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A negative role for *MyD88* in the resistance to starvation as revealed in an intestinal infection of *Drosophila melanogaster* with the Gram-positive bacterium *Staphylococcus xylosus*

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ABSTRACT

Drosophila melanogaster is a useful model to investigate mucosal immunity. The immune response to intestinal infections is mediated partly by the Immune deficiency (IMD) pathway, which only gets activated by a type of peptidoglycan lacking in several medically important Gram-positive bacterial species such as *Staphylococcus*. Thus, the intestinal host defense against such bacterial strains remains poorly known. Here, we have used *Staphylococcus xylosus* to develop a model of intestinal infections by Gram-positive bacteria. *S. xylosus* behaves as an opportunistic pathogen in a septic injury model, being able to kill only flies immunodeficient either for the Toll pathway or the cellular response. When ingested, it is controlled by IMD-independent host intestinal defenses, yet flies eventually die. Having excluded an overreaction of the immune response and the action of toxins, we find that flies actually succumb to starvation, likely as a result of a competition for sucrose between the bacteria and the flies. Fat stores of wild-type flies are severely reduced within a day, a period when sucrose is not yet exhausted in the feeding solution. Interestingly, the Toll pathway mutant *MyD88* is more resistant to the ingestion of *S. xylosus* and to starvation than wild-type flies. *MyD88* flies do not rapidly deplete their fat stores when starved, in contrast to wild-type flies. Thus, we have uncovered a novel function of *MyD88* in the regulation of metabolism that appears to be independent of its known roles in immunity and development.

Introduction

Drosophila melanogaster feeds on decaying fruits and vegetables and thus lives in a microbe-rich environment. As a result of constant interactions with its septic environment, *Drosophila* has evolved a sophisticated host defense that generally allows it to contain potentially hazardous microorganisms. The phagocytosis of microbes by circulating hemocytes and the secretion of antimicrobial peptides (AMPs), respectively the cellular and humoral immune responses, constitute the major *Drosophila* defense mechanisms against infections (Ferrandon et al. 2007; Lemaitre and Hoffmann 2007). AMPs are either secreted systemically or can be produced locally by contact epithelia (Akhouayri et al. 2011; Ferrandon et al. 1998; Han et al. 2004; Onfelt Tingvall et al. 2001; Tzou et al. 2000).

Following a septic injury, AMPs are secreted by the fat body, a functional analog of the mammalian liver, into the fly hemolymph. Microbes are either recognized through their structural components or alternatively by the enzymatic activity of microbial virulence factors (Gottar et al. 2006). On the basis of differences in the chemical properties of microbial structural compounds, *Drosophila* is able to distinguish between different categories of microbes and, to some extent, activate the relevant antimicrobial response (reviewed in Ferrandon et al. 2007). Gram-negative bacteria, for instance, are recognized by the pattern recognition receptors (PRRs), Peptidoglycan recognition protein-LC (PGRP-LC) and PGRP-LE. These receptors sense Gram-negative bacteria and Gram-positive bacilli through their meso-diaminopimelic acid-containing peptidoglycans (DAP-type PGNs) and subsequently activate the Immune deficiency (IMD) pathway, which ultimately leads to the nuclear localization of Relish, a NF-κB family transcription factor. Nuclear Relish transcribes AMP genes such as *Diptericin*, *Drosocin*, *Attacins*, and *Cecropins* that are active against this

Abbreviations: AMP, antimicrobial peptides; CGD, chronic granulomatous disease; DIF, Dorsal-related immunity factor; FOXO, forkhead box O; GNBP3, Gram-negative protein 3; IMD, Immune deficiency; ISC, intestinal stem cell; IRC, Immune-regulated catalase; JAK/STAT, Janus kinase/Signal transducer and activator of transcription; LT50, median lethal time; NADPH, nicotinamide adenine dinucleotide phosphate; PFA, paraformaldehyde; PGN, peptidoglycan; PGRP, Peptidoglycan recognition protein; ROS, reactive oxygen species; PRR, pattern recognition receptor; SPZ, Spätzle; TLR, Toll like receptor; UV, ultra violet.

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category of microbes. Fungi and some Gram-positive bacteria are sensed *via* their β-(1,3)glucans or *via* their lysine type peptidoglycans (Lys-type PGNs). This recognition event involves respectively Gram-negative protein 3 (GNBP3) for fungi and a complex comprising the secreted proteins PGRP-SA, PGRP-SD, and GNBP1 for the Gram-positive bacteria. The detection of these microbes activates proteolytic cascades that ultimately lead to the cleavage of the Spätzle cytokine (SPZ) into a ligand of the transmembrane Toll receptor. Toll activation triggers the nuclear localization of DIF in a DmelMyD88-dependent manner. The NF-κB transcription factor DIF in turn transcribes AMP genes encoding antifungal peptides such as Drosomycin and Metchnikowin. Interestingly, the only AMP active against Gram-positive bacteria is Defensin, the expression of which can be induced by IMD and Toll pathway activation (Dimarcq et al. 1994; Imler and Bulet 2005; Nehme et al. 2011). The Toll pathway is required in the host defense against some Gram-positive bacterial and fungal infections. Additionally, it plays a role in development, as it is required maternally for setting up the embryonic dorso-ventral axes. Indeed, most Toll pathway mutants are sterile.

The *Drosophila* gut is equipped with physical and chemical barriers that contain microbes within the digestive tract. The peritrophic matrix is the first line of defense restricting the microbes to the lumen and prevents their direct contact with epithelial cells (Kuraishi et al. 2011). It thus fulfills a function analogous to that of mucus in vertebrates. AMPs are also secreted by the epithelial cells. However, this local AMPs secretion is IMD pathway-, and not *Toll* pathway-dependent (Liehl et al. 2006; Nehme et al. 2007; Ryu et al. 2006). A finely regulated induction of reactive oxygen species (ROS) is also triggered against ingested microorganisms (Bae et al. 2010; Ha et al. 2005a,b). In addition to its resistance to microbes, *Drosophila* has developed endurance mechanisms to withstand and repair the damages caused by pathogenic bacteria. Gut homeostasis, in this case, is maintained by the compensatory proliferation of intestinal stem cell (ISC) (Biteau and Jasper 2011; Buchon et al. 2009a,b, 2010; Cronin et al. 2009; Jiang et al. 2009, 2011). Most of these studies, however, have been performed using Gram-negative bacterial species.

The human intestine harbors hundreds of bacterial species (Qin et al. 2010). Any change in balanced interactions between intestinal microbes and the host immune system can lead to inflammatory disorders (Chassaing and Darfeuille-Michaud 2011; Wells et al. 2011). Moreover, Firmicutes, a phylum that mostly consists of Gram-positive bacteria, is a major microbial population inhabiting the human intestine. In mammals, many physiological complications like obesity (Kallus and Brandt 2012), insulin resistance (De Bandt et al. 2011), and Toll like receptors (TLRs)-mediated inflammation have been found to be associated with an abnormal proportion of Firmicutes (Caricilli et al. 2012; Serino et al. 2012). The *Drosophila* microbiota is made up of only 5–30 bacterial species. Interestingly, the microbiota is mainly dominated by Firmicutes, such as *Enterococcus faecalis* and *Lactobacillus plantarum*, and Proteobacter like *Acetobacter pomorum* (Cox and Gilmore 2007; Ren et al. 2007; Roh et al. 2008; Shin et al. 2011; Storelli et al. 2011; Wong et al. 2011).

Staphylococcus xylosus is a Lys-type PGN containing Gram-positive bacterium that belongs to the phylum Firmicutes. It is a commensal of mucus and skin in mammals (Hariharan et al. 2011; Kloos et al. 1976; Kloos and Schleifer 1986; Nagase et al. 2002). *S. xylosus* can be found in various niches like polluted water (Kessie et al. 1998), animal fodder and grains (Pioch et al. 1988), soil and various surfaces (Shale et al. 2005). It can form biofilms (Planchon et al. 2006; Planchon et al. 2009) and can adapt to various environmental conditions. *S. xylosus* is a natural component of raw meat and milk. It is used as a starter medium in the meat and milk fermentation industry (Kloos and Schleifer 1986; Talon et al. 2002). Moreover, the zinc-dependent metallolipase produced by *S. xylosus* is extensively used by the biotransformation industry (Bertoldo et al. 2011).

S. xylosus is normally considered to be a nonpathogenic *Staphylococcus* but some strains are opportunistic in humans and animals (Bingel 2002; Bradfield et al. 1993; Fthenakis et al. 1994; Jackson et al. 2001; Miedzobrodzki et al. 1989). In humans *S. xylosus* has been found associated with endocarditis (Conrad and West 1984), septicemia (Koksal et al. 2009), acute pyelonephritis (Tselenis-Kotsowilis et al. 1982) and chronic granulomatous disease (CGD) (Gozalo et al. 2010). CGD is caused by genetic disorders in humans that affect one component of NADPH oxidase and lead to recurrent bacterial and fungal infections (Roos et al. 2007). Indeed, *S. xylosus* was reported to be the major cause of death for mice deficient in NADPH oxidase (Gozalo et al. 2010). Genetic variation observed between 24 different strains of *S. xylosus* divided them into two distinct groups based on their potential to become opportunistic pathogens (Dordet-Frisoni et al. 2007a).

Our knowledge about the *Drosophila* gut defense responses against Lys-type PGN Gram-positive bacteria is very limited. Indeed, the major AMP response described in the intestinal epithelium is controlled by the IMD pathway, which cannot be activated by these bacteria. We therefore used a *S. xylosus* strain Argentoratum originally isolated from microsporidia-infected fly stocks found in our laboratory in Strasbourg. In this work, we find that *S. xylosus* behaves as a classical Gram-positive bacterium upon septic injury. Second, we show that flies fed on a *S. xylosus* containing solution succumb faster than uninfected controls. We then report that the Toll pathway mutant *MyD88* were more resistant to an oral challenge with *S. xylosus* as compared to wild-type flies and to starvation. Our data suggest a potential link between innate immune genes and lipid metabolism.

Materials and methods

Fly strains

Flies were reared at 25 °C on standard corneal-agar medium. *DD1 cn bw* flies (DD1 is a X chromosome carrying both *pDipt-LacZ* and a *pDrom-GFP* reporter transgene (Jung et al. 2001)) were used as wild-type control for *Dif* mutants flies (Rutschmann et al. 2000a) while A5001 were wild-type controls for $MyD88^{c03881}$ (Tauszig-Delamasure et al. 2002), *key* (Rutschmann et al. 2000b), and rescue strains with a UAS-MyD88^{+} transgene (Tauszig-Delamasure et al. 2002). The $MyD88^{kra[56]}$ allele (Charatsi et al. 2003) was used as well as a deletion that removes the *MyD88* locus, Df(2R)wun^{GL}. The NP1-Gal4 driver line was obtained from DGRC, Japan. All crosses to generate transgenic rescue fly lines were performed at 25 °C.

Bacterial strains and growth conditions

S. xylosus strain named Argentoratum was isolated from moribund Oregon flies at UPR9022, IBMC. The flies were later found to be infected by microsporidia (*Tubulinosema ratisbonensis* (Niehus et al. 2012)) as well. Another *S. xylosus* strain C2a is described in (Dordet-Frisoni et al. 2007a,b). Colonies naturally resistant to streptomycin (100 μg/mL) were selected to establish stock in 20% glycerol stored at −80 °C. Before infection bacteria were grown at 37 °C overnight in LB containing Streptomycin (100 μg/mL).

Pricking assay

Bacteria were pelleted to the equivalent of an optical density of about 200 at 600 nm (OD_{600}) from an overnight culture grown at 37 °C in Luria–Bertani broth (LB). A tungsten needle was either directly dipped in this pellet or the bacteria were first diluted to an optical density of 6 before challenging flies (20 flies/survival