bacteria *L. plantarum* colonization is induced by PON1 and does not induce PGRP-LE mediated defense response. -It naturally colonizes, induces intestinal regeneration, and facilitates *Drosophila* development.[69]
**Listeria monocytogenes*	-Listeriosis[70]	-Genetic screens in *Drosophila* identify host autophagy and bacterial factors required for resistance and susceptibility to *L. monocytogenes* infection, as well as, the metabolic changes in the host during infection.[71,72]
Micrococcus luteus	-Meningitis -Pneumonia -Arthritis[73]	-*M. luteus* is NOT pathogenic in flies. Nevertheless its phagocytosis can be studied in *Drosophila*.[74]
**Staphylococcus aureus*	-Pneumonia[75] -Necrotizing fasciitis[75]	-*Drosophila* models of *S. aureus* infection show the interplay of peptidoglycan recognition and evasion of this recognition by D-alanylated wall teichoic acid bound to peptidoglycan.[76-78]
Staphylococcus xylosus		-*MyD88* mutant flies are more resistant to starvation and to *S. xylosus* intestinal infection than wild-type flies.[79]

Asterisk indicates human-related species that have been studied in flies.

Table 1. Microbes studied in *Drosophila melanogaster* (continued)

Microbes	Human diseases caused	Lessons from *Drosophila melanogaster*
Gram-positive bacteria (continued)		
**Streptococcus pneumoniae*	-Pneumonia[80] -Meningitis[80]	-*Drosophila* phagocytes exhibit an immunological memory.[81] -Circadian rhythms modulate the *Drosophila* defense against *S. pneumoniae*.[82]
Fungi		
**Aspergillus fumigatus*	-Aspergillosis[83]	-Drug screens in immunocompromised flies against various strains of *A. fumigatus* can reveal the efficacy of combinatorial drug treatments.[84]
Beauveria bassiana		-Inhibits the activity of phenol oxidases, which are the main melanization enzymes[85] -Cold stress increases resistance to *B. bassiana* infection.[86] -Female *Wolbachia*-infected flies are more resistant to *B. bassiana* infection[55]
**Candida albicans* **Candida glabrata*	-Superficial and systemic infections[87]	-Toll-dependent defense responses contribute to resistance although to a different extent against systemic *C. albicans* and *C. glabrata*.[88] -SAP proteases of *C. albicans* compromise the intestinal barrier function and contribute to pathology.[87]
Candida silvativa		-The N-terminal part of the major phagocytic receptor, Eater, binds several microbes including *C. silvativa*[89]
**Cryptococcus neoformans*	-Meningoencephalitis[90]	-Alternative routes of infection reveal the existence of intestinal defense pathways other than Imd and Toll as critical for host defense.[91,92] -Host cell autophagy contributes to pathogenesis.[90]
**Cunninghamella bertholletiae*	-Invasive mucormycosis[93]	-*Drosophila* models of infection show that iron availability in the growth media and iron availability in the host affect the virulence of *C. bertholletiae* isolates.[93,94]
**Fusarium moniliforme*	-Infects fatally immunosuppressed hosts[95]	-Test of antifungal treatments[95] -Pathogenic when injected to wild-type flies.[16]
Metarhizium anisopliae		-The fungal peptide Destruxin A suppresses humoral immune responses in *Drosophila*.[96] -The proteolytic activity of *Metarhizium anisopliae* PR1A triggers the expression of *Drosomycin* in *psh*-dependent manner[97]
Pneumocystis *(P. murina*, *P.carinii*, and *P. jirovecii)*	-*P. jirovecii* cause pneumonia in humans -*P. murina* and *P. carinii* are rodent pathogens	-Toll-deficient flies are resistant to infection with *Pneumocystis* spp.[98]
**Rhizopus oryzae*	-Infects fatally immunosuppressed hosts[93]	-Tarcolimus and posaconazole show promise in combinatorial treatments.[99]
**Scedosporium apiospermum* **Scedosporium prolificans*	-Infect fatally immunosuppressed hosts[95]	-Antifungal drug testing in Toll-deficient flies[95]
Viruses		
Cricket paralysis virus (CrPV)		-CrPV increases and decreases respectively the host and viral mRNA translation during infection[100]
*Dengue virus (DENV)	-Dengue fever (dengue hemorrhagic fever and dengue shock syndrome)[101,102]	-An RNAi response is triggered by DENV to control infection[101] -Additional factors conserved between *Drosophila* and humans have been found to control infection and those could be further explored in mammals.[102]
Drosophila C virus (DCV)		-Identification of factors involved in different viral-life cycle stages.[103] -Infected flies induce the peptidoglycan receptor protein PGRP-SA and upregulate AMP encoding genes[104]
Drosophila X virus (DXV)		-Infection of flies leads in the upregulation AMP encoding genes[104]
*Epstein-Barr virus (EBV)	-Several cancers[105,106] -Autoimmune diseases[107]	-*Drosophila* is a model host system for identifying human genes, such as tumor suppressors that are targeted by BRLF1 and are relevant to EBV-mediated tumorigenesis.[105,106]
Flock house virus (FHV)		-Induces apoptosis of *Drosophila* Line-1 cells by depleting *Drosophila* Inhibitor-of-Apoptosis protein DIAP1.[108] -Viral siRNAs might cause FHV persistent infections.[109]

Asterisk indicates human-related species that have been studied in flies.

Table 1. Microbes studied in *Drosophila melanogaster* (continued)

Microbes	Human diseases caused	Lessons from *Drosophila melanogaster*
Viruses (continued)		
*Hepatitis B virus (HBV)	-Hepatitis -Cirrhosis -Hepatocellular carcinoma[110]	-*Drosophila* S2 cells were used as an expression system for viral protein preparation[110]
*Human cytomegalovirus (HCMV)	-Birth defects[111]	-Viral protein expression in *Drosophila* blocks embryogenesis[111]
*Human immunodeficiency virus 1 (HIV-1)	-Acquired immunodeficiency syndrome (AIDS)	-Vpu inhibits Toll and induces JNK pathway, depending on the tissue in which it is expressed.[112,113]
*Influenza A virus	-Flu pandemics[114]	-Adaptation of the virus for growth in *Drosophila* cells facilitates the identification of host genes that affect viral replication and aberrant host cell programming.[115,116]
Nora virus		-Mainly found in the intestine of infected flies[117] -Infection is not affected by mutations in the RNAi, Toll, or JAK-STAT pathways[118] although these and other pathways are induced upon infection[119]
*SARS coronavirus (SARS-CoV)	-Atypical pneumonia[120,121]	-*Drosophila* transgenic models of SARS-CoV indicate genetic interactions of the viral apoptotic proteins 3a and M with cytochrome c and the AKT pathway, respectively.[120-122]
Sigma virus (SIGMAV)		-Induces expression of the peptidoglycan receptor protein genes PGRP-SB1 and PGRP-SD and some, but not all, AMP genes[104] -Toll and Imd signaling are not significantly induced by Sigma virus infection[104]
*Simian vacuolating virus 40 (SV40)	-Oncogenic properties[123]	-The interaction of tumor antigen ST with PF2A and the concomitant centromere duplication may drive oncogenesis by SV40.[123]
*Sindbis virus (SINV)	-Sindbis fever, arthralgia, and rush[124]	-NRAMP family proteins are used by the SINV alphavirus to enter *Drosophila* and mammalian cells.[124] -ERK pathway induction is pivotal for *Drosophila* and mosquito host intestinal defense.[125]
*Vaccinia virus (VACV)	-Used as a vaccine for smallpox prevention[126]	-Useful model for identifying cellular factors required for viral entry[127]
*Vesicular stomatitis virus (VSV)	-Oncolytic virus[128]	-Similarly to mammalian TLR7, Toll-7 induces autophagy to suppress VSV infection in an NFκB-independent manner.[129] -Toll-7 recognizes the viral capsid, as opposed to viral RNA recognition by the mammalian TLR7.[130]
*West Nile virus (WNV)	-Highly pathogenic: fever, meningitis, encephalitis[131,132]	-Non-coding WNV RNA can induce and suppress RNAi in *Drosophila* and mammals.[131,133]

Asterisk indicates human-related species that have been studied in flies.

NFκB pathways of *Drosophila,* as well as the highly conserved in mammals' JAK-STAT pathway.[135] Viruses that infect *Drosophila* may also induce RNA interference and autophagy.[139] The many studies that have established the paradigm of innate immunity in flies provide one framework in which to analyze host-pathogen interactions with the added dimensions of specific virulence factor, regeneration and tolerance mechanisms.[135,140-142]

Infections in flies enable the study of infected tissues and organs without the ethical concerns that accompany mammalian hosts. Moreover, flies are amenable to anti-infective treatments and a great number of genetic tools based on the *Drosophila* genome are now available.[2,134] Prominent among those is the ability to conditionally inactivate every single gene using fly strains expressing gene specific RNAi constructs.[138]

During the last years flies played a critical role in identifying virulence factors of various opportunistic pathogens.[16] Some microbes use to a large extent similar virulence mechanisms to infect flies and mammals, and many virulence factors effective against mammals are also responsible for pathogenicity in flies.[143,144] As a result, a big array of microbes has been studied in fruit flies, including many important human-related microbes (**Table 1**). We discuss the most extensively studied of the human pathogens in the following sections (**Fig. 1**).

Modeling Human Microbial Diseases in *D. melanogaster*

Human wound, systemic, and intestinal infections can be easily recapitulated in *Drosophila* by pricking, injecting, and feeding flies, respectively, with the pathogens of interest[145] (**Fig. 1**). The method of thoracic or abdominal needle pricking involves the use of a metal needle dipped into a bacterial suspension.[145] If flies are pricked in the thorax, wounding is primarily imposed to the thoracic cuticular epithelium and the underlying muscle.[137,145,146] Upon inoculation at the wound site, the bacteria may proliferate locally and disseminate throughout the body of the fly, leading to both local and systemic tissue damage and immune response.[137,145,146] A second method is the "injector pumping" that

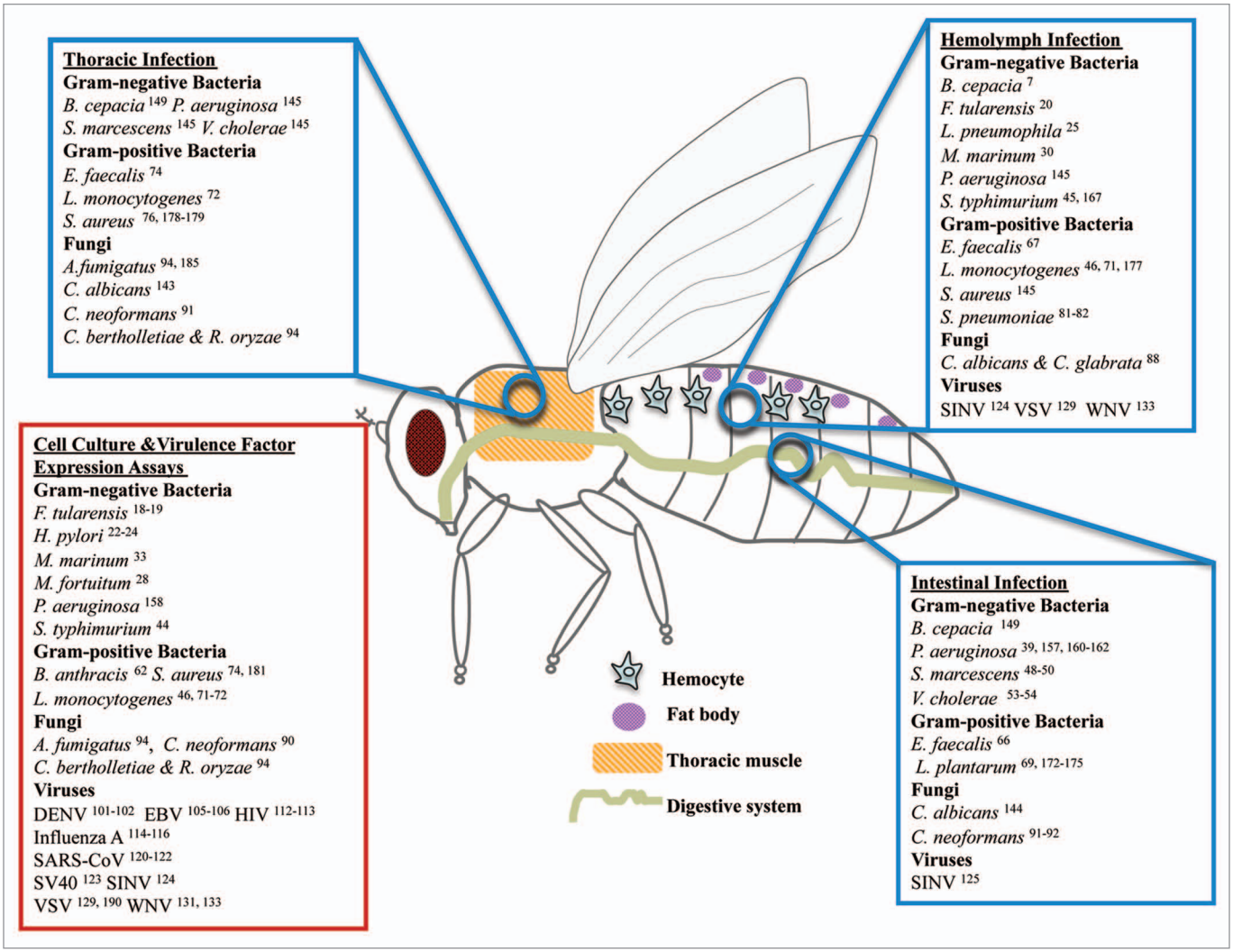

Figure 1. Human microbes extensively studied in *Drosophila*. Human microbes studied during their interaction with *Drosophila* in wound (thoracic pricking), systemic (hemolymph injection), or intestinal (feeding) infection assays. While depicted in adult flies, many hemolymph and intestinal infections are studied in larvae. In addition, microbial virulence factors have been expressed in live *Drosophila* tissues or *Drosophila* tissue culture cells have been studied upon infection with various human microbes.

produces primarily systemic inoculation by distributing microbes throughout the fly body.[145] Using this method adult flies or larvae can be easily injected with precise doses of the microbes of interest directly into the hemolymph, bypassing the wound site barrier.

Using *Drosophila* feeding assays to mimic mammalian intestinal infection various microbes can be introduced into the fly intestine.[145] This method provides the advantage, of the facile assessment of intestinal regeneration orchestrated by evolutionary conserved signaling pathways, including the JNK, Hippo, EGFR, and JAK-STAT signaling pathways.[147] Moreover, microbial genes can be individually studied by being expressed as transgenes in flies. This is a valuable technique necessary for studying human microbes that are unable to establish an infection in flies, expressing nonetheless virulence factors potentially harmful to both flies and mammals. Finally, infection of *Drosophila* hemocyte-like cell lines provides a means for high-throughput studies of microbe–immune cell interactions.

Many human bacterial, fungal, and viral pathogens have been studied in *Drosophila*. Some of them can be highly pathogenic in flies, while others are relatively harmless.[134,148] In the following sections we focus on *Drosophila* studies describing mechanisms of pathogenesis as potential targets against human pathogens.

Lessons from *Drosophila* Studies of Human Pathogens

Gram-negative and gram-positive bacteria, fungi, and viruses are grouped in separate subsections for systematic purposes.

Gram-negative bacteria

Burkholderia cepacia

Colonization with bacterial species of the *B. cepacia* complex (Bcc) is associated with serious respiratory infections in immunocompromised patients, such as cystic fibrosis and wounded individuals.[6] *B. cepacia* complex does not appear to kill *Drosophila* in

feeding assays.[149] However, in wound infection (pricking) assays it is highly lethal and appropriate for screening Bcc mutants for virulence attenuation.[149] Mutant flies for *eiger*, the *Drosophila* TNFα homolog, die faster than wild type flies when injected with *B. cepacia*.[7] On the contrary, there is no increase in the mortality of flies mutant for melanization, although melanization-deficient flies bear on average more bacteria.[8]

Conclusion: TNFα pathway might act against Bcc wound infections in humans. Interestingly, melanization seems to reduce the ability of bacteria to grow (increases resistance) in flies, but also reduces the tolerance of flies to Bcc infection, presumably because melanization induces immunopathology.

Francisella tularensis

F. tularensis is the causative agent of tularemia which, is a zoonotic disease affecting many hosts including humans.[17] Most strains require biosafety level 3 handling due to the potential aerosol transmission. Flies and other arthropods, transmit *F. tularensis* to small mammals, such as rabbits.[150] *D. melanogaster* has been established as a good arthropod model for studying tularemia.[150,151] For example, out of 394 mutants assessed for defects in intracellular proliferation, 135 were defective is both *Drosophila* S2 cells and human macrophages.[18] Two virulence factors conserved in mammals, the PI4 kinase PI4KCA and the ubiquitin hydrolase USP22, are required for proliferation within the cytosol while a third, the ubiquitin ligase CDC27, is important for the escape of *F. tularensis* into the cytosol of the host cells.[19] In addition, 249 mutant strains of *F. tularensis* subsp. *novicida*, potentially relevant to mammalian cell pathogenesis, were tested in adult flies.[17] This subspecies is attenuated in virulence in mammals yet lethal to flies allowing experimentation in a reduced biosafety level environment. Twenty percent of the genes tested in mice also contributed to adult fly pathogenesis.[17] In a similar Transponson Site Hybridization (TraSH) screen the transcription factor oxyR and the DNA repair proteins uvrB, recB, and ruvC were found to contribute to virulence.[20] These virulence factors resist oxidative stress and counteract the melanization that *Drosophila* uses as an immune response to infection. On the other hand, *F. tularensis* subsp. *novicida* is very sensitive to the antimicrobial peptides produced by the Imd-regulated immune response of the infected flies,[20] despite the ability of *Francisella* lipid A and Kdo core but not of O-antigen to confer resistance against *Drosophila* antimicrobial peptides.[21]

Conclusion: *F. tularensis* uses common and many virulence factors to proliferate within *Drosophila* and mouse cells. Nevertheless, the factors required for virulence in adult flies might be different from those inferred from in vitro or studies, and further studies are necessary to validate their significance.

Helicobacter pylori

Helicobacter pylori is a causative agent of peptic ulcers, atrophic gastridis and gastric carcinoma.[152] Virulent strains can inject the CagA effector protein into the host cells.[22] Expression of this virulence factor in *Drosophila*, promotes apoptosis or tumorigenesis through the activation of the JNK signaling and the activation of receptor tyrosine kinase (RTK) pathway genes, such as Gab adapters.[22,23] Similarly, *Drosophila* transgenic models show that CagA activates myosin regulatory light chain (MLC), leading to the rapid disruption of epithelial integrity.[24]

Conclusion: JNK, RTKs, and MLC are activated in response to CagA in a tissue-dependent manner. Thus orthotopic activation of CagA in *Drosophila* stomach like tissues, for instance, midgut copper cells, might be recommended to validate these mechanisms of action.

Legionella pneumophila

L. pneumophila can cause severe pneumonia in humans called Legionnaire disease.[25] The bacteria direct the formation of their replication vacuole by injecting many effector proteins into the host cells via the Dot/Icm type IV secretion system,[153] a mechanism that is conserved in *Drosophila*.[25] In an RNA interference screen using *Drosophila* cells *Legionella* protein complex Cdc48/p97 was found necessary for the subcellular localization of bacterial effector proteins into the host cells.[153] Another screen, which combined bacterial mutagenesis with *Drosophila* cell RNA interference, uncovered the role in pathogenesis of bacterial effectors, previously considered as redundant for bacterial replication inside host cells.[154]

Conclusion: The Dot/Icm system and the pertinent secreted effectors of *L. pneumophila* are pivotal for pathogenicity in both flies and mammals. Importantly, some bacterial effectors are required for full infectivity in *Drosophila* cells only in specific host genetic backgrounds.

Mycobacterium marinum and Mycobacterium fortuitum

M. marinum, a close relative to *M. tuberculosis*, causes human skin infections that may spread deeper, resulting in arthritis or osteomyelitis.[29] Injection of only 5 colony forming units of *M. marinum* suffices to kill 50% of flies.[30] Infected flies undergo a "wasting" process characterized by hyperglycemia and the loss of metabolic stores, similarly to what happens in humans. This process is partially induced by the transcription factor FOXO, which nevertheless does not affect bacterial load.[155] Thus FOXO controls fly tolerance to *M. marinum* infection. In addition, infection with *M. marinum* does not induce the expression of antimicrobial peptides by *Drosophila*, as it is customary during other bacterial infections.[30] This means that flies—similarly to human lung cells infected with *M. tuberculosis*—fail to recognize and clear the bacteria or that bacteria actively suppress immune responses.[30] Strikingly, host cell autophagy activation is necessary process for successful antimycobacterial drug action in infected flies and mammalian macrophages.[31] And the highly conserved ubiquitin ligase *parkin* contributes to host defense against *Mycobacteria* and other intracellular pathogens in flies and mice.[32] Moreover, lysosomal enzyme β-hexosaminidase is sufficient to control *M. marinum* growth in S2 cells and mouse macrophages.[33] Furthermore, fly cell infection with *M. fortuitum,* which is also pathogenic to humans, is a useful model for the identification of conserved host factors, for example the CD36 family gene *peste*, that are required for *M. fortuitum* recognition and uptake by fly and human cells.[28]

Conclusion: Innate immunity and autophagy stimulants and anabolic and antimycobacterial drugs can be tested in flies against *M. marinum* and other mycobacterial infections.

Pseudomonas aeruginosa

P. aeruginosa is a major agent of lethal infections in cystic and burn wound patients.[38] Many of its virulence factors show exceptional conservation by contributing to pathogenesis in flies and mice.[156] *P. aeruginosa* redox-active phenazine pyocyanin induces *Drosophila* intestinal stem cells overproliferation as a defense response to infection, which nevertheless may lead to tumor formation in genetically predisposed flies.[39] In an oral infection model, in which the bacteria spread systemically to kill the fly, the quorum sensing regulator RhlR is required for full virulence.[157] In a wound infection model, transgenic flies expressing Paraoxonase 1 (PON1) are more resistant to *P. aeruginosa* wound infection, because PON1 can neutralize the quorum sensing regulator LasI.[158]

Interestingly, *P. aeruginosa* may interact with avirulent or beneficial bacteria in the fly alimentary canal to enhance its pathogenicity against *Drosophila*.[159] In the fly gut *P. aeruginosa* senses gram-positive bacteria peptidoglycan to induce its infectivity and virulence against eukaryotic and prokaryotic cells.[160] In addition, it may suppress the NFκB and JNK mediated innate immune response during wound infection but it may induce JNK signaling during intestinal infection to promote intestinal regeneration or tumor cell growth and dissemination.[38,161,162] *P. aeruginosa* actively limits the expression of *Drosophila* skeletal muscle genes at the site of wound infection and the expression of glutathione-S-transferase S1 (GstS1) in flies, a JNK-mediated response that is also conserved in mouse wound infections.[137] This wound site response is a resistance mechanism that inhibits bacterial growth and dissemination.[137] Interestingly, low expression levels of GstA4, the GstS1 homolog in mice and humans, proved later on to be a factor of susceptibility to wound infection in mice and humans.[163]

Recently, formation of *P. aeruginosa* biofilms was noticed upon infection in the *Drosophila* crop.[40] In this model biofilm formation correlates negatively with the virulence of the different strains. That is, mutants with decreased biofilm formation are significantly more virulent than hyperbiofilm strains, because the former disseminate more easily to the fly hemolymph and immune response is decreased, facilitating the progression of infection.[40]

The *P. aeruginosa*–fly model has still many aspects of infection to teach us because the *Drosophila* genotypic variation affects bacterial load and survival post-infection independently, suggesting that there are mechanisms of tolerance to infection which have not been studied.[164] Furthermore, evolutionary selection for traits that allow better survival of *Drosophila* to *P. aruginosa* infection reveal a correlation between organismal development and host defense, plus the importance of genes with dual involvement in developmental and immune pathways.[165] Thus pleiotropy might be a mechanism for the observed correlation.

Conclusion: *P. aeruginosa* modulates the local host defense responses in a tissue-dependent manner and may contribute to epithelial inflammation and cancer in genetically predisposed organisms. Moreover, *Drosophila* studies show that there is an inverse correlation between biofilm formation and acute virulence and the ability of other microbial species to enhance *P. aeruginosa* virulence.

Salmonella Typhimurium

S. Typhimurium is highly virulent due to its many virulence factors.[43] It can cause inflammatory diarrhea (gastroenteritis) in calves and humans and a typhoid-like disease in mice.[43] AvrA is among the effector proteins that *S.* Typhimurium secretes into the mammalian cells. Expression of AvrA in *Drosophila* suppresses apoptosis by inhibiting the JNK pathway, a conserved mechanism used by *S.* Typhimurium to restrict its elimination.[44] Consistently, AvrA was found suppressing innate immune response and inflammation in the mouse intestine.[166]

When injected into the hemocoel of *Drosophila*, *S.* Typhimurium is lethal[167] and similarly to most lethal infections it induces anorexia in flies. Anorexia in turn increases the fly's tolerance to *S.* Typhimurium infection.[168] Similarly, during *S.* Typhimurium infection, *eiger*, the only known TNF family member in the fly is required in the fat body to reduce the bacterial load via melanization.[45] *Eiger* mutant flies nevertheless survive the infection better because they are anorexic.[45] However, the relationship between diet restriction and host defense is not universal and should be evaluated on a pathogen-specific basis. Furthermore, the *Drosophila* p38 mitogen-activated protein (MAP) kinase (Dmp38b), a homolog of the mammalian p38 MAP kinase family, protects the host against *S.* Typhimurium, because it increases the phagocytic capacity of hemocytes.[46]

Conclusion: The JNK and the p38 MAP kinases may drive humoral and the cellular innate immune response respectively against *S.* Typhimurium, while the secreted effector protein AvrA may inhibit JNK to promote infection. Nevertheless, TNF pathway inhibition induces anorexia, which seems to contribute to host tolerance.

Serratia marcescens

S. marcescens is an entomopathogenic bacterium able to infect many hosts, including humans.[48] It is a significant cause of hospital-acquired infections with high mortality rates, especially in neonatal intensive care units as it may cause pneumonia, meningitis or other serious infections.[47] *Drosophila* intestinal infection with *S. marcescens* causes a local immune response but bacteria can also traverse the intestinal epithelium and gain access to the host's body cavity.[48] A genome-wide in vivo *Drosophila* RNAi screen using *S. marcescens* infected flies identified the JAK-STAT pathway as an important inducer of intestinal regeneration and a negative regulator of host defense to intestinal infection.[49] On the contrary, Imd/NFκB signaling activation upon infection induces host defense.[48,49] Moreover, bacteria that escape to the hemolymph are contained by phagocytes.[48] Ingested bacteria that translocate to the hemolymph are detected by the systemic humoral immune system only when phagocytosis is blocked.[48] Importantly, flies lacking the gene *subdued*, a member of the mammalian calcium-activated chloride channels-TMEM16 family, accumulate more bacteria and succumb faster than wild-type flies upon *S. marcescens* oral infection, indicating a role of this gene in the *Drosophila* resistance to infection.[50]

Conclusion: *S. marcescens* may cause intestinal pathologies and concomitant lethality, in accordance to the propensity of bacteria to damage mammalian epithelia. While phagocytosis and NFκB pathway induction promotes host defense, JAK-STAT

pathway-induced intestinal regeneration appears to exacerbate infection.

Vibrio cholerae

V. cholerae is the etiological agent of cholera, a life-threatening diarrheal disease. Humans are usually infected through ingestion of contaminated water, because this bacterium primarily exists in marine environments. *V. cholerae* polysaccharide (VPS)-dependent biofilm is highly activated upon entry into the arthropod intestine and is specifically required for colonization of the arthropod rectum.[169] Interestingly, intestinal infection of *D. melanogaster* with *V. cholera* mimics to a great extent the human disease cholera.[52]

KerV, a virulence factor conserved among pathogenic *Proteobacteria*, contributes to *V. cholerae* pathogenesis in *Drosophila*.[61] Furthermore, mutations in the pro-apoptotic Eiger/TNF signaling pathway increase the susceptibility of the fly to *V. cholerae* infection, suggesting that this pathway promotes host defense against this bacterium.[170] *V. cholerae* inhibits intestinal regeneration in infected flies, but Imd/NFκB pathway and *mustard* mutants counteract this inhibition, maintain higher levels of intestinal stem cell division, and survive better during *V. cholerae* infection.[53] Cholera toxin-driven inhibition of Rab11/exocyst-mediated trafficking of host proteins induces junctional damage, weight loss, and dye leakage in the *Drosophila* gut and other pathologies conserved in human intestinal epithelial cells, and ligated mouse ileal loops.[54]

Conclusion: Suppression of intestinal stem cell division is likely a virulence strategy of *V. cholerae* because accelerated epithelial regeneration may protect the host against *V. cholerae*. Also the barrier-disrupting effects of cholera toxin may act in parallel with Cl^- secretion to drive the pathophysiology of cholera.

Gram-positive bacteria

Bacillus anthracis

Bacillus anthracis is the etiological agent of anthrax, and can infect many mammals, including humans.[62] There are three factors secreted by this bacterium which contribute to its high virulence: the lethal factor (LF), theoedema factor (EF), and the protective antigen (PA).[62] PA contributes to the entrance of LF and EF into the host cells.[62] Expression of LF and EF in *Drosophila* during development, cooperatively inhibit the last step of endocytosis, namely endocytic recycling, by blocking the Rab11/Sec15 exocyst.[62] The role of LF and EF in endocytosis proved to be conserved in a human cell line.[62] Another *Bacillus anthracis*-secreted factor the hemolytic/cytolytic protein anthrolysin O binds and kills mouse and human macrophage-like, but not *Drosophila* S2 cells, because flies contain mainly ergosteror instead of cholesterol in their cell membranes.[171]

Conclusion: Endocytic recycling and cell membrane cholesterol are targets of *B. anthracis* toxins in flies and probably in humans.

Enterococcus faecalis

Enterococci, including *E. faecalis*, are commensal organisms of the gastrointestinal tract. Interestingly, *E. faecalis* appears to naturally colonize the *Drosophila* intestine and is the leading cause of many nosocomial infections. *E. faecalis* strains that express the virulence factor cytolysin are significantly more virulent to both flies and mammals.[66] Septic injury with *E. faecalis* activates phagocytosis in addition to the antimicrobial peptide production in *Drosophila*.[74] *E. faecalis* phagocytosis is regulated by the receptor Eater and is critical for the *Drosophila* host defense.[74] *E. faecalis* quorum regulatory system genes LrgAB and SprE, and bacteriocin EF1097 were found to contribute to infection toxicity in *Drosophila*.[67]

Conclusion: *E. faecalis* shows exceptional similarities in natural colonization of *Drosophila* and humans, a property that places *Drosophila* in a suitable position to assess its quorum sensing factors that relate to pathogenicity.

Lactobacillus plantarum

L. plantarum is a gram-positive commensal bacterium in humans suggested to protect the intestinal epithelium barrier function.[68] Recent studies demonstrate that *L. plantarum* can colonize germ-free *Drosophila* larval gut and remains associated with it long after the initial colonization.[69] A mechanism used by *L. plantarum* to establish itself in the gut is the recognition by PGRP-LE and the subsequent lack of inhibition of the Imd/NFκB pathway.[172] On the contrary, PGRP-LE senses entomopathogenic *Erwinia carotovora* and induces the Imd/NFκB pathway to defend the host from infection.[172]

Several *L. plantarum* strains stimulate larval development upon nutrient scarcity and adults emerge faster than in the germ-free flies.[69] Importantly, colonization with *L. plantarum* protects the fly from virulent *P. aeruginosa* and *S. marcescens* oral infection.[173] In addition, expression of human PON1, previously found to inhibit *P. aeruginosa* quorum sensing, is shown to increase *L. plantarum* colonization in the fly gut;[174] yet another mechanism to inhibit *P. aeruginosa* infection.

Interestingly, NADPH oxidase 1-dependent ROS generation and consequent cellular proliferation in intestinal stem cells are induced upon ingestion of *L. rhamnosus* and *L. plantarum* in mice and *Drosophila* respectively.[175] Although in disparate phylogenic clades, *L. rhamnosus* and *L. plantarum* seemingly have evolved the ability to induce cellular ROS and intestinal generation within their adapted host.

Conclusion: Unlike pathogenic bacteria, *L. plantarum* colonization is induced by PON1 and does not induce PGRP-LE mediated defense response. Due to its ability to naturally colonize, induce intestinal regeneration and facilitate larval development, *L. plantarum* studies in flies can be directly relevant to human health.

Listeria monocytogenes

L. monocytogenes, is an opportunistic anaerobic intracellular pathogen that causes listeriosis, which is presented by non-specific flu-like symptoms and gastroenteritis.[70] In a *Drosophila* cell culture RNAi screen many host factors were identified required for intracellular pathogenesis and factors that specifically affect access to the cytosol by *L. monocytogenes*.[176] Induction of autophagy in *Drosophila* requiring the autophagy-related factors Atg5 and Atg1 is crucial to prevent the intracellular growth of *L. monocytogenes* and promote host survival.[71] *Drosophila* genes conferring tolerance to infection were found to be specific to the different stages of infection.[8] For example, p38 MAPK-dependent phagocytic encapsulation of bacteria resulted in enlarged phagocytes that trap *L. monocytogenes* conferring tolerance to infection.[46]

L. monocytogenes virulence genes are expressed at 25 °C, and not only at temperatures higher than 30 °C as previously thought.[72] Moreover, similar bacterial genes, such as *actA* and *prfA*, are used in *Drosophila* and mammalian cells for the intracellular replication and cell to cell spreading of *L. monocytogenes*.[72] In addition, flies infected with *L. monocytogenes* exhibit a shift in their metabolism manifested primarily as changes in their lipid, carbohydrate, and amino acid levels.[177]

Conclusion: Genetic screens in *Drosophila* identify host autophagy, phagocytosis, and bacterial factors required for resistance and tolerance to *L. monocytogenes* infection, as well as the metabolic changes in the host during infection.

Staphylococcus aureus

S. aureus has been characterized as a nosocomial pathogen, but can also infect healthy individuals.[75] *S. aureus* infections can be life-threatening because they can cause pneumonia and necrotizing fasciitis.[75] *Drosophila* is used for studying the virulence determinants of *S. aureus* strains,[178,179] and the response to antibiotic treatment upon infection.[180] *Drosophila* infection by *S. aureus* can be controlled by phagocytosis mediated by the Eater receptor.[74] Toll pathway recognizes peptidoglycan from many gram-positive bacteria and contributes to resistance against *S. aureus*.[181] Wound infection of *Drosophila* with *S. aureus* shows that D-alanylation of wall teichoic acid alters peptidoglycan recognition by the Toll innate immune pathway[76] because D-alanylated wall teichoic acid binds covalently to peptidoglycan.[77,78]

Conclusion: *Drosophila* models of *S. aureus* infection show the interplay of peptidoglycan recognition and evasion of this recognition by D-alanylated wall teichoic acid bound to peptidoglycan.

Streptococcus pneumoniae

S. pneumoniae is a human pathogen that can cause serious pathologies, including community-acquired pneumonia and meningitis.[80] Flies injected with 3000 bacterial cells into the hemolymph are usually killed within 2 d.[81] However, flies challenged with a lethal dose after being primed with heat-killed bacteria resist infection.[81] Phagocyte activation is critical for immune priming.[81] Nevertheless, this long-lasting effect is not universal and needs to be evaluated individually for each microbial species.[182] Furthermore, flies infected with *S. pneumoniae* lose circadian rhythms several days before dying.[82] Consistently, flies lacking the central clock proteins *timeless* or *period* have higher sensitivity to *S. pneumoniae* but also to *L. monocytogenes* infection.[82] Interestingly, survival during a *L. monocytogenes* infection is determined by phagocytosis and melanization; while only phagocytosis determines survival during a *S. pneumoniae* infection.[183] A trade-off in phagocytosis is evident, because increased phagocytosis is beneficial to the host during *S. pneumoniae* infection but detrimental during *L. monocytogenes* infection.[183] This might be because the former is an extracellular and the latter an intracellular pathogen.

Conclusion: *Drosophila* phagocytes are protective and exhibit an immunological memory, while circadian rhythms modulate the *Drosophila* defense against *S. pneumoniae*. Whether similar mechanisms take place in humans will be important to explore.

Fungi

Aspergillus fumigatus

A. fumigatus is the major cause of invasive aspergillosis in immunocompromised individuals and adult flies, although other *Aspergillus* species are also pathogenic.[83] The virulence of *A. fumigatus* has a multifactorial nature.[184] In 1996 Lemaitre, Hoffmann, and collaborators found that the Toll pathway is required in *Drosophila* to respond to *A. fumigatus* infection.[185] While non-pathogenic to wild-type flies, this fungus is lethal to *Toll*-deficient flies,[83,185] which can also be used to screen for antifungal drugs combinatorially in vivo. For example, combinatorial treatments with voricanazole and terbinafine have been shown to have a synergistic effect against infection.[84] In addition, Toll-deficient flies have been used in combination with zebrafish to show that *A. fumigatus* secondary metabolites contribute to fungal virulence and phagocyte function respectively.[186]

Conclusion: Drug screens in immunocompromised flies against various strains of *A. fumigatus* can reveal the efficacy of combinatorial drug treatments.

Candida albicans and Candida glabrata

C. albicans is the predominant fungal pathogen in humans causing invasive infections and most commonly death in immunocompromised patients.[187] *C. albicans,* and to a lesser extend the microbiologically disctinct *Candida glabrata*, can cause superficial infections in several organs using tissue site-specific virulence factors, but also bloodstream infections in immunocompromised and inflammatory bowel diseases patients.[188] In immunocompromised patients the systemic dissemination is thought to occur from the gut to the bloodstream.[144]

The pathogenicity of *C. albicans* can be studied by systemically infecting Toll-deficient flies or by feeding wild-type *Drosophila* larvae, because in both systems the virulence ranking of several clinical strains is the same between mice and *Drosophila*.[143,144] *Drosophila* intestinal infection with *C. albicans* results in an extensive JNK-mediated death of gut cells and the expression of antimicrobial peptides in the fat body.[144] Moreover, *Candida* pathogens secrete aspartyl proteinases (SAPs), which are critical molecules that allow them to degrade barrier tissues by hydrolysing proteins such as collagen, fibronectin and keratin in order to obtain nutrition at the site of the infection.[87] In addition, the secretion of SAP4 and SAP6 from *Candida* is necessary for the activation of systemic Toll-dependent immunity.[144] Although Toll pathway controls fungal infection with both *C. albicans* and *C. glabrata*, the two species differ in their ability to activate protective melanization.[88]

Conclusion: Toll-dependent defense responses contribute to resistance although to a different extent against systemic *C. albicans* and *C. glabrata*. SAP proteases of *C. albicans* compromise the intestinal barrier function and contribute to pathology.

Cryptococcus neoformans

C. neoformans is another opportunistic fungal pathogen that can cause serious infections in immunocompromised patients, such as those with HIV/AIDS.[90] In addition, systemic *Cryptococcus* infection is associated with meningoencephalitis.[90] *Drosophila* S2 cells can be used in combination with RNA

interference technology for identifying host defense factors and mechanisms, for example, the exploitation of host autophagy by *C. neoformans* to survive and disseminate upon infection.[90] Moreover, Toll pathway is critical for host defense when *C. neoformans* is introduced into the hemolymph of *Drosophila*, but Toll and Imd pathways are dispensable for host defense against intestinal infections.[91] Further studies showed that there are alternative, NFκB-independent, immune responses acting in the *Drosophila* intestine against many intestinal pathogens.[92]

Conclusion: Alternative routes of infection reveal the existence of intestinal defense pathways other than the Imd and Toll as critical for host defense, while host cell autophagy contributes to pathogenesis.

Cunninghamella bertholletiae and Rhizopus oryzae

C. bertholletiae and *R. oryzae* are filamentous fungi that cause invasive mucormycosis, and are associated with high rates of mortality, especially in immunocompromised patients, such as those with hematological malignancies.[93] In a *Drosophila* model of mucormycosis the virulence of *C. bertholletiae* isolates is affected by iron content the nutrient media in which fungi grow.[93] Similarly, corticosteroid drugs and deferoxamide that affect iron availability in the host also affect wild-type *Drosophila* infection with *C. bertholletiae*.[94] In addition, tarcolimus and posaconazole have been shown to have combinatorial efficacy against *R. oryzae* in flies and mice.[99]

Conclusion: *Drosophila* models of infection show that iron availability in the growth media and iron availability in the host affect the virulence of *C. bertholletiae* isolates. Tarcolimus and posaconazole show promise in combinatorial treatments against *R. oryzae*.

Viruses

Dengue virus (DENV)

Dengue virus can cause dengue fever which can develop into dengue hemorrhagic fever and dengue shock syndrome.[101,102] Infection of *Drosophila* S2 cells with four DENV serotypes (DENV1–4) induces an RNAi response. Knocking down the RNAi pathway results in 10- to 100-fold enhancement of replication of all strains tested.[101] In addition, a genome-wide RNA interference screen in *Drosophila* cells identified candidate host factors implicated in the propagation of DENV.[102] Eighty-two of these have human homologs, while 42 were previously known to affect virus replication in human cells.[102]

Conclusion: An RNAi response is triggered by DENV to control infection. Additional factors conserved between *Drosophila* and humans have been found to control infection and those could be further explored in mammals.

Epstein–Barr virus (EBV)

Epstein–Barr virus is associated with many different cancers,[105,106] but also with several autoimmune diseases.[107] Viral gene expression in *Drosophila* is used to identify host cell proteins that can modulate the functions of EBV immediate-early genes BRLF1 and BZLF1, which are essential for the EBV replication.[105,106] BRLF1 expression in fly tissues inhibits known tumor suppressor genes and as a consequence induces overproliferation.[106] Furthermore, many *Drosophila* genes with known human homologs are required for EBV induced cell proliferation.[106]

Conclusion: *Drosophila* is a model host system for identifying human genes, such as tumor suppressors that are targeted by BRLF1 and are relevant to EBV-mediated tumorigenesis.

Human immunodeficiency virus (HIV)

HIV is the cause of the acquired immunodeficiency syndrome (AIDS) and there is no vaccine against it. High HIV-1 replication in the host-cells is achieved by accessory proteins, including the viral protein U (Vpu).[112] Vpu expression in the *Drosophila* fat body results in the inhibition of Cactus degradation counteracting Toll pathway activation.[112] In addition, Vpu expression in the *Drosophila* wing primordia triggers apoptosis via JNK pathway signaling.[113]

Conclusion: Vpu inhibits Toll and induces JNK pathway, depending on the tissue in which it is expressed. Thus orthotopic expression of viral proteins in immune cells and barrier epithelia might be required for the study of responses elicited by Vpu.

Influenza A virus

Influenza is caused by negative-strand RNA viruses of the family *Orthomyxoviridae*. It is highly contagious and sometimes deadly.[114] Using a modified virus able to replicate in *Drosophila* cells 3 genes and their human homologs (ATP6 V0D1, COX6A1, and NXF1) were found to control viral replication.[114] In addition, expression of the influenza virus M2 gene in *Drosophila* led to the identification of V1V0 ATPase as a potentiator of M2-mediated aberrant cell development to the host cell.[115,116]

Conclusion: Adaptation of the virus for growth in *Drosophila* cells facilitates the identification of host genes that affect influenza A virus replication and aberrant host cell programming.

SARS coronavirus (SARS-CoV)

The severe acute respiratory syndrome–coronavirus (SARS-CoV) is the etiological agent of the 2003 atypical pneumonia outbreak.[120,121] The SARS-CoV3a locus encodes a 274 a.a. potassium channel protein, which is detected in the patient's cells.[122] This protein is usually localized on the cell surface of virus-infected cells.[122] *Drosophila* expressing the 3a protein is suitable for the investigation of its apoptotic function and genetic interaction with host factors, such as cytochrome c.[120,122] Likewise, expression of the SARS-CoV Membrane (M) structural protein in *Drosophila* induces apoptosis via the inhibition of the AKT pathway.[121]

Conclusion: *Drosophila* transgenic models of SARS-CoV indicate genetic interactions of the viral apoptotic proteins 3a and M with cytochrome c and the AKT pathway, respectively.

Simian vacuolating virus 40 (SV40)

Simian vacuolating virus 40 belongs to the family of DNA tumor viruses.[123] Such viruses induce host cell proliferation in order to promote their replication.[123] Expression of the viral oncogene tumor antigen ST in *Drosophila* tissues and mammalian cells leads to its interaction with PF2A and the induction of centromere duplication.[123]

Conclusion: The interaction of tumor antigen ST with PF2A and the concomitant centromere duplication may drive oncogenesis by SV40.

Sindbis virus (SINV)

Sindbis virus is a mosquito-borne alphavirus that can cause fever, arthralgia and rush in humans.[124] Natural resistance-associated macrophage protein (NRAMP), a host cell surface iron transporter with 12 transmembrane domains, is used by SINV to

enter *Drosophila* cells in culture and in adult flies.[124] Consistently, SINV entry and infection of the mammalian cells is mediated by the NRAMP homolog, NRAMP2.[124]

Interestingly, arboviruses and food nutrients induce the ERK pathway, which in turn restricts viral infection in the *Drosophila* intestine. That is, SINV and vesicular stomatitis virus become infective upon genetic or pharmacological inhibition of the ERK pathway. Strikingly, vertebrate insulin, which activates ERK in the mosquito gut during a blood meal, restricts viral infection of the insect intestinal epithelium.[125]

Conclusion: NRAMP family proteins are used by the SINV α virus to enter *Drosophila* and mammalian cells. ERK pathway induction is pivotal for *Drosophila* and mosquito host intestinal defense.

Vesicular stomatitis virus (VSV)

Vesicular stomatitis virus, a member of rhabdovirus family,[189] is a highly promising agent for cancer treatment, since it selectively infects and kills cancer cells.[128] Recognition of this single stranded RNA virus by the *Drosophila* pattern recognition receptor Toll-7, similarly to mammalian TLR7,[130] results in the activation of antiviral autophagy, which is NFκB-independent.[129] Consistent with this, flies deficient for Toll-7 are more susceptible to VSV infection.[129] Other studies in both adult flies and *Drosophila* S2 cells also show that activation of autophagy in *Drosophila* decreases the replication of VSV.[190] Interestingly, the host cells recognize a preformed component of the virus and induce autophagy before the initiation of viral replication.[190]

Conclusion: Similarly to mammalian TLR7, *Drosophila* Toll-7 induces autophagy to suppress VSV infection in an NFκB-independent manner. Nevertheless, Toll-7 recognizes the viral capsid, as opposed to viral RNA recognition by the mammalian TLR7. Thus, similarly to Toll, Toll-7 pathway appears conserved in mammals, but only downstream of the receptor.

West Nile virus (WNV)

West Nile virus is emerging as a highly virulent human pathogen.[131,132] It belongs to neurotropic mosquito-borne flaviviruses[131,132] causing fever, meningitis and encephalitis. Similarly to VSV infection, WNV infection induces RNAi as a defense mechanism in *Drosophila*.[133] WNV infection of adult *Drosophila* also supports the idea of a triggered protective RNAi response upon infection.[133] Importantly, non-coding WNV and other flavivirus RNA can suppress the RNAi defense mechanism in mammalian and *Drosophila* cells.[131]

Conclusion: WNV can induce and suppress RNAi in *Drosophila* and mammals.

Shortcomings of *Drosophila* Models of Microbial Infections

Drosophila can be used to investigate many mechanisms underlying microbial infections in humans, but there are also limitations in its use due to the evolutionary distance between flies and mammals. Thus a gold standard in studying human pathogens in flies is to verify findings in mammalian models of infection. Focusing on conserved aspects of host immunity and physiology increases the chance that any mechanism of pathogenesis identified in *Drosophila* will have a direct impact in humans. For example, the *Drosophila* melanization, while clearly contributing to host defense, it does not appear conserved in mammals. Thus, caution should be taken when interpreting findings related to the fly melanization in terms of human infectious diseases.

Some aspects of wound healing and inflammation cannot be modeled in *Drosophila*, because particular cells and tissues found in mammals are missing from flies. For example, flies lack an adaptive immune response as we know it in humans, thus they are inappropriate for studying the impact of the known adaptive immunity on tissue repair and inflammation.[191] Also fibrosis and scarring cannot be easily investigated in *Drosophila* because there are no myofibroblasts and no connective tissues to induce fibrosis.[191] Additionally, flies lack structural orthologs of many mammalian effector molecules, including chemokines, which are crucial for cell communication and regulation of inflammation during infection.[138,191] Furthermore, due to the absence of lamina propria from the *Drosophila* intestine, which includes connective tissue, myofibroblasts, and immune cells, it is only possible to study regenerative inflammatory signals of the intestinal epithelium, trachea, and muscle.[2] For example, the local tissue-emanating signals in *Drosophila* that control regeneration of the intestinal epithelium upon damage or infection.[147]

Additional limitations may also be posed by the wrong choice of infection methods. For example, when *Drosophila* is injected directly into the hemolymph with various bacteria, flies can be killed even by bacterial strains that are considered nonpathogenic in mammals.[192] Thus, this technique might fail to distinguish between virulent and non-virulent bacteria,[192] in which case pathogenicity cannot be studied and alternative modes of infection should be tried. Accordingly, infection modes that mimic intestinal or wound infections might be more appropriate for highly virulent microbes, such as *P. aeruginosa*, *E. faecalis*, and *S. aureus* that initially exert their virulence locally on soft tissues. Importantly, while major differences in host survival to infection and bacterial load are mostly independent of the general genetic background, less extensive differences are not.[145] In the latter case isogenic fly strains should be compared or more than one wild-type and mutant fly strains for the same gene should be assessed.

Finally, while some mammalian viruses can be recognized by and can enter *Drosophila* cells, others need to be previously modified. Therefore in many cases only viral proteins can be assessed via transgene expression in fly tissues. While transgenic flies can produce valuable results they do not necessarily recapitulate the complexity of the whole virus and can only provide insights on specific aspects of the infection.

Conclusions

A better understanding of host–microbe interactions is critical for the development of successful treatments. *Drosophila* represents a very useful invertebrate model host for studying many human microbes. Similarly to humans, host–pathogen interactions in flies are far more complex than the induction of distinct immune responses directed against gram-negative or gram-positive bacteria and fungi or viruses. This is because microbial strain-specific

virulence factors—identified in *Drosophila* and other hosts—and host factors control, not only innate immune responses, but also muscle homeostasis, intestinal regeneration, predisposition for cancer, and tolerance to infection. Prominent among those is the role of intestinal regeneration as a protective response induced by pathogens, such as *P. aeruginosa,* but also beneficial bacteria, such as *L. plantarum*. Interestingly, *V. cholerae* appears to have the ability to suppress regeneration and *S. marcescens* appears to benefit from the induction of regeneration. Clearly, future studies can shed more light into this exciting area of research.

Regarding the modeling of disease in flies, the route of infection plays a pivotal role in the interaction. Microbial injection into the hemolymph, for example, bypasses many of the host barrier defenses, and it might be appropriate to study systemic infections, but not highly virulent microbes able to bypass *Drosophila* barrier defenses. Finally, microbes that do not inflict disease in wild type or even in immunocompromised flies can still be studied if their virulence factors are genetically expressed preferentially orthotopically in fly tissues homologous to those relevant to human pathophysiology.

Much of the knowledge gained from *Drosophila* studies of human microbes is and will continue to be important for biomedical research because most infection models strive to recapitulate conserved aspects of human disease. Despite the existence of rough guidelines, there is no strict formula of success in modeling human disease in flies. Thus, validation of any new findings conventionally necessitates the use of mammalian models of infectious disease.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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