INFECTION AND IMMUNITY, Jan. 2011, p. 449–458 Vol. 79, No. 1
0019-9567/11/$12.00 doi:10.1128/IAI.00785-10

Porphyromonas gingivalis-Host Interactions in a *Drosophila melanogaster* Model[▽]

Christina O. Igboin,[1] Kevin P. Tordoff,[2] Melvin L. Moeschberger,[2] Ann L. Griffen,[3] and Eugene J. Leys[1]*

Section of Oral Biology[1] and Section of Pediatric Dentistry,[3] College of Dentistry, and Division of Epidemiology and Biostatistics, College of Medicine and Public Health,[2] The Ohio State University, Columbus, Ohio 43210

Received 20 July 2010/Returned for modification 25 August 2010/Accepted 25 October 2010

***Porphyromonas gingivalis* is a Gram-negative obligate anaerobe that has been implicated in the etiology of adult periodontitis. We recently introduced a *Drosophila melanogaster* killing model for examination of *P. gingivalis*-host interactions. In the current study, the *Drosophila* killing model was used to characterize the host response to *P. gingivalis* infection by identifying host components that play a role during infection. *Drosophila* immune response gene mutants were screened for altered susceptibility to killing by *P. gingivalis*. The Imd signaling pathway was shown to be important for the survival of *Drosophila* infected by nonencapsulated *P. gingivalis* strains but was dispensable for the survival of *Drosophila* infected by encapsulated *P. gingivalis* strains. The *P. gingivalis* capsule was shown to mediate resistance to killing by *Drosophila* antimicrobial peptides (Imd pathway-regulated cecropinA and drosocin) and human beta-defensin 3. *Drosophila* thiol-ester protein II (Tep II) and Tep IV and the tumor necrosis factor (TNF) homolog Eiger were also involved in the immune response against *P. gingivalis* infection, while the scavenger receptors Eater and Croquemort played no roles in the response to *P. gingivalis* infection. This study demonstrates that the *Drosophila* killing model is a useful high-throughput model for characterizing the host response to *P. gingivalis* infection and uncovering novel interactions between the bacterium and the host.**

Porphyromonas gingivalis is a Gram-negative, obligate anaerobe that has been strongly implicated as a pathogen in adult (chronic) periodontitis (23, 29), a polymicrobial inflammatory disease that affects the gingiva and other tooth-supporting structures. In order to characterize *P. gingivalis*-host interactions a number of animal infection models have been developed, the most common of which are rodent models (6, 20, 25, 28, 40, 44). Rodent models have been used to identify *P. gingivalis* components that are involved in pathogenesis (26, 32, 43, 46, 48, 52, 56, 57, 67, 73) and to characterize the host response to *P. gingivalis* infection (3, 6, 7, 13, 22, 31, 34, 35, 41, 74).

The use of the fruit fly *Drosophila melanogaster* has been well established for examining host-pathogen interactions (5, 16, 19, 21, 53, 55, 65, 66). Numerous studies have demonstrated the high degree of conservation between the *Drosophila* immune system and the mammalian innate immune system (reviewed in reference 49). Like the mammalian innate immune system, the *Drosophila* immune system detects the presence of invading microbes by using pattern recognition receptors (PRRs), which recognize pathogen-associated molecular patterns (PAMPs) and trigger an immune response that is specific for the class of invading microbe. Other mammalian immune response features that are conserved in *Drosophila* include signaling pathways (e.g., Toll/interleukin-1 receptor [IL1R], tumor necrosis factor receptor [TNFR]), antimicrobial peptides (AMPs), macrophage-like blood cells, complement C3/α_2-macroglobulin (C3/α_2M) superfamily proteins, cytokines, reactive oxygen and nitrogen species, and iron-sequestering proteins. The use of *Drosophila* as a model host offers several important advantages. The absence of an adaptive immune response makes the model useful for studying pathogen interactions with the host's innate immune response in isolation. The *Drosophila* genome sequence is known, and the Berkeley *Drosophila* Genome Project has successfully inactivated 40% of the currently annotated *Drosophila* genes (8), with ongoing efforts to eventually inactivate all genes. *Drosophila* is genetically amenable, and well-developed genetic technologies are available that facilitate the identification of host factors that promote or fight infection (4, 12, 53, 70). Additionally, their short generation time, ease of use, and affordability allow for the use of sample sizes that are large enough to permit statistical analysis of the data.

We have recently developed a *Drosophila* killing model for examining *P. gingivalis*-host interactions (37). We observed that *P. gingivalis* is pathogenic in *Drosophila* and that differences in the virulence of *P. gingivalis* strains can be observed with the *Drosophila* killing model. Multiple *P. gingivalis* components are involved in the killing of *Drosophila*, and they are also involved in virulence in mammals. Additionally, our data suggest that *P. gingivalis* killing of *Drosophila* involves mechanisms that are host mediated. The objective of the current study was to use the *Drosophila* killing model to characterize the host response to *P. gingivalis* infection. Specifically, *Drosophila* immune response gene mutants were screened to identify host factors that play a role during infection. The *Drosophila* Imd pathway was found to be important for the immune response against infection by unencapsulated *P. gingivalis*, and in a novel finding the *P. gingivalis* capsule was shown to be

* Corresponding author. Mailing address: Section of Oral Biology, College of Dentistry, The Ohio State University, 3185 Postle Hall, 305 W. Twelfth Avenue, Columbus, OH 43210. Phone: (614) 292-5273. Fax: (614) 247-6945. E-mail: leys.1@osu.edu.

▽ Published ahead of print on 1 November 2010.

TABLE 1. Bacterial and *Drosophila* strains used in this study

Strain or stock	Description or genotype	Source
P. gingivalis strains		
W83	Lab strain	Margaret Duncan
381	Lab strain	Joseph Zambon
W50	Lab strain; renamed W50UK for these studies; wt for GPC	Mike Curtis (1)
GPC	Capsule mutant	Mike Curtis (1)
E. coli DH5α		Invitrogen
D. melanogaster		
Canton S	Wild type	
key^{1}	Kenny null; *yw DD1;cn bw key*^{1}	Neal Silverman (60)
DIG 672	*yw dpt-lacZ Drs-GFP*	Bruno Lemaitre
15936	Tep IV null; $y^{1}\ w^{67c23}$;*P{EPgy2}TepIV*^{EY04656}	Bloomington Stock Center (8)
20939	Croquemort null; $y^{1}\ w^{67c23}$;*P{EPgy2}crq*^{EY14489}	Bloomington Stock Center (8)
f02756	Tep II null; *PBac{WH}TepII*^{f02756}	Exelexis Stock Center (8)
eiger^{3}	*w[1118]egr[3]*	Masayuki Miura (36)
w^{1118}	*w[1118]*; wt for *eiger*^{3}	Masayuki Miura (36)
BOS 32	Df(3R)D605/TM3 Ser,GFP	Christine Kocks (45)
BOS 34	Df(3R)Tl-I e1/TM3 Ser,GFP	Christine Kocks (45)
Eater	Eater null; Df(3R)D605/Df(3R)TI-l e1	This study, and as previously described (45)

involved in mediating resistance to antimicrobial peptides. *Drosophila* thiol-ester proteins II and IV and cytokine Eiger (TNF homolog, Janus kinase [JNK] pathway ligand) were also involved in the response against *P. gingivalis* infection.

MATERIALS AND METHODS

Bacterial and *Drosophila* strains and growth conditions. Bacterial and *Drosophila* strains used in this study are described in Table 1. *P. gingivalis* strains were grown on brucella blood agar (BBA; Anaerobe Systems) at 37°C in an anaerobic chamber (85% N_2, 10% H_2, 5% CO_2). *Drosophila* stocks were maintained and propagated at 26°C in standard culture vials containing corn flour-molasses medium. Only 3- to 5-day-old female flies were used in experiments. Transheterozygous *eater* null progeny [Df(3R)D605/Df(3R)TI-l e1] were generated as previously described (45) by crossing the deficiency lines B32 [Df(3R)D605/TM3 Ser,GFP] and B34 [Df(3R)Tl-I e1/TM3 Ser,GFP].

Infection of adult female *Drosophila*. Bacterial strains were grown in 40 ml of Trypticase soy broth (TSB) for 24 h at 37°C. *Escherichia coli* was grown aerobically with shaking, while *P. gingivalis* was supplemented with hemin (5 μg/ml) and vitamin K (1 μg/ml) and incubated anaerobically. The bacteria were harvested at 3,150 × *g* for 8 min and diluted in TSB to an optical density at 600 nm (OD_{600}) of 2.0 (1.09×10^{11} CFU ml^{-1} of *P. gingivalis*; 1×10^{11} CFU ml^{-1} of *E. coli* DH5α). The bacteria were introduced into the hemocoel (body cavity) of CO_2-anesthetized *Drosophila* through the thorax, using a 30-gauge (G) needle dipped into 500 μl of bacterial culture or sterile TSB for mock infections (vector controls [VC]). The *Drosophila* flies were returned to the original culture vials, and the number of surviving animals at time 0 h was recorded. The animals were incubated at 30°C, and the number of dead animals was recorded every 12 h for 7 days. All experiments were repeated.

β-Galactosidase assay and microscopy. *Drosophila* were killed 4 h postinfection by immersion in 95% ethanol then rinsed once in 1× phosphate-buffered saline (PBS, pH 7.4), and holes were made in the cuticle of the dead animals by using a 30-G needle to facilitate penetration of the fixative (10). Fixing the carcasses and assaying for β-galactosidase activity were performed as previously described (9), with modifications. *Drosophila* carcasses were fixed in 5% paraformaldehyde in 1× PBS for 10 min, rinsed three times (10 min, 10 min, and 20 min) in 1× PBS, and incubated at 37°C for 24 h in staining solution (1.8 mM magnesium chloride, 0.9× PBS [pH 7.3], 5 mM potassium ferrocyanide, 5 mM potassium ferricyanide, 1.2 mg/ml 5-bromo-4-chloro-3-indolyl-beta-D-galactopyranoside [X-Gal] in dimethylformamide). After staining, the carcasses were washed in 1× PBS for 2 h and immediately viewed under a microscope. Bright-field images were taken of representative carcasses by using a Nikon Eclipse E600 microscope equipped with a Nikon DXM 1200 digital camera.

AMP susceptibility assay. *P. gingivalis* strains W50UK and GPC were grown in 40 ml of TSB with hemin (5 μg/ml), vitamin K (1 μg/ml), and anaerobic incubation until mid-log phase (13 to 17 h) and then harvested by centrifugation at 3,150 × *g* for 11 min. The bacteria were washed once in 2 volumes of 1× PBS and diluted to approximately 4×10^{6} CFU/ml in 1× PBS with 1% TSB. Fifty microliters of this suspension was mixed in Eppendorf tubes with various concentrations of cecropin A from *Hyalophora cecropia* (Bachem, Torrance CA), drosocin from *Drosophila melanogaster* (Biosynthesis, Lewisville, TX), human beta-defensin-3 (hβd3; Aaron Weinberg, Case Western Reserve University), or 1× PBS for controls, in a final volume of 100 μl. The mixtures were incubated anaerobically at 37°C for 3 h, after which serial dilutions were spread on BBA plates and incubated anaerobically at 37°C for 6 days. Colony counts were determined, and the results are expressed as percentages of the colony counts of bacteria not exposed to AMPs. The experiments were performed with duplicate samples (triplicates for controls) on four independent occasions.

Statistical methods. (i) Sample size. Power calculations based on pilot data estimated that a sample size of 136 *Drosophila* animals per group would be sufficient to detect a relative risk of mortality (RR) of at least 2.0 at an α-level of 0.05 with 90% power when comparing different infections. A sample size of 150 animals per group was used in all experiments. Depending on the number of experimental groups involved, each experiment was divided into four or five parts for feasibility. The experiments were repeated for a total sample size of 300 *Drosophila* animals per group.

(ii) Data analysis. Survival data were analyzed using the SAS statistical software package (SAS Institute, Cary, NC). A Cox proportional hazards (P-H) model was fitted to the survival data. Likelihood ratio tests were performed, and RR values were obtained from the fitted Cox P-H model and adjusted for the individual "experiments" and "parts." RR values with P values of <0.05 were considered significant.

The results of the antimicrobial peptide susceptibility assays are presented as means ± standard deviations of four independent experiments. Statistical analyses were performed via an unpaired, two-tailed t test using JMP software (SAS, Cary, NC). A P value of <0.05 was considered significant.

RESULTS

The rationale used to select *Drosophila* immune response components to test for a role in the response to *P. gingivalis* infection was as follows: Lemaitre et al. compiled a list of 370 *Drosophila* genes (50) that are induced in response to septic injury (17), known to function in immunity, or encode proteins with homology to immune response components of other organisms. From this list, we identified genes that encode proteins with homology to mammalian proteins for which we could predict a role in the interaction with *P. gingivalis* and for which *Drosophila* mutants are readily available. Using this ra-

TABLE 2. *Drosophila* immune response components tested for a role in the immune response to infection with various *P. gingivalis* strains

Drosophila immune response component (comparison strains)	*P. gingivalis* infection strain	RR (comparison strain vs wt)	*P* value[a]	Component involved?
Imd signaling pathway (key^1 vs wt)	W83 (encapsulated)	1	0.8	No
	381 (unencapsulated)	1.4	**0.006**	Yes
	W50UK (encapsulated)	1	0.7	No
	GPC (W50UK mutant, unencapsulated)	2.2	**<0.0001**	Yes
Tep II ($tepII^{-/-}$ vs wt)	W83	1.68	**<0.0001**	Yes
	381	2.45	**<0.0001**	Yes
Tep IV ($tepIV^{-/-}$ vs wt)	W83	1.5	**<0.0001**	Yes
	381	1.5	**0.0006**	Yes
Eiger ($egr^{-/-}$ vs wt)	W83	1.32	**0.009**	Yes
	381	1.47	**0.007**	Yes
Croquemort ($crq^{-/-}$ vs wt)	W83	1.1	0.4	No
	381	0.9	0.4	No
Eater ($eater^{-/-}$ vs wt)[b]	W83		>0.9999	No
	381		>0.9957	No

[a] RR values for which *P* was <0.05 are shown in bold.
[b] Because $eater^{-/-}$ animals are naturally more susceptible to death by injury than wt animals, the reported *P* values are for comparisons of the RR of $eater^{-/-}$ versus wt flies infected with W83 or 381 to the RR of mock-infected $eater^{-/-}$ versus wt flies.

tionale we selected six *Drosophila* immune response components (Tables 1 and 2) to test for a role in the response to *P. gingivalis* infection. Although the selected components play important and varied roles in the *Drosophila* immune response, they represent only a small fraction of the *Drosophila* immune response components. The *Drosophila* immune response gene mutants have all been previously characterized. Groups of wild-type (wt) and immune response-defective *Drosophila* animals were infected with *P. gingivalis* strain W83, strain 381, or mock infected, and the survival of the animals was compared. Based on results presented in our other report also appearing in this issue (37), between 5.10×10^3 CFU and 3.17×10^4 CFU of *P. gingivalis* were inoculated into the animals. RR values for pairwise comparisons of the survival of immune response-deficient *Drosophila* versus wt *Drosophila* animals are shown in Table 2. An RR value greater than 1 indicates that the *Drosophila* immune response gene mutant was more likely than the wt *Drosophila* animals to die from the *P. gingivalis* infection.

Imd signaling pathway. The Imd signaling pathway is homologous to the mammalian TNF receptor signaling pathway (39, 49), although they differ at the level of activation/detection. The Imd pathway is a major regulator of *Drosophila* immune response genes (18), most notably AMP genes. In fat body cells (the functional equivalent of the liver), the detection of Gram-negative-type peptidoglycan (PPG) by peptidoglycan recognition protein LC (PGRP-LC) activates the pathway. Activation of the Imd pathway triggers a signal transduction cascade that culminates in the activation of the NF-κB family protein Relish, which then activates the transcription of AMPs, e.g., diptericin, and other immune response genes. In key^1 *Drosophila* the *kenny* gene, which encodes a subunit of the IκB kinase complex, is inactivated, which inactivates the Imd pathway (60). key^1 *Drosophila* flies therefore tend to be highly susceptible to infections with Gram-negative bacteria, e.g., *E. coli* (27, 60).

Survival curves of infected and mock-infected wt and key^1 flies are shown in Fig. 1. key^1 *Drosophila* were significantly more likely to die than wt *Drosophila* from an *Escherichia coli* DH5α infection, as previously reported (60). No difference was observed in the survival of wt and key^1 *Drosophila* animals infected with *P. gingivalis* strain W83 (Fig. 1A; Table 2). To determine whether the capsule that surrounds strain W83 plays a role in the nullification of the Imd pathway, the survival rates of wt and key^1 animals infected with the acapsular *P. gingivalis* strain 381 were compared (Fig. 1B). key^1 *Drosophila* flies were more likely to die than wt *Drosophila* flies from an infection with strain 381 (Fig. 1B; Table 2). To further examine the role of the *P. gingivalis* capsule in the nullification of the Imd pathway, *Drosophila* flies were infected with *P. gingivalis* strain W50UK or its isogenic capsule mutant, GPC (1), and the survival rates of the animals were compared (Fig. 1C). Strain W50UK is highly similar to strain W83 (51). No difference was observed in the survival of wt and key^1 *Drosophila* flies infected with the encapsulated strain W50UK; however, key^1 *Drosophila* flies were significantly more likely to die than wt *Drosophila* flies from the GPC (acapsular mutant) infection (Fig. 1C; Table 2).

To determine whether the *P. gingivalis* capsule can hide the bacterium's peptidoglycan, thereby preventing activation of the Imd pathway, *Drosophila* animals that express β-galactosidase under the control of the diptericin promoter (*dipt-LacZ*) were used to monitor activation of the Imd pathway in response to W50UK, GPC, and mock infection. Images of representative animals are shown in Fig. 2. Animals infected with strain W50UK (encapsulated) as well as animals infected with strain GPC (acapsular mutant) displayed robust β-galactosidase activity at 4 h postinfection, while mock-infected animals (VC) displayed little or no β-galactosidase activity. The Imd pathway was activated by both the encapsulated and capsule mutant strains of W50UK.

To determine whether the *P. gingivalis* capsule can provide

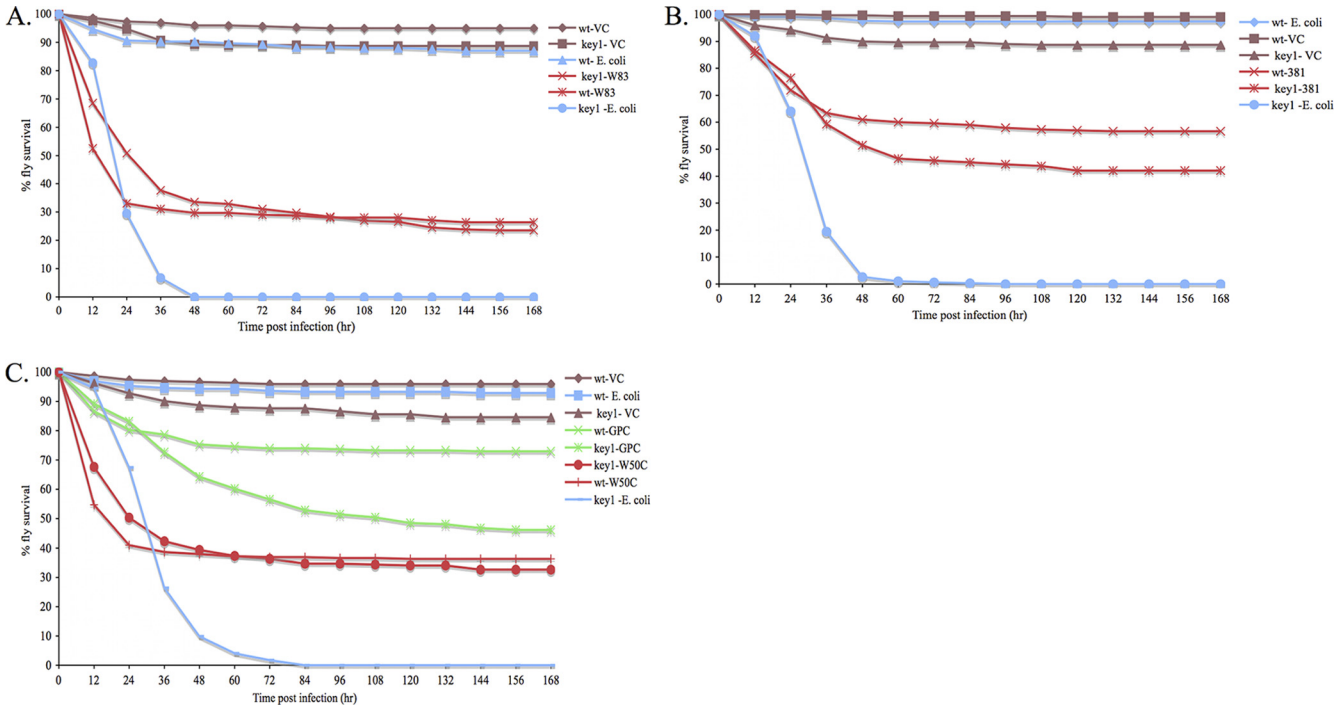

FIG. 1. Survival rates of *P. gingivalis*-, *E. coli* DH5α-, and mock-infected wt and *key*^{1} *Drosophila* animals. wt and *key*^{1} *Drosophila* flies were infected with the indicated bacteria. The legend labels are of the form *Drosophila* strain-infection agent. (A) Survival of W83-, *E. coli* DH5α-, and mock-infected *Drosophila* animals. (B) Survival of 381-, *E. coli* DH5α-, and mock-infected *Drosophila* animals. (C) Survival of W50UK-, GPC (capsule mutant)-, *E. coli* DH5α-, and mock-infected *Drosophila* animals.

resistance to Imd pathway-regulated AMPs, strains W50UK and GPC were exposed to a range of concentrations of cecropin A (from *Hyalophora cecropia*) and drosocin (from *Drosophila melanogaster*) up to their reported physiological concentrations (49, 61), and the survival rates of the bacteria were compared. Cecropin A from *Hyalophora cecropia* was used because it has an identical activity spectrum as cecropin A from *Drosophila* (61) and is readily available. The relative survival rates of W50UK versus GPC bacteria after exposure to cecropin A and drosocin are shown in Fig. 3A and B, respectively. Strain GPC was significantly ($P < 0.05$) more sensitive than strain W50UK to killing by cecropinA and drosocin, although only at the physiological concentration for drosocin. Strain GPC was also more sensitive than W50UK to killing by hβd3 (Fig. 4C) ($P < 0.05$).

Teps II and IV. Teps I to IV are members of the complement C3/α_2M superfamily of proteins (2, 11, 47, 49). Teps are expressed by *Drosophila* fat body cells and plasmatocytes (47) in response to Upd3 (cytokine) signaling via the JAK/STAT pathway. Proteins in this family are involved in the opsonization of pathogens for phagocytosis (complement) and the inhibition of a broad spectrum of proteases (α_2M). Three Teps (I, II, and IV) are strongly upregulated in response to bacterial challenge (47).

Tep II and Tep IV were tested for a role in the *Drosophila* immune response to *P. gingivalis* infection. Survival curves of infected and mock-infected wt and *tep*$^{-/-}$ animals are shown in Fig. 4. *tepII*$^{-/-}$ *Drosophila* flies were more likely to die than wt *Drosophila* flies from infections with *P. gingivalis* strains W83 and 381 (Fig. 4A; Table 2). Similarly, *tepIV*$^{-/-}$ *Drosophila* flies were more likely to die than wt *Drosophila* flies from infections with *P. gingivalis* strains W83 and 381 (Fig. 4B; Table 2). The data suggest a role for Tep II and Tep IV in the *Drosophila* immune response to *P. gingivalis* infection.

Eiger. Eiger is the sole TNF superfamily homolog in *Drosophila* (42, 54), which upon binding to its receptor Wengen activates the JNK signaling pathway (36, 42, 54). Eiger was tested for a role in the *Drosophila* immune response to *P. gingivalis* infection. Survival curves of infected and mock-infected wt and *egr*$^{-/-}$ animals are shown in Fig. 5. *egr*$^{-/-}$ *Drosophila* animals were more likely to die than wt *Drosophila* animals from infections with *P. gingivalis* strains W83 and 381 (Fig. 5; Table 2), demonstrating a role for Eiger in the *Drosophila* immune response to *P. gingivalis* infection.

Croquemort. Croquemort is a CD36 superfamily protein that is expressed on *Drosophila* plasmatocytes (24). It is a scavenger receptor that has been implicated in the phagocytosis of *S. aureus* (71). No differences were observed in the survival of *crq*$^{-/-}$ versus wt *Drosophila* animals infected with *P. gingivalis* strains W83 and 381 (Table 2), suggesting that Croquemort plays no role in the *Drosophila* immune response to *P. gingivalis* infection.

Eater. Eater is an epidermal growth factor (EGF) domain-containing scavenger receptor that is expressed on *Drosophila* plasmatocytes and is involved in the phagocytosis of Gram-positive (*Staphylococcus aureus*) and Gram-negative (*E. coli*) bacteria (45). The most similar mammalian protein to Eater is SREC-I (scavenger receptor expressed by endothelial cells I). Mock-infected *eater*$^{-/-}$ *Drosophila* animals were more likely to die than mock-infected wt *Drosophila* animals (data not

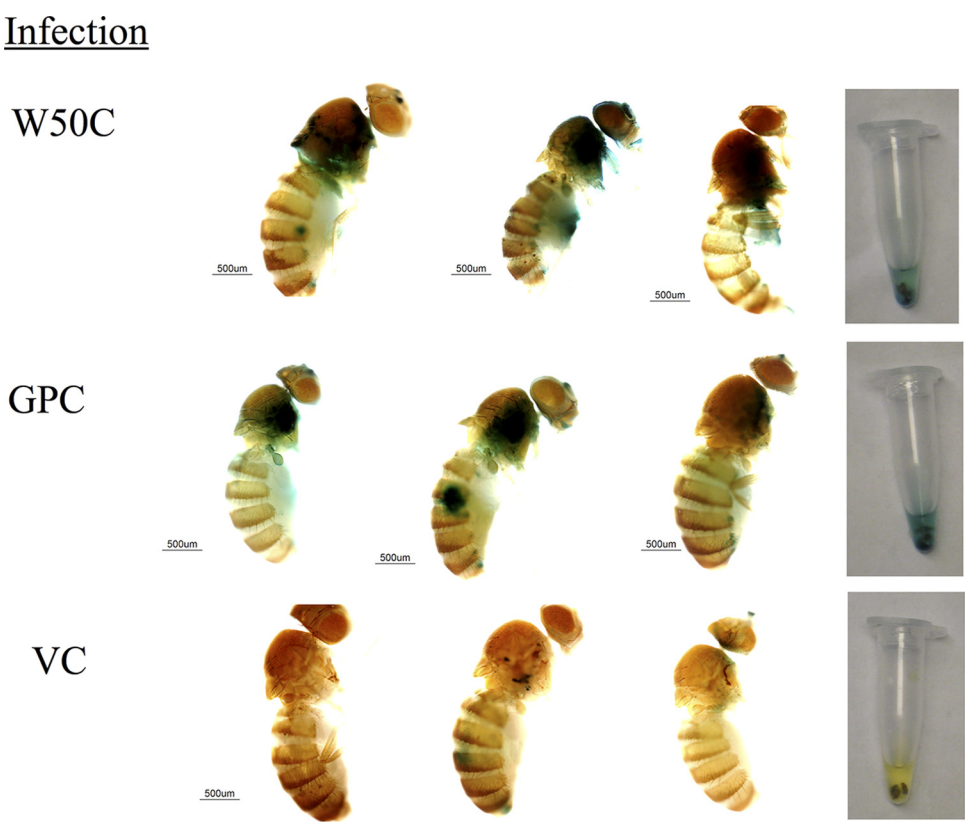

FIG. 2. Imd signaling pathway activation by *P. gingivalis* infection, as measured using a *dipt-LacZ* reporter. *dipt-LacZ* animals were infected with strain W50UK, strain GPC, or mock (VC) infected and killed 4 h postinfection. Representative animals from each of the infection groups and the tubes in which the assays were performed are shown.

shown), suggesting that the *eater*$^{-/-}$ animals are defective in wound healing. The differences in the survival of *eater*$^{-/-}$ versus wt *Drosophila* animals infected with *P. gingivalis* strains W83 and 381 were similar to the differences in survival of mock-infected *eater*$^{-/-}$ versus wt animals (Table 2), suggesting no role for Eater in the *Drosophila* immune response to *P. gingivalis* infection.

DISCUSSION

We recently developed a *Drosophila melanogaster* killing model for examining *P. gingivalis*-host interactions (37), and in the current study the killing model was used to characterize the host response to *P. gingivalis* infection. The *Drosophila* model has been widely used to examine the host responses to many other bacterial pathogens. For example, Brandt et al. observed that the *Drosophila* cytokine Eiger (TNF homolog) contributes to *Salmonella enterica* serovar Typhimurium-induced pathology (12), and Mansfield et al. observed that the Toll pathway is important for the *Drosophila* immune response against *Listeria monocytogenes* infection (53).

In this study the *Drosophila* Imd signaling pathway was shown to be important for the immune response against unencapsulated strains of *P. gingivalis* but ineffective for the immune response against encapsulated strains of the bacterium. *key*^{1} *Drosophila* flies infected with the encapsulated *P. gingivalis* strain W83 survived as well as wt *Drosophila* flies with the same infection, indicating that the Imd signaling pathway is dispensable for the immune response against W83 infection. This is unlike *E. coli* DH5α infection in *Drosophila*, for which a functional Imd pathway is necessary and sufficient to control the infection (27, 60). We hypothesized that the capsule, which is present on strain W83 and absent from *E. coli* DH5α, may be involved in nullification of the Imd pathway by the bacterium. To test this hypothesis, the survival of wt and *key*^{1} *Drosophila* flies infected with *P. gingivalis* strain W50UK (highly similar if not identical to strain W83) versus its isogenic capsule mutant (GPC) were compared. Similarly to the W83 infection, infection with W50UK did not result in significant differences in the survival of *key*^{1} and wt *Drosophila* animals; however, *key*^{1} *Drosophila* animals were significantly more susceptible than wt *Drosophila* animals to killing by the capsule mutant GPC. To summarize, when the capsule is removed from *P. gingivalis* strain W50UK the Imd signaling pathway becomes relevant for the *Drosophila* immune response against the infection. Thus, the capsule plays a role in the nullification of the Imd pathway by encapsulated *P. gingivalis* strains (e.g., W83 and W50UK). The presence of a capsule on *P. gingivalis* could shield the bacterium's PPG from detection by PGRP-LC, thus preventing activation of the pathway. The use of a *dipt-LacZ* reporter to monitor Imd pathway activation in response to W50UK and GPC (capsule mutant) infections demonstrated that the pathway was activated in response to both infections. Thus, the *P. gingivalis* capsule does not shield the bacterium's PPG from

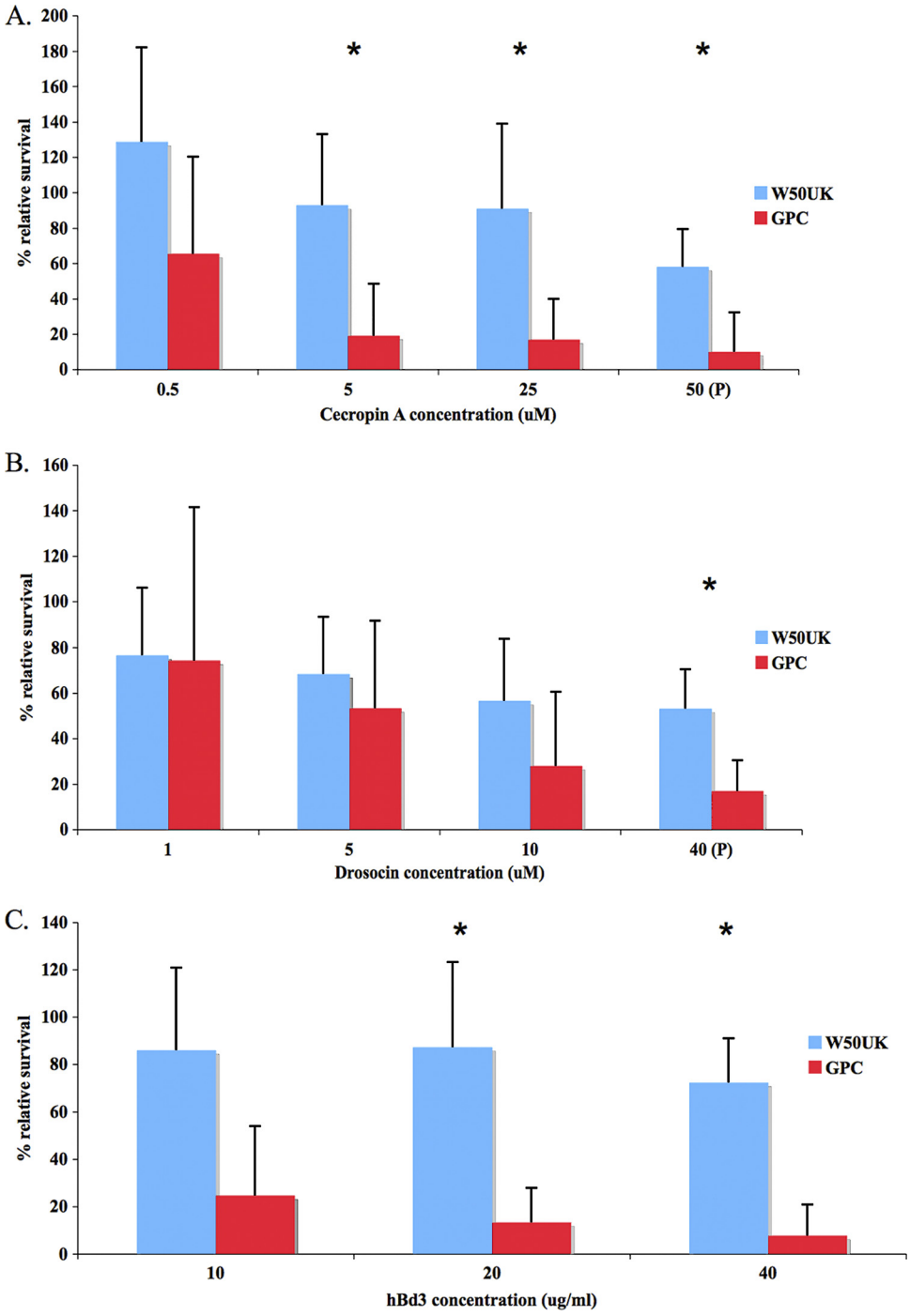

FIG. 3. Survival of *P. gingivalis* strains W50UK and GPC after exposure to insect and human antimicrobial peptides. P, physiological concentration (49, 60). Survival rates of bacteria after exposure to different concentrations of cecropin A (A), drosocin (B), or hβd3 (C) are shown. Each point represents the mean and standard deviation of eight samples from four independent experiments, and statistically significant ($P < 0.05$) survival differences between W50UK and GPC are indicated by asterisks.

detection by PGRP-LC. It is also possible that the *P. gingivalis* capsule protects the bacterium from the actions of Imd pathway-regulated immune effectors. For example, the capsule could act as a physical barrier to *Drosophila* AMPs, limiting their interaction with the *P. gingivalis* cell membrane and thereby protecting the bacteria from AMP-induced killing, while the absence of a capsule could render *P. gingivalis* more sensitive to killing by AMPs. The sensitivities of strains W50UK and GPC to killing by the Imd-regulated AMPs cecropin A and drosocin were therefore tested. Cecropins kill bacteria by permeabilizing the cell membrane, which causes lysis (69). Drosocin functions intracellularly, entering bacterial

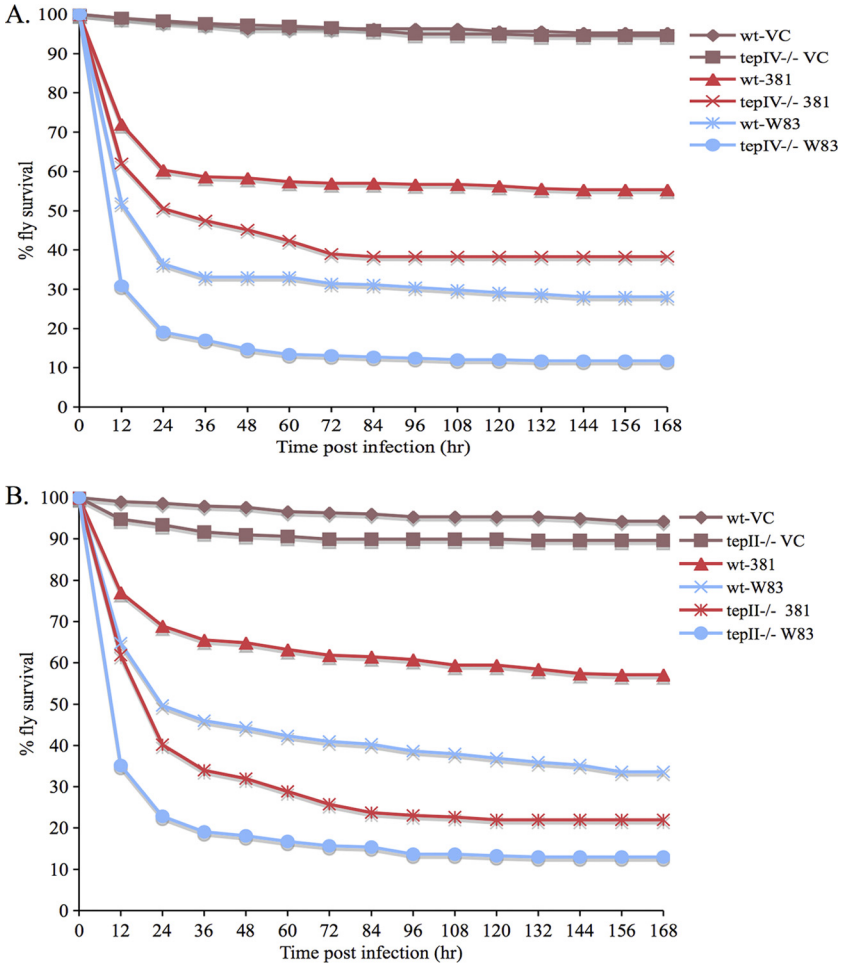

FIG. 4. Survival curves of wt, *tep II*$^{-/-}$ and *tep IV*$^{-/-}$ *Drosophila* flies infected with *P. gingivalis* strains W83 or 381. wt and *tep*$^{-/-}$ *Drosophila* flies were infected with strain W83, strain 381, or mock infected (VC). Labels are of the form *Drosophila* strain-infection agent. (A) Survival of wt and *tep II*$^{-/-}$ *Drosophila* flies; (B) survival of wt and *tep IV*$^{-/-}$ *Drosophila* flies.

cells via lipopolysaccharide-mediated entry and kills them by interfering with the actions of DnaK (58). Strain W50UK was more resistant than its capsule mutant GPC to killing by the antimicrobial peptides, demonstrating that the *P. gingivalis* capsule is involved in the resistance to killing by *Drosophila* Imd-regulated AMPs. These results are physiologically relevant, as the AMP concentrations that were tested are within the range reported to be present in *Drosophila* hemolymph (49, 61). The findings are also relevant to humans, as strain W50UK was also more resistant than GPC to killing by hβd3, which has the same mode of action as cecropin A. The involvement of the *P. gingivalis* capsule in mediating resistance to host AMPs is a novel finding; however, similar protective effects of bacterial capsules against host AMPs have been observed with *Klebsiella pneumoniae* (14) and *Neisseria meningitidis* (68). It is important to note that in addition to AMPs the Imd pathway activates multiple immune response genes and processes (an estimated 30% of *Drosophila* immune response genes are regulated by the pathway). Therefore, *P. gingivalis* capsule interaction with and nullification of Imd pathway-regulated responses are likely to be multifaceted. The Imd pathway is a major regulator of *Drosophila* immune response genes (18), and although it is activated in response to infection by encapsulated *P. gingivalis*, it is dispensable for the survival of the animals. Therefore, Imd activation by encapsulated *P. gingivalis* could be directly toxic and/or energetically wasteful to *Drosophila* and could contribute to the pathology induced by the bacterium in this model. Schneider et al. suggested that Eiger signaling induced upon infection by some intracellular bacteria, which does not help fight infection, results in lethality via mechanisms that may be directly toxic or energetically wasteful (66). As heat-killed *P. gingivalis* kills *Drosophila* as readily as live *P. gingivalis* (37), nullification of the Imd pathway by the *P. gingivalis* capsule likely involves mechanisms that do not require bacterial viability.

This study demonstrated that the C3/α_2M superfamily proteins Tep II and Tep IV are involved in the *Drosophila* immune response against *P. gingivalis* infection. Tep II appears to play a bigger role in the response to infection with strain 381 versus strain W83 (Fig. 4A). It has been suggested that Tep II functions as an α_2M and that alternative splicing (38) serves to increase the diversity of proteases that can be inhibited by this protein (11). At least one Tep II isoform and Tep IV have been demonstrated to function as opsonins in the phagocytosis of *E.*

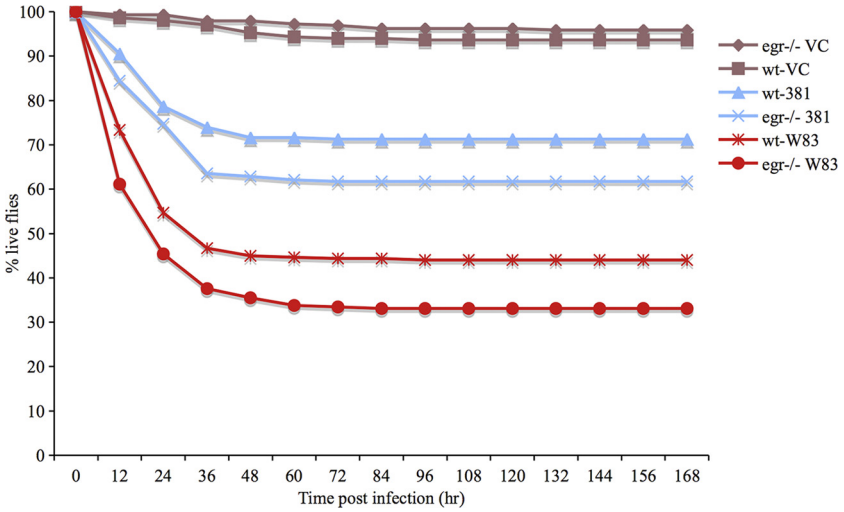

FIG. 5. Survival curves for wt and $egr^{-/-}$ *Drosophila* flies infected with *P. gingivalis* strain W83 or 381. wt and $egr^{-/-}$ *Drosophila* flies were infected with strain W83, strain 381, or mock infected (VC). Labels are of the form *Drosophila* strain-infection agent.

coli and *S. aureus*, respectively (70). It is likely that Tep IV functions as an opsonin in the response to *P. gingivalis* infection, while Tep II may function as both an opsonin and a protease inhibitor by virtue of its multiple isoforms. It has been demonstrated that purified human α_2M can inhibit the activity of *P. gingivalis* arginine-specific proteases *in vitro* (30, 59) and that C3 can bind to (15, 63) and promote the phagocytosis of (64) *P. gingivalis in vitro*. The results of this study provide strong evidence of an *in vivo* role for C3/α_2M family proteins in the host immune response against *P. gingivalis* infection.

This study demonstrated that Eiger (TNF homolog) is involved in the *Drosophila* immune response against *P. gingivalis* infection. Schneider et al. showed that Eiger fights infection by extracellular pathogens (66) and suggested that Eiger signaling increases the potency of *Drosophila* phagocytes against these microbes. Eiger also contributes to pathology during infections by some facultative intracellular pathogens (12, 66).

There are a number of transgenic mouse strains with defined immune response gene mutations that have been used to study *P. gingivalis*-host interactions (3, 7, 13, 33, 34, 62, 72, 74). However, the powerful genetics of *Drosophila* has made it more feasible to generate a large group of animals with defined mutations in immune response genes and other genes. The availability of a large number of *Drosophila* immune response mutants will facilitate large-scale screening to identify host components that play a role during *P. gingivalis* infection. Also, as the *Drosophila* immune response continues to be characterized, additional components and pathways that could potentially interact with the bacterium will be identified. Additionally, well-developed *Drosophila* genetic tools, e.g., microarray and RNA interference libraries are available for genome-wide analysis of *P. gingivalis-Drosophila* interactions. As with any animal model, the *Drosophila* killing model has its limitations. *Drosophila* is not a natural host for *P. gingivalis*, and due to the chronic, polymicrobial, multifactorial nature of adult periodontitis, the *Drosophila* killing model does not mimic the natural disease process. *P. gingivalis* interactions with the host's adaptive immune system cannot be studied in *Drosophila*, which lacks this system. While the *Drosophila* model cannot be used to identify all host components that are involved in the immune response to *P. gingivalis* infection, the results of this study clearly show that important host factors that are involved in the immune response to *P. gingivalis* infection can be identified and novel findings about the interaction between the bacterium and the host can be made using this model.

The results of this study demonstrate that *Drosophila melanogaster* is a powerful model system for characterizing the host response to *P. gingivalis* infection. We have identified several *Drosophila* immune system components that are important in the response to *P. gingivalis* infection. The interaction between *P. gingivalis* and *Drosophila* is clearly multifaceted, and there are likely additional host factors involved. Future studies involving genome-wide examination of the *Drosophila* response to *P. gingivalis* infection should provide new insights into the interaction between the bacterium and the host.

ACKNOWLEDGMENTS

We thank Amanda Simcox (Ohio State University) for helpful advice, discussions, demonstrations, and for the kindly supplied *Drosophila* husbandry materials. We thank Aaron Weinberg and Zhinmin Feng (Case Western Reserve University) for providing hβd3 and for helpful discussions. We thank Gregory Young (Ohio State University) for help with statistical analyses.

This study was supported by grant DE10467 from the National Institutes of Health.

REFERENCES

1. **Aduse-Opoku, J., J. M. Slaney, A. Hashim, A. Gallagher, R. P. Gallagher, M. Rangarajan, K. Boutaga, M. L. Laine, A. J. Van Winkelhoff, and M. A. Curtis.** 2006. Identification and characterization of the capsular polysaccharide (K-antigen) locus of Porphyromonas gingivalis. Infect. Immun. **74:**449–460.
2. **Agaisse, H., and N. Perrimon.** 2004. The roles of JAK/STAT signaling in Drosophila immune responses. Immunol. Rev. **198:**72–82.
3. **Alayan, J., S. Ivanovski, E. Gemmell, P. Ford, S. Hamlet, and C. S. Farah.** 2006. Deficiency of iNOS contributes to Porphyromonas gingivalis-induced tissue damage. Oral Microbiol. Immunol. **21:**360–365.
4. **Apidianakis, Y., M. N. Mindrinos, W. Xiao, G. W. Lau, R. L. Baldini, R. W. Davis, and L. G. Rahme.** 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.

5. **Apidianakis, Y., L. G. Rahme, J. Heitman, F. M. Ausubel, S. B. Calderwood, and E. Mylonakis.** 2004. Challenge of Drosophila melanogaster with Cryptococcus neoformans and role of the innate immune response. Eukaryot. Cell **3:**413–419.
6. **Baker, P. J., R. T. Evans, and D. C. Roopenian.** 1994. Oral infection with Porphyromonas gingivalis and induced alveolar bone loss in immunocompetent and severe combined immunodeficient mice. Arch. Oral Biol. **39:**1035–1040.
7. **Baker, P. J., L. Howe, J. Garneau, and D. C. Roopenian.** 2002. T cell knockout mice have diminished alveolar bone loss after oral infection with Porphyromonas gingivalis. FEMS Immunol. Med. Microbiol. **34:**45–50.
8. **Bellen, H. J., R. W. Levis, G. Liao, Y. He, J. W. Carlson, G. Tsang, M. Evans-Holm, P. R. Hiesinger, K. L. Schulze, G. M. Rubin, R. A. Hoskins, and A. C. Spradling.** 2004. The BDGP gene disruption project: single transposon insertions associated with 40% of Drosophila genes. Genetics **167:**761–781.
9. **Bellen, H. J., C. J. O'Kane, C. Wilson, U. Grossniklaus, R. K. Pearson, and W. J. Gehring.** 1989. P-element-mediated enhancer detection: a versatile method to study development in Drosophila. Genes Dev. **3:**1288–1300.
10. **Bello, B., D. Resendez-Perez, and W. J. Gehring.** 1998. Spatial and temporal targeting of gene expression in Drosophila by means of a tetracycline-dependent transactivator system. Development **125:**2193–2202.
11. **Blandin, S., and E. A. Levashina.** 2004. Thioester-containing proteins and insect immunity. Mol. Immunol. **40:**903–908.
12. **Brandt, S. M., M. S. Dionne, R. S. Khush, L. N. Pham, T. J. Vigdal, and D. S. Schneider.** 2004. Secreted bacterial effectors and host-produced Eiger/TNF drive death in a Salmonella-infected fruit fly. PLoS Biol. **2:**e418.
13. **Burns, E., G. Bachrach, L. Shapira, and G. Nussbaum.** 2006. Cutting edge. TLR2 is required for the innate response to Porphyromonas gingivalis: activation leads to bacterial persistence and TLR2 deficiency attenuates induced alveolar bone resorption. J. Immunol. **177:**8296–8300.
14. **Campos, M. A., M. A. Vargas, V. Regueiro, C. M. Llompart, S. Alberti, and J. A. Bengoechea.** 2004. Capsule polysaccharide mediates bacterial resistance to antimicrobial peptides. Infect. Immun. **72:**7107–7114.
15. **Cutler, C. W., R. R. Arnold, and H. A. Schenkein.** 1993. Inhibition of C3 and IgG proteolysis enhances phagocytosis of Porphyromonas gingivalis. J. Immunol. **151:**7016–7029.
16. **D'Argenio, D. A., L. A. Gallagher, C. A. Berg, and C. Manoil.** 2001. Drosophila as a model host for Pseudomonas aeruginosa infection. J. Bacteriol. **183:**1466–1471.
17. **De Gregorio, E., P. T. Spellman, G. M. Rubin, and B. Lemaitre.** 2001. Genome-wide analysis of the Drosophila immune response by using oligonucleotide microarrays. Proc. Natl. Acad. Sci. U. S. A. **98:**12590–12595.
18. **De Gregorio, E., P. T. Spellman, P. Tzou, G. M. Rubin, and B. Lemaitre.** 2002. The Toll and Imd pathways are the major regulators of the immune response in Drosophila. EMBO J. **21:**2568–2579.
19. **Dionne, M. S., N. Ghori, and D. S. Schneider.** 2003. Drosophila melanogaster is a genetically tractable model host for Mycobacterium marinum. Infect. Immun. **71:**3540–3550.
20. **Eke, P. I., V. O. Rotimi, and B. E. Laughon.** 1996. Experimental model for Porphyromonas gingivalis infection in animals. Afr. J. Med. Med. Sci. **25:**31–39.
21. **Elwell, C., and J. N. Engel.** 2005. Drosophila melanogaster S2 cells: a model system to study Chlamydia interaction with host cells. Cell. Microbiol. **7:**725–739.
22. **Evans, R. T., B. Klausen, N. S. Ramamurthy, L. M. Golub, C. Sfintescu, and R. J. Genco.** 1992. Periodontopathic potential of two strains of Porphyromonas gingivalis in gnotobiotic rats. Arch. Oral Biol. **37:**813–819.
23. **Ezzo, P. J., and C. W. Cutler.** 2003. Microorganisms as risk indicators for periodontal disease. Periodontol. 2000 **32:**24–35.
24. **Franc, N. C., J. L. Dimarcq, M. Lagueux, J. Hoffmann, and R. A. Ezekowitz.** 1996. Croquemort, a novel Drosophila hemocyte/macrophage receptor that recognizes apoptotic cells. Immunity **4:**431–443.
25. **Genco, C. A., C. W. Cutler, D. Kapczynski, K. Maloney, and R. R. Arnold.** 1991. A novel mouse model to study the virulence of and host response to Porphyromonas (Bacteroides) gingivalis. Infect. Immun. **59:**1255–1263.
26. **Gibson, F. C., III, and C. A. Genco.** 2001. Prevention of Porphyromonas gingivalis-induced oral bone loss following immunization with gingipain R1. Infect. Immun. **69:**7959–7963.
27. **Gottar, M., V. Gobert, T. Michel, M. Belvin, G. Duyk, J. A. Hoffmann, D. Ferrandon, and J. Royet.** 2002. The Drosophila immune response against Gram-negative bacteria is mediated by a peptidoglycan recognition protein. Nature **416:**640–644.
28. **Grenier, D., and D. Mayrand.** 1987. Selected characteristics of pathogenic and nonpathogenic strains of Bacteroides gingivalis. J. Clin. Microbiol. **25:**738–740.
29. **Griffen, A. L., M. R. Becker, S. R. Lyons, M. L. Moeschberger, and E. J. Leys.** 1998. Prevalence of Porphyromonas gingivalis and periodontal health status. J. Clin. Microbiol. **36:**3239–3242.
30. **Gron, H., R. Pike, J. Potempa, J. Travis, I. B. Thogersen, J. J. Enghild, and S. V. Pizzo.** 1997. The potential role of alpha 2-macroglobulin in the control of cysteine proteinases (gingipains) from Porphyromonas gingivalis. J. Periodontal Res. **32:**61–68.
31. **Gyurko, R., G. Boustany, P. L. Huang, A. Kantarci, T. E. Van Dyke, C. A. Genco, and F. C. Gibson III.** 2003. Mice lacking inducible nitric oxide synthase demonstrate impaired killing of Porphyromonas gingivalis. Infect. Immun. **71:**4917–4924.
32. **Hamada, N., K. Watanabe, T. Tahara, K. Nakazawa, I. Ishida, Y. Shibata, T. Kobayashi, H. Yoshie, Y. Abiko, and T. Umemoto.** 2007. The r40-kDa outer membrane protein human monoclonal antibody protects against Porphyromonas gingivalis-induced bone loss in rats. J. Periodontol. **78:**933–939.
33. **Holzhausen, M., L. C. Spolidorio, R. P. Ellen, M. C. Jobin, M. Steinhoff, P. Andrade-Gordon, and N. Vergnolle.** 2006. Protease-activated receptor-2 activation: a major role in the pathogenesis of Porphyromonas gingivalis infection. Am. J. Pathol. **168:**1189–1199.
34. **Houri-Haddad, Y., W. A. Soskolne, E. Shai, A. Palmon, and L. Shapira.** 2002. Interferon-gamma deficiency attenuates local P. gingivalis-induced inflammation. J. Dent. Res. **81:**395–398.
35. **Huang, J. H., Y. Y. Lin, Y. Y. Lai, and S. W. Hu.** 2006. Lethal outcome caused by Porphyromonas gingivalis A7436 in a mouse chamber model is associated with elevated titers of host serum interferon-gamma. Oral Microbiol. Immunol. **21:**100–106.
36. **Igaki, T., H. Kanda, Y. Yamamoto-Goto, H. Kanuka, E. Kuranaga, T. Aigaki, and M. Miura.** 2002. Eiger, a TNF superfamily ligand that triggers the Drosophila JNK pathway. EMBO J. **21:**3009–3018.
37. **Igboin, C. O., M. L. Moeschberger, A. L. Griffen, and E. J. Leys.** 2011. *Porphyromonas gingivalis* virulence in a *Drosophila melanogaster* model. Infect. Immun. **79:**439–448.
38. **Jiggins, F. M., and K. W. Kim.** 2006. Contrasting evolutionary patterns in Drosophila immune receptors. J. Mol. Evol. **63:**769–780.
39. **Kaneko, T., and N. Silverman.** 2005. Bacterial recognition and signalling by the Drosophila IMD pathway. Cell. Microbiol. **7:**461–469.
40. **Kastelein, P., T. J. van Steenbergen, J. M. Bras, and J. de Graaff.** 1981. An experimentally induced phlegmonous abscess by a strain of Bacteroides gingivalis in guinea pigs and mice. Antonie Van Leeuwenhoek **47:**1–9.
41. **Katz, J., D. C. Ward, and S. M. Michalek.** 1996. Effect of host responses on the pathogenicity of strains of Porphyromonas gingivalis. Oral Microbiol. Immunol. **11:**309–318.
42. **Kauppila, S., W. S. Maaty, P. Chen, R. S. Tomar, M. T. Eby, J. Chapo, S. Chew, N. Rathore, S. Zachariah, S. K. Sinha, J. M. Abrams, and P. M. Chaudhary.** 2003. Eiger and its receptor, Wengen, comprise a TNF-like system in Drosophila. Oncogene **22:**4860–4867.
43. **Kesavalu, L., S. C. Holt, and J. L. Ebersole.** 1996. Trypsin-like protease activity of Porphyromonas gingivalis as a potential virulence factor in a murine lesion model. Microb. Pathog. **20:**1–10.
44. **Klausen, B.** 1991. Microbiological and immunological aspects of experimental periodontal disease in rats: a review article. J. Periodontol. **62:**59–73.
45. **Kocks, C., J. H. Cho, N. Nehme, J. Ulvila, A. M. Pearson, M. Meister, C. Strom, S. L. Conto, C. Hetru, L. M. Stuart, T. Stehle, J. A. Hoffmann, J. M. Reichhart, D. Ferrandon, M. Ramet, and R. A. Ezekowitz.** 2005. Eater, a transmembrane protein mediating phagocytosis of bacterial pathogens in Drosophila. Cell **123:**335–346.
46. **Kumagai, Y., K. Konishi, T. Gomi, H. Yagishita, A. Yajima, and M. Yoshikawa.** 2000. Enzymatic properties of dipeptidyl aminopeptidase IV produced by the periodontal pathogen Porphyromonas gingivalis and its participation in virulence. Infect. Immun. **68:**716–724.
47. **Lagueux, M., E. Perrodou, E. A. Levashina, M. Capovilla, and J. A. Hoffmann.** 2000. Constitutive expression of a complement-like protein in toll and JAK gain-of-function mutants of Drosophila. Proc. Natl. Acad. Sci. U. S. A. **97:**11427–11432.
48. **Laine, M. L., and A. J. van Winkelhoff.** 1998. Virulence of six capsular serotypes of Porphyromonas gingivalis in a mouse model. Oral Microbiol. Immunol. **13:**322–325.
49. **Lemaitre, B., and J. Hoffmann.** 2007. The host defense of Drosophila melanogaster. Annu. Rev. Immunol. **25:**697–743.
50. **Lemaitre, L.** 2007. Drosophila genes potentially involved in responses to microbial infection. http://www.cgn.cnrs-gif.fr/immunity/drosophila_immunity_genes.html.
51. **Leys, E. J., J. H. Smith, S. R. Lyons, and A. L. Griffen.** 1999. Identification of Porphyromonas gingivalis strains by heteroduplex analysis and detection of multiple strains. J. Clin. Microbiol. **37:**3906–3911.
52. **Malek, R., J. G. Fisher, A. Caleca, M. Stinson, C. J. van Oss, J. Y. Lee, M. I. Cho, R. J. Genco, R. T. Evans, and D. W. Dyer.** 1994. Inactivation of the Porphyromonas gingivalis fimA gene blocks periodontal damage in gnotobiotic rats. J. Bacteriol. **176:**1052–1059.
53. **Mansfield, B. E., M. S. Dionne, D. S. Schneider, and N. E. Freitag.** 2003. Exploration of host-pathogen interactions using Listeria monocytogenes and Drosophila melanogaster. Cell. Microbiol. **5:**901–911.
54. **Moreno, E., M. Yan, and K. Basler.** 2002. Evolution of TNF signaling mechanisms: JNK-dependent apoptosis triggered by Eiger, the Drosophila homolog of the TNF superfamily. Curr. Biol. **12:**1263–1268.
55. **Nehme, N. T., S. Liegeois, B. Kele, P. Giammarinaro, E. Pradel, J. A. Hoffmann, J. J. Ewbank, and D. Ferrandon.** 2007. A model of bacterial intestinal infections in Drosophila melanogaster. PLoS Pathog. **3:**e173.

56. **O'Brien-Simpson, N. M., R. A. Paolini, B. Hoffmann, N. Slakeski, S. G. Dashper, and E. C. Reynolds.** 2001. Role of RgpA, RgpB, and Kgp proteinases in virulence of Porphyromonas gingivalis W50 in a murine lesion model. Infect. Immun. **69:**7527–7534.
57. **O'Brien-Simpson, N. M., R. D. Pathirana, R. A. Paolini, Y. Y. Chen, P. D. Veith, V. Tam, N. Ally, R. N. Pike, and E. C. Reynolds.** 2005. An immune response directed to proteinase and adhesin functional epitopes protects against Porphyromonas gingivalis-induced periodontal bone loss. J. Immunol. **175:**3980–3989.
58. **Otvos, L., Jr., I. O., M. E. Rogers, P. J. Consolvo, B. A. Condie, S. Lovas, P. Bulet, and M. Blaszczyk-Thurin.** 2000. Interaction between heat shock proteins and antimicrobial peptides. Biochemistry **39:**14150–14159.
59. **Rangarajan, M., M. A. Scragg, and M. A. Curtis.** 2000. Bait region cleavage and complex formation of human α2M with a Porphyromonas gingivalis W50 protease is not accompanied by enzyme inhibition. Biol. Chem. **381:**57–65.
60. **Rutschmann, S., A. C. Jung, R. Zhou, N. Silverman, J. A. Hoffmann, and D. Ferrandon.** 2000. Role of Drosophila IKK gamma in a toll-independent antibacterial immune response. Nat. Immunol. **1:**342–347.
61. **Samakovlis, C., D. A. Kimbrell, P. Kylsten, A. Engstrom, and D. Hultmark.** 1990. The immune response in Drosophila: pattern of cecropin expression and biological activity. EMBO J. **9:**2969–2976.
62. **Sasaki, H., Y. Okamatsu, T. Kawai, R. Kent, M. Taubman, and P. Stashenko.** 2004. The interleukin-10 knockout mouse is highly susceptible to Porphyromonas gingivalis-induced alveolar bone loss. J. Periodontal Res. **39:**432–441.
63. **Schenkein, H. A.** 1989. Failure of Bacteroides gingivalis W83 to accumulate bound C3 following opsonization with serum. J. Periodontal Res. **24:**20–27.
64. **Schenkein, H. A., H. M. Fletcher, M. Bodnar, and F. L. Macrina.** 1995. Increased opsonization of a prtH-defective mutant of Porphyromonas gingivalis W83 is caused by reduced degradation of complement-derived opsonins. J. Immunol. **154:**5331–5337.
65. **Schneider, D., and M. Shahabuddin.** 2000. Malaria parasite development in a Drosophila model. Science **288:**2376–2379.
66. **Schneider, D. S., J. S. Ayres, S. M. Brandt, A. Costa, M. S. Dionne, M. D. Gordon, E. M. Mabery, M. G. Moule, L. N. Pham, and M. M. Shirasu-Hiza.** 2007. Drosophila Eiger mutants are sensitive to extracellular pathogens. PLoS Pathog. **3:**e41.
67. **Shi, X., S. A. Hanley, M. C. Faray-Kele, S. C. Fawell, J. Aduse-Opoku, R. A. Whiley, M. A. Curtis, and L. M. Hall.** 2007. The rag locus of Porphyromonas gingivalis contributes to virulence in a murine model of soft tissue destruction. Infect. Immun. **75:**2071–2074.
68. **Spinosa, M. R., C. Progida, A. Tala, L. Cogli, P. Alifano, and C. Bucci.** 2007. The Neisseria meningitidis capsule is important for intracellular survival in human cells. Infect. Immun. **75:**3594–3603.
69. **Steiner, H., D. Andreu, and R. B. Merrifield.** 1988. Binding and action of cecropin and cecropin analogues: antibacterial peptides from insects. Biochim. Biophys. Acta **939:**260–266.
70. **Stroschein-Stevenson, S. L., E. Foley, P. H. O'Farrell, and A. D. Johnson.** 2006. Identification of Drosophila gene products required for phagocytosis of Candida albicans. PLoS Biol. **4:**e4.
71. **Stuart, L. M., J. Deng, J. M. Silver, K. Takahashi, A. A. Tseng, E. J. Hennessy, R. A. Ezekowitz, and K. J. Moore.** 2005. Response to Staphylococcus aureus requires CD36-mediated phagocytosis triggered by the COOH-terminal cytoplasmic domain. J. Cell Biol. **170:**477–485.
72. **Tsukuba, T., S. Yamamoto, M. Yanagawa, K. Okamoto, Y. Okamoto, K. I. Nakayama, T. Kadowaki, and K. Yamamoto.** 2006. Cathepsin E-deficient mice show increased susceptibility to bacterial infection associated with the decreased expression of multiple cell surface Toll-like receptors. J. Biochem. **140:**57–66.
73. **Yoshimura, M., Y. Nakano, Y. Yamashita, T. Oho, T. Saito, and T. Koga.** 2000. Formation of methyl mercaptan from L-methionine by Porphyromonas gingivalis. Infect. Immun. **68:**6912–6916.
74. **Yu, J. J., M. J. Ruddy, G. C. Wong, C. Sfintescu, P. J. Baker, J. B. Smith, R. T. Evans, and S. L. Gaffen.** 2007. An essential role for IL-17 in preventing pathogen-initiated bone destruction: recruitment of neutrophils to inflamed bone requires IL-17 receptor-dependent signals. Blood **109:**3794–3802.

Editor: R. P. Morrison