CHAPTER TWO

Drosophila as a Model for Human Diseases—Focus on Innate Immunity in Barrier Epithelia

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Abstract

Epithelial immunity protects the host from harmful microbial invaders but also controls the beneficial microbiota on epithelial surfaces. When this delicate balance between pathogen and symbiont is disturbed, clinical disease often occurs, such as in inflammatory bowel disease, cystic fibrosis, or atopic dermatitis, which all can be in part linked to impairment of barrier epithelia. Many innate immune receptors, signaling pathways, and effector molecules are evolutionarily conserved between human and *Drosophila*. This review describes the current knowledge on *Drosophila* as a model for human diseases, with a special focus on innate immune-related disorders of the gut, lung, and skin.

The discovery of antimicrobial peptides, the crucial role of Toll and Toll-like receptors, and the evolutionary conservation of signaling to the immune systems of both human and *Drosophila* are described in a historical perspective. Similarities and differences between human and *Drosophila* are discussed; current knowledge on receptors, signaling pathways, and effectors are reviewed, including antimicrobial peptides, reactive oxygen species, as well as autophagy. We also give examples of human diseases for which *Drosophila* appears to be a useful model. In addition, the limitations of the *Drosophila* model are mentioned. Finally, we propose areas for future research, which include using the *Drosophila* model for drug screening, as a validation tool for novel genetic mutations in humans and for exploratory research of microbiota–host interactions, with relevance for infection, wound healing, and cancer.

1. INTRODUCTION

The fruit fly *Drosophila melanogaster* has been used as a research model for over a century. *Drosophila* was one of the first multicellular organisms to have its genome sequenced and well annotated (Adams et al., 2000). Releasing the human genome sequence a few years later revealed that 75% of disease-related genes in human have functional orthologs in flies (Lloyd & Taylor, 2010; Reiter, Potocki, Chien, Gribskov, & Bier, 2001). This strengthened the role of *Drosophila* as a model to study biological processes with relation to human diseases.

Much of today's general knowledge about innate immunity has developed from research that was initially carried out in *Drosophila* and other

insects. The basis for this successful approach of using an invertebrate model for studies of human immune responses is motivated by the similarity and evolutionary conservation of fundamental aspects of the underlying processes. This can be exemplified with the well-conserved signaling pathways that regulate innate immune responses, gut epithelium regeneration, and wound healing. Also, cellular immune responses, such as phagocytosis and autophagy, are evolutionarily conserved; bactericidal and fungal effector mechanisms, such as production of antimicrobial peptides (AMPs) and reactive oxygen species (ROS), are shared, as well as some antiviral responses (Lamiable & Imler, 2014). The fact that insects lack an adaptive immune system in the form it is present in vertebrates has simplified the dissection of innate immunity per se by genetic and molecular analyses. Furthermore, humans and flies both have a commensal microbial flora and can be infected partly by the same pathogenic bacteria, fungi, and viruses, and both are hosts for protozoa and nematode infections. This has not only enabled discoveries of many crucial components of innate immune responses against these pathogens but also disclosed *Drosophila* as a useful model for human diseases, where host–microbe interaction plays an important role, such as intestinal inflammation and tumorigenesis. In addition, the *Drosophila* model has been used for unraveling microbial pathogenesis and virulence mechanisms of various microbes in gut, lung, and skin. More recently, the fly is being used as a primary or complementary whole-animal target for chemical drug screening to discover novel antibiotics. For this, the large number of flies that easily can be tested in high-throughput screens make it a cost-effective and logistic choice, not the least for incorporating the replacement, reduction, and refinement (3R) principles of alternatives in vertebrate animal drug testing regimes.

The genetic and molecular tool box for *Drosophila* is excellent. In addition to large collections of well-characterized mutants, a plethora of genetically modified flies has been engineered, which enables detailed manipulation of gene activity both temporally and spatially. This provides great possibilities for functional analysis of genes and pathways that have been linked to a human disease but where the molecular mechanism is unknown. One of the major advantages with a genetic model such as *Drosophila* is that well-planned genetic screens almost always give unexpected and unbiased results, which in essence is a hallmark of new discoveries. While the human genome usually carries multiple gene copies for regulatory proteins, the fly genome typically contains single genes, meaning less redundancy and more straightforward genetic analyses of gene function in

genetically modified flies. For studies of host–microbe interaction and immunity, whole animal responses can easily be followed in flies by a multitude of measures of tolerance, resistance, or fatal outcome of the interactions (Ayres & Schneider, 2012). In addition, it is simple to create germ-free flies, or flies with mono-association of specific commensal or pathogenic microbes.

For which biological questions or human immune-related diseases is the *Drosophila* model a less appropriate choice? First, for diseases where adaptive immunity is dominating in humans, the fly cannot give a complete picture and some questions may not even be realistic to address. This includes diseases with strong B-cell responses, such as rheumatoid arthritis and antibody-dependent diseases; T-cell responses, such as autoimmune diseases and multiple sclerosis, and immunological disorders of T- and NK-cells. That said, research in the fly has often provided completely unexpected insight into immune processes that initially have been considered to be vertebrate specific. Second, many viruses are highly host specific and cannot infect *Drosophila* without prior manipulation, while infection with insect viruses can be used for answering general questions on innate antiviral responses. Third, some human pathogens use the human body temperature of 37°C in combination with serum factors to trigger expression of virulence factors. Regular *Drosophila* husbandry uses temperatures of 18–29°C, while 37°C for longer periods is lethal. Thus, infection with such pathogens will only cause harmless interactions in the fly. A fourth point is that host–microbe interactions in the gastrointestinal tract of mammals involve obligate anaerobic bacteria, and those are not found in the fly gut.

In the first part of this review, we will describe the systemic innate immune response in *Drosophila* in a historical perspective, as the discoveries made in *Drosophila* were crucial for today's understanding of innate immunity in humans, and paved the way for the important characterization of Toll-like receptors (TLRs) in mammals. We will also highlight other conserved signaling pathways involved in different aspects of the immune response. The second part will focus on *Drosophila* as a disease model to study host–microbe interaction and innate immunity in epithelial tissues, with a focus on bacterial and fungal infections in gut, lung/trachea, and skin/epidermis. Finally, we will emphasize for which human diseases *Drosophila* already has been used or could be a good model to answer fundamental questions on disease mechanisms, with possible impact for prevention and treatment in humans in the future.

2. EVOLUTIONARY CONSERVATION OF INNATE IMMUNITY

2.1 The Innate Immune System of *Drosophila*

The immune system of *Drosophila* is multifaceted and involves many cellular and humoral processes, which show high similarity or direct evolutionary relationships with the ones observed in humans. While all cannot be covered here, we refer to broad general reviews (Buchon, Silverman, & Cherry, 2014; Lemaitre & Hoffmann, 2007) and to more specialized reviews describing responses to viral infections (Lamiable & Imler, 2014), cellular immunity including phagocytosis (Honti, Csordas, Kurucz, Markus, & Ando, 2014; Parsons & Foley, 2015); hemocyte development, with parallels to the two myeloid systems in vertebrates (Gold & Bruckner, 2014), and coagulation and clotting systems (Theopold, Krautz, & Dushay, 2014). In addition to these well-conserved immune system processes, *Drosophila* and other insects mount a strong melanization reaction upon wounding or infection that is not found in mammals (Tang, 2009); as well as encapsulation of large intruders, such as parasitic wasp eggs, which can be considered functional equivalents of vertebrate granulomas (Honti et al., 2014). Extracellular serine proteinase cascades are crucial in activating many of the immune processes in *Drosophila*, such as the Toll pathway, melanization reaction, and hemolymph clotting reaction, the latter with functional analogy to the activation of the human complement system (Loof, Schmidt, Herwald, & Theopold, 2011).

2.2 The Discovery of Antimicrobial Peptides

A milestone in the history of innate immunity was the pioneering discovery made by Boman, Nilsson, and Rasmuson (1972) of an inducible, humoral antibacterial defense system in *Drosophila* and other insects. Following purification, primary structure determination, and activity measurements, it became clear that insects synthesize several families of peptides and proteins, such as cecropins, attacins, and lysozyme, with bacteriostatic or lytic activities (Hultmark, Steiner, Rasmuson, & Boman, 1980; Steiner, Hultmark, Engstrom, Bennich, & Boman, 1981). The term "antibacterial peptides" was coined, referring to secreted peptides/proteins with direct effects on bacterial membranes, leading to lysis or growth inhibition. It was later changed to AMPs to include also peptides with antifungal activity. Within few

years, seven different gene families encoding AMPs were isolated from *Drosophila*, and many also in other insects (reviewed in Hultmark, 1993; Imler, 2014).

Mammalian peptides with analogous functions called defensins and cathelicidins were subsequently isolated from rabbit macrophages and human neutrophils, bone marrow, and testis (Agerberth et al., 1995; Ganz et al., 1985; Selsted, Brown, DeLange, & Lehrer, 1983; Selsted, Harwig, Ganz, Schilling & Lehrer, 1985; reviewed in Ganz, 2003). The membrane-activity and killing mechanisms of some of the AMPs have been well characterized, while the function of others still is not completely understood (Shai, 1999). In addition, a large number of immunomodulatory peptides, called host defense peptides or innate defense regulators, which do not kill microbes directly but show different modulatory effects on both innate and adaptive immune responses have been identified in mammals (Scott et al., 2007). These peptides have attracted much attention as possible drugs for host-directed therapies, as reviewed in Mansour, Pena, and Hancock (2014).

2.3 The Role of *Drosophila* Toll and IMD Pathways in Innate Immunity

In the beginning of the 1990s, the genes encoding several insect AMPs were found to harbor κB-like DNA sequence elements in their upstream regions (Reichhart et al., 1992; Sun et al., 1991), and then shown to be required for AMP gene expression in vivo in response to microbial challenge (Engström et al., 1993; Kappler et al., 1993). The κB motif was a known target sequence for the mammalian nuclear factor kappaB (NF-κB) transcription factor in regulation of immunoglobulin gene expression in B-cells (Sen & Baltimore, 1986). Thus, this was one of the first indications of evolutionarily conserved mechanisms in regulation of innate immune responses between *Drosophila* and mammals. Also, parallels between the *Drosophila* Toll pathway and the mammalian IL-1 pathway were gradually becoming evident (Gay & Keith, 1991; Heguy et al., 1992; Schneider et al., 1991). At that time, only one *Drosophila* NF-κB-type transcription factor, called Dorsal, had been described in for its role in dorsoventral pattern formation in the *Drosophila* embryo (Anderson & Nusslein-Volhard, 1984; Steward, 1987). Despite Dorsal's capacity to activate AMP gene expression in reporter assays (Reichhart et al., 1993), *dorsal* mutants were still capable of producing AMPs in response to infection (Lemaitre, Meister, et al., 1995). Instead, another NF-κB-type transcription factor, named Dorsal-related immunity factor (Dif), was isolated and found to be a potent activator of many AMP genes

(Ip et al., 1993; Petersen et al., 1995). Later it was shown that Dif is the predominant transactivator upon antifungal infection and that *Dif* mutant flies are susceptible to fungal and Gram-positive bacterial infections (Rutschmann et al., 2000). Meanwhile, a third NF-κB-type transcription factor, called Relish (Dushay et al., 1996), was cloned and shown to be the predominant downstream activator of the IMD pathway, as described further later.

During embryo development, the Toll pathway regulates NF-κB/Dorsal activity. Thus, it was tested if Toll could be involved also in regulation of the NF-κB factors Dorsal and Dif during an immune response. Linking a constitutively active form of Toll with AMP gene expression in larvae (Ip et al., 1993) and in cell culture (Rosetto et al., 1995) in response to microbial elicitors attracted additional attention to Toll as a likely immunoregulatory factor. Final proof for the importance of Toll and the downstream pathway in immune response activation came when it was shown that flies with mutations in several Toll pathway components were killed by fungal infection (Lemaitre et al., 1996).

Consequently, a search for mammalian orthologs of Toll started, which led to the cloning of the first human TLR 1 year later (Medzhitov et al., 1997). Subsequently, five human TLRs were cloned (Rock et al., 1998), and the important immune function of the TLRs and the involvement in sensing microbial ligands were demonstrated in mice mutant for the *Tlr4* locus (Poltorak et al., 1998; Takeuchi et al., 1999). The number of known mammalian TLRs has now increased to 13; 10 of them (TLR1–10) are expressed in human and mice and the 3 remaining (TLR11–13) only in mice. The intracellular signaling cascade of the *Drosophila* Toll pathway and mammalian TLR pathways is evolutionarily conserved and was recently reviewed in Lindsay and Wasserman (2014).

Several lines of evidence were indicating that more than one signaling pathway is involved in regulation of AMP gene expression in *Drosophila*. The breakthrough came with the isolation of a mutant for the *immune deficiency* (*imd*) gene, which was affecting the expression of several AMP genes, but had little effect on expression of the antifungal peptide Drosomycin (Lemaitre, Kromer-Metzger, et al., 1995). In addition, it was shown that loss-of-function mutations in several Toll pathway genes still could mount expression of Diptericin in response to infection with Gram-negative bacteria (Lemaitre et al., 1996). Thus, it became clear that flies could discriminate between infection with different classes of microorganisms (Lemaitre et al., 1997). In addition, flies with mutations in both *Toll* and *imd* were

sensitive to infections by most classes of microorganisms, and also became susceptible to nonpathogenic microbes (Gottar et al., 2002). Importantly, transgene expression of a single AMP was shown to be sufficient for rescue and survival of such *Toll/imd* pathway double mutants, confirming the crucial role of AMPs for *Drosophila* immunity (Tzou et al., 2002).

Following the identification of *imd* mutant flies, the efforts of many labs led to identification of numerous components and regulators of the so-called IMD signaling pathway. The IMD pathway in *Drosophila* is homologous to the mammalian tumor necrosis factor receptor (TNFR) pathway and many signaling components are conserved between *Drosophila* and human (recently reviewed by Kleino & Silverman, 2014; Myllymaki et al., 2014).

The main downstream activator of the IMD pathway, the NF-κB-type transcription factor Relish was isolated as an immune-inducible gene itself (Dushay et al., 1996) and shown to be crucial for humoral immunity, as *Relish* mutant flies were extremely sensitive to infection (Hedengren et al., 1999). Like the mammalian NF-κB transcription factors p100 and p105, Relish is localized in the cytoplasm in an inactive form with a C-terminal domain containing multiple copies of ankyrin repeats (Stöven et al., 2000). Activation of Relish involves phosphorylation and cleavage to release the N-terminal fragment (REL-68), which translocates to the nucleus for DNA binding and transcriptional activation (Erturk-Hasdemir et al., 2009; Stöven et al., 2000, 2003). Whole genome expression analysis later revealed that most genes regulated by the IMD pathway in *Drosophila* utilize Relish for activation (De Gregorio et al., 2002).

More recently, factors that act downstream of the IMD pathway by recruitment of chromatin remodeling complexes have been identified, such as Akirin (Goto et al., 2008). It acts as a Relish cofactor for a subset of its target genes by SWI/SNF-Brahma complex (Bonnay et al., 2014). The mouse homolog, Akirin2, has been shown to play a similar role in bridging NF-κB and SWI/SNF complexes during activation of both innate and adaptive immune responses, such as activation of proinflammatory gene expression in mouse macrophages (Tartey et al., 2015, 2014).

2.4 Pattern Recognition Receptors

The microbial elicitors and the pattern recognition receptors (PRRs) upstream of both the Toll and IMD pathway were unknown until the beginning of this millennium. Bacterial lipopolysaccharide (LPS) was considered as a potent activator of the *Drosophila* pathways in analogy with the

mammalian TLR-4 signaling pathway. The isolation of peptidoglycan recognition proteins (PGRPs) (Kang et al., 1998; Yoshida et al., 1996) and Gram-negative binding proteins (GNBPs) (Lee et al., 1996) from different insects indicated, however, that peptidoglycan and β-glucans are the true microbial elicitors, which was subsequently experimentally confirmed (Kaneko et al., 2004; Mishima et al., 2009; Takahasi et al., 2009). The PGRP gene family in *Drosophila* consists of 13 genes encoding 19 PGRPs, including secreted, transmembrane, and intracellular variants (Werner et al., 2000). Many parallel studies demonstrated that different PGRPs and GNBPs act as specific PRRs upstream of either the Toll or IMD pathway (Choe et al., 2002; Gobert et al., 2003; Gottar et al., 2002; Michel et al., 2001; Ramet, Manfruelli, et al., 2002), thereby triggering responses to different classes of microbes, as reviewed in Aggrawal and Silverman (2007). Some PGRPs bind to peptidoglycan and act as true PRRs, while others have catalytical amidase activity and act as immune scavengers (Mellroth et al., 2003), as recently reviewed in Kurata (2014) and Royet (2011).

Importantly, the PGRP family is conserved from insects to mammals. In mammals, four PGRP genes have been characterized: PGLYRP1–4, and they have all been shown to be bactericidal. In general, mammalian PGLYRPs are expressed in barrier epithelia with direct contact with commensal or environmental bacteria and, therefore, seem to play a role in protecting the host from enhanced inflammation, tissue damage, and colitis (reviewed by Royet, 2011).

In spite of the similarities between the *Drosophila* Toll pathway and mammalian TLR signaling, important differences were noticed. All mammalian TLRs have been shown to act as direct PRRs for bacterial-, viral-, and parasitic-produced ligands; and also to some host cell products (Kawai & Akira, 2011). In contrast, *Drosophila* Toll is a cytokine receptor, which is activated by a cleaved form of the endogenous polypeptide Spätzle (Valanne et al., 2011; Veillard et al., 2016; Weber et al., 2003). Thus, the role of Spätzle as an immune-stimulating ligand seemed to be unique to insects and absent in vertebrates. However, a recent study indicated that nerve growth factor β (NGFβ), which is a cystine knot protein and a putative vertebrate ortholog of Spätzle, plays an important role in immunity to *Staphylococcus aureus* (Hepburn et al., 2014). NGFβ is released by macrophages in response to *S. aureus* via activation of NOD-like receptors (NLRs) and shown to stimulate a broad range of responses and activities in macrophages and neutrophils. In addition, mutations in human NGFβ or in its receptor TRKA, and knockdown of *trkA* in zebrafish, were associated with

susceptibility to *S. aureus* infections. This suggests an evolutionarily conserved role of cystine knot proteins in innate immunity against pathogenic Gram-positive bacteria, such as *S. aureus*.

An intense research field involving mammalian PRRs focuses on the role of inflammasomes in a number of diseases related to inflammatory disorders (Guo et al., 2015). Inflammasomes consist of multimeric protein complexes, which serve as PRRs and include NLRs and absent in melanoma 2 (AIM)-like receptors (ALRs). Stimulation of these receptors leads to oligomerization of the relevant NLRs/ALRs, followed by activation of caspase-1, and subsequent generation of active forms of the proinflammatory cytokines IL-1β and IL-18 (Vanaja et al., 2015). Inflammasome activation will also trigger a specific form of cell death called pyroptosis. Thus, activation of inflammasomes by host-derived factors can both initiate and exaggerate inflammatory reactions. Therefore, it is not surprising that autoinflammatory and autoimmune diseases have been linked to activation of inflammasomes in humans, including neurodegenerative diseases and metabolic disorders. Although inflammatory-like reactions have been observed in *Drosophila* in response to sterile tissue damage and tumor growth (Krautz et al., 2014; Shaukat et al., 2015), direct fly homologs of the NLR/ALR components of the mammalian inflammasome have not been identified (Martinon et al., 2009). Thus, genetic and mechanistic studies of inflammasome activation and function are presently not feasible in the *Drosophila* model. However, continued studies of inflammatory reactions in the fly may lead to discovery of other shared components and pathways, which regulate responses to tissue damage and inflammation in both the fly and humans.

2.5 The Role of Other Evolutionarily Conserved Signaling Pathways in Immunity

In addition to Toll and IMD pathways, a number of other conserved signaling pathways play important roles in *Drosophila* immune response processes directly or indirectly. The *Drosophila* Jun N-terminal kinase (dJNK) pathway is homologous to mammalian tumor necrosis factor (TNF) pathway. The first indications of JNK playing a role in the immune defense in *Drosophila* came from activation of dJNK by microbial elicitors (Sluss et al., 1996). It was later reported that the *Drosophila* IMD pathway bifurcates into two branches, activating Rel and Jun target genes, respectively (Boutros et al., 2002), in a similar manner as the TNFR pathway in mammals (Dai et al., 2012). In relation to infection and immunity, the *Drosophila* JNK pathway thus plays a role in regulation of AMP gene expression (Delaney et al., 2006;

Kallio et al., 2005; Kim et al., 2005), melanization through crystal cell rupture (Bidla et al., 2007), and bacteria-induced stem cell activation in the gut epithelium (Buchon, Broderick, Chakrabarti, & Lemaitre, 2009).

The Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway is well conserved between human and *Drosophila* (Li & Watowich, 2014; Myllymaki & Ramet, 2014). The *Drosophila* pathway has only one JAK (*Hop*) and one STAT (*STAT92E*) and therefore confers less redundancy compared to mammals that have multiple JAKs and STATs. The *Drosophila* JAK/STAT pathway is activated by cytokine-like proteins Os/Upd, Upd2, and Upd3 that bind to the single receptor Domeless (*Dome*). The pathway is involved in many processes linked to immunity, especially cellular immunity such as hematopoiesis, encapsulation, and lymph gland responsiveness (Hanratty & Dearolf, 1993; Sorrentino et al., 2004). In response to bacterial injury, hemocytes secrete Upd3, which stimulates JAK/STAT signaling in fat body cells, leading to immune gene expression (Agaisse et al., 2003). In addition, the JAK/STAT pathway is involved in gut epithelial responses to infection and is required for bacteria-induced stem cell proliferation in the gut epithelium (Osman et al., 2012). Just as in mammals, the *Drosophila* JAK/STAT pathway also participates in the control of viral infection (Lamiable & Imler, 2014; Myllymaki et al., 2014) and in tumorigenesis (Amoyel et al., 2014). In a recent *Drosophila* study, methotrexate was discovered as a strong inhibitor of the JAK/STAT pathway and suggested as a novel treatment for myeloproliferative neoplasm in humans (Thomas et al., 2015).

In addition to the signaling systems mentioned earlier, a wealth of studies has shown involvement of other pathways in immunity both in humans and in *Drosophila*. In flies these include the Duox pathway (Bae et al., 2010), insulin pathway (Becker et al., 2010), the Wingless/Wnt pathway (Gordon et al., 2005), the Pvr pathway (Bond & Foley, 2009), the p38 pathway (Chen et al., 2010; Davis et al., 2008), and the Hippo pathway (Liu et al., 2016). Furthermore, there is growing evidence for positive and negative cross talk between these and the Toll and IMD pathways. Similarly, mammalian TLR and TNF pathways were shown to interact (Kawai & Akira, 2011).

3. INNATE IMMUNITY IN BARRIER EPITHELIA

3.1 Epithelia as Physical and Chemical Barriers

Surface epithelia constitute physical and chemical barriers that separate internal tissues and organs from the surrounding environment. The epithelial

linings of the skin, lungs, gut, and genitalia are normally exposed to a very broad range of microorganisms, including commensals as well as potentially harmful microbes. Therefore, these barrier epithelia serve important functions in protecting the organism from invasion of other organisms and protection against toxic and harmful molecules.

Epithelial cells also create a chemical barrier by releasing AMPs, chemokines, and cytokines. Human α-defensins are expressed in polymorphonuclear leukocytes (PMNs), Paneth cells in the gut, and epithelial cells in the genital tract. Human β-defensins are widely expressed in epithelial cells in all mucosal tissues, including intestine, lung, and skin (Ganz, 2003). Theta-defensins are only expressed in nonhuman primates and the genes are truncated in humans (Cole et al., 2004). The expression of AMPs in humans is tightly regulated and can be constitutive and/or induced by cytokines or microbial compounds, and even downregulated by certain virulent bacteria, like *Shigella* spp. (Gudmundsson et al., 2010). The barrier epithelia of *Drosophila* larvae and flies maintain basic expression levels of AMPs. It was shown, using transgenic flies carrying fluorescent reporter genes, that each epithelial surface expresses several AMPs (Tzou et al., 2000). In addition, local infection triggers increased expression of these AMPs in barrier epithelia, as reviewed in Davis and Engstrom (2012).

Thus, it is likely that both human and fly epithelia produce cocktails of AMPs to protect against invasion by pathogenic microbes. An alternative function for AMPs in barrier epithelia would be that they shape the local microbial community and promote certain commensals to become predominant. Such selected microbial communities may then in fact serve as a first line of defense by competing with pathogenic microbes. A protective role of the microbiota resident in epithelial surfaces has in fact been demonstrated in both insects and humans. It was shown in *Drosophila* that germ-free larvae are more susceptible to infection by pathogenic fungi, such as *Candida albicans*, than in the presence of the normal microbial flora (Glittenberg et al., 2011). In humans, this phenomenon is best illustrated by *Clostridium difficile*-associated diarrhea, which often occurs after antibiotic treatment. Thus, it is clear that a healthy microbiota protects against pathogens by occupying a niche in the intestinal mucosa (Britton & Young, 2014).

The regulatory networks controlling tissue specificity in epithelia of both fly and human AMPs are relatively poorly described, in comparison to the well-studied pathways regulating responses to systemic infection. Improved knowledge of the cues that control endogenous AMP expression should enable the development of novel approaches to strengthen the epithelial

barriers and to boost responses to infection. With the high degree of evolutionary conservation of transcription factors and signaling between *Drosophila* and mammals, it is likely that studies of effector mechanisms and their regulation in *Drosophila* barrier epithelia will continue to provide important knowledge to the benefit of understanding these regulatory networks in humans.

3.2 Impact and Relevance of Innate Epithelial Infections in Humans

3.2.1 Bacterial Infections and Immunity

Bacterial infections in humans are a common clinical problem in all disciplines, ranging from primary care to advanced surgery in university clinics. In particular, the emerging resistance against common antibiotics has become a real threat to many surgical procedures. Spread of *Staphylococci* resistant to methicillin (MRSA), Enterobacteriaceae with extended spectrum of β-lactamases (ESBL), and carbapenemase-producing Enterobacteriaceae (CPE) constitute real clinical challenges due to their resistance to first and second line treatments (Pitout & Laupland, 2008; Watson, 2011). Infections with these bacteria require treatment with expensive drugs, which are not accessible in all countries, and thus, the infections cannot be treated properly. In fact, bacterial strains being resistant against colistin, the last treatment resort, were recently discovered in China (Stoesser et al., 2016). Combined, this new situation requires novel approaches to prevent and treat infections with multidrug-resistant bacteria. One such approach would be to harness the power of the innate immune system and to use *Drosophila* to screen for novel compounds, or screen existing drugs for new purposes, which then rapidly can be incorporated in clinical treatment regimes. In addition, *Drosophila* could be utilized to study the virulence of multidrug-resistant bacteria, an area that just recently has been addressed.

3.2.2 Fungal Infections and Immunity

Fungal infections are of huge medical importance, but the knowledge about fungal immune responses is not as developed as the knowledge for bacterial and viral infections. Fungal infections in humans are becoming a significant problem due to rising numbers of immune-compromised individuals. Drug resistance is also increasing and there is a large need for novel treatments and prevention against severe fungal infections.

The skin and mucosal surfaces of humans are inhabited by commensal yeasts and fungi, such as *Candida* and *Malassezia* species, and the skin and mucosa is an entry point for invasive fungal diseases (Underhill & Pearlman, 2015). The lungs also constitute an important route of infection as they are exposed to airborne spores of common molds, such as *Aspergillus* and *Fusarium*. Immunosuppression and genetic immune-deficiencies severely increase the risk for developing chronic and/or invasive fungal infections. Although T-cell responses are necessary for full defense against fungi, the innate immune system plays an important role (Lionakis et al., 2011). Recognition of yeast and fungi in humans depends on lectins that recognize fungal β-glucans. A large number of receptors for lectins exist, such as the C-type lectin receptor (CLR) clusters Dectin-1 and Dectin-2. These promote phagocytosis and production of inflammatory cytokines. This and related topics on recognition and responses to fungal infections in humans have been well covered in recent reviews (Sancho & Reis e Sousa, 2012; Underhill & Pearlman, 2015).

Fungal immune responses in *Drosophila* are also based on the recognition of β-glucans as described earlier, and on sensing of fungal virulence factors (Gottar et al., 2006). Signaling via the Toll pathway to the Rel factor Dif promotes expression of antifungal peptides, such as drosomycin, metchnikowin, and cecropin (reviewed in Lindsay & Wasserman, 2014; Uvell & Engstrom, 2007) and the recently characterized bomanins (Clemmons et al., 2015; Uttenweiler-Joseph et al., 1998). Phagocytosis and encapsulation by hemocytes are also important for antifungal defense in *Drosophila* (Lemaitre & Hoffmann, 2007).

3.2.3 *Human Microbial Pathogens and Virulence Mechanisms Studied in* Drosophila

Wild-type strains of *Drosophila* are easy to grow in a time-efficient manner and have successfully been used for virulence tests of human pathogenic bacteria that cause systemic infections in flies, such as *Mycobacterium marinum* (Dionne et al., 2003), *Salmonella typhimurium* (Brandt et al., 2004), *Serratia marcescens* (Cronin et al., 2009), *Francisella tularensis* (Ahlund et al., 2010), and *S. aureus* (Wu et al., 2012). Similarly, a number of human fungal pathogens are lethal when injected into wild-type *Drosophila*, such as *C. albicans* (Davis et al., 2011; Glittenberg et al., 2011) and *Cryptococcus* (Thompson et al., 2014). However, several human fungal pathogens, such as *Candida glabrata* and *Aspergillus fumigatus*, do not cause lethal infections in fully immune-competent *Drosophila*. For these fungi, flies/larvae with mutations

in the IMD or Toll pathways have been used as hosts, as reviewed in Panayidou et al. (2014).

So far, relatively small size drug screens for antibacterial or antifungal chemicals have been carried out in *Drosophila*, primarily identifying the response to a combination of a few known or new antibiotics (Ben-Ami et al., 2013; Lionakis & Kontoyiannis, 2005; Oh et al., 2013, 2014; Thompson et al., 2014). These studies serve, however, as good proof of principles, indicating the great potential in using *Drosophila* as a primary in vivo target for high-throughput screening efforts for novel pharmaceuticals, and for retesting drugs already approved for human use (Tzelepis et al., 2013).

4. EPITHELIAL IMMUNITY IN THE GASTROINTESTINAL TRACT OF HUMANS AND *DROSOPHILA*

The gastrointestinal systems of *Drosophila* and human share many similarities in structural and cellular architecture of the gut epithelium, and its barrier functions include gut epithelial immunity and host–microbe interactions. With the unique possibilities for genetic manipulation, *Drosophila* has become an important model for studies of the underlying mechanisms regulating gut development, epithelial regeneration and stem cell activity, metabolism, and immunity. This is likely to bring more light into many unsolved questions of human gastrointestinal diseases that are caused by disturbances in these processes, such as intestinal barrier function, Crohn's disease, and colon cancer (Frosali et al., 2015; Merga et al., 2014).

4.1 Similarities and Differences in Human and Fly Gut Structure and Immune Systems

Both the human and *Drosophila* digestive systems are highly compartmentalized tubular structures with different anatomical/morphological, transcriptomic, and functional immune specialization (Buchon, Osman, et al., 2013; Lemaitre & Miguel-Aliaga, 2013; Marianes & Spradling, 2013; Mowat & Agace, 2014). The *Drosophila* gut is structurally divided into the foregut, midgut, and hindgut (Fig. 1), and the midgut serves the same functions as the human stomach, small intestine, and colon in food digestion and nutrient absorption. The *Drosophila* midgut epithelium is a single cell layer with two differentiated cell types, absorptive enterocytes (ECs), and enteroendocrine (EE) cells, which are renewed from intestinal stem cells (ISCs) via a nondividing transient cell types called enteroblasts (EBs) and

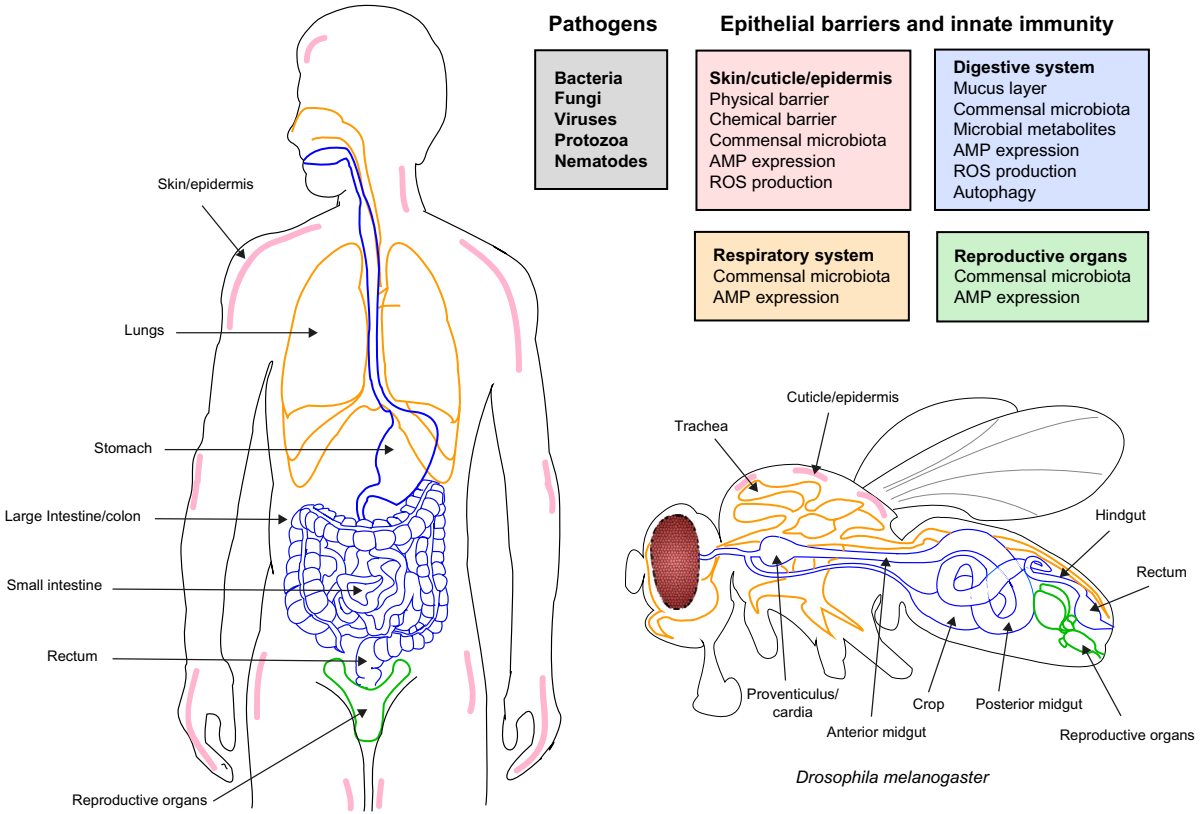

Fig. 1 See legend on opposite page.

pre-EE cells (Micchelli & Perrimon, 2006; Ohlstein & Spradling, 2006; recently reviewed in Li & Jasper, 2016). The epithelium of the human small intestine is more three-dimensional with finger-like villi and deep crypts, while the colon lacks the protruding villi and has a smooth epithelium (Mowat & Agace, 2014). The ISCs reside in the crypts and after cell division the transient cells move upward, further proliferate, and finally differentiate into absorptive ECs, goblet cells, or EE cells. The ISCs can also differentiate into Paneth cells, which reside at the bottom of the crypts and escape from

Fig. 1 Epithelial barriers and innate immunity. Overview of analogous organ systems in human and *Drosophila* that are exposed to common types of pathogens and share evolutionarily conserved defense reactions to prevent and fight such infections. In addition, all barrier epithelia harbor commensal bacteria that stimulate host immune competence and also protect the host by competing with more harmful microbes. The skin/epidermis of humans and cuticle/epidermis of insects serve as physical and chemical barriers that prevent infection. Insults that breach this barrier trigger AMP production, and in combination with other humoral and cellular reactions, promote local protection. The respiratory systems consist of tubular epithelial organs, which in flies directly transport oxygen throughout the body cavity, while the lungs in humans are connected to the vascular system. Nevertheless, the lungs and trachea share many immune defense reactions, such as constitutive and inducible expression of AMPs. The gastrointestinal system of humans and flies is functionally analogous in their digestive and excretory functions and shares a similar overall regionalized structure. While the human gut epithelium is covered by a thick protective mucus layer, the *Drosophila* foregut and hindgut are of ectodermal origin and their epithelia are covered by an impermeable cuticle. The fly midgut, which is analogous to the human stomach, small intestine, and colon, is covered by the peritrophic matrix and a thin mucus layer that together serve a similar function as the human mucus layer, to separate the cellular epithelium from bacteria and toxic compounds present in the gut lumen. The midgut of both human and fly is surrounded by visceral musculature, which is innervated and in the fly also supplied via fine tracheoles (Lemaitre & Miguel-Aliaga, 2013). The fly system also comprises the crop, which is a sack-like structure for food storage and detoxification. The intestinal epithelium of both human and flies consists of differentiated epithelial cells, the enterocytes (ECs), and enteroendocrine (EE) cells. The regeneration of the gut epithelium from intestinal stem cells that divide asymmetrically to form transient amplifying (TA) cells in human and analogous enteroblasts (EB) in flies, which then further differentiate into ECs and EEs, shows surprisingly high degree of evolutionary conservation, with homologous signaling pathways being involved. The excretory system of flies consists of the malphigian tubules, which are analogous to human renal organs/kidney, connected to the midgut/hindgut junction and of the ileum that regulates osmolarity by absorption of water and ions (Lemaitre & Miguel-Aliaga, 2013). The epithelia of the reproductive organs in both human and fly express AMPs but will not be further described in this review.

the upward migration (van der Flier & Clevers, 2009). Both the *Drosophila* and human intestine are surrounded by visceral musculature.

Human intestinal cells are covered by the glycocalyx (mucus), which is a thick and viscous fluid composed of negatively charged mucins. The mucus layer keeps the microbiota at a distance from the epithelial cells, and a deficient mucus layer leads to intestinal inflammation. The *Drosophila* gut epithelium is covered by a thin chitinous peritrophic matrix that serves the same function as the human glycocalyx, to separate the cellular epithelium from the contents of the gut lumen. The human gut differs from that of the fly in the presence of a lamina propria that contains cells from the adaptive immune system (Mowat & Agace, 2014). These immune cells play important roles in regulating intestinal immunity by producing cytokines and immunoglobulins of the IgA-type. Recently, a novel group of immune cells, the innate lymphoid cells (ILCs), have been intensively studied and found to coordinate many of the immune activities in the intestines of mice and humans (Mowat & Agace, 2014). Since *Drosophila* lacks adaptive immunity, these pathways are not possible to study in the fly system.

The enteric nervous system plays a key role for the physiologic response in the human intestine by releasing neurotransmitters in response to physiological stimuli, including bacterial metabolites (Kabouridis & Pachnis, 2015). Even though the fly enteric nervous system is different from the human counterpart, many aspects are actually conserved, which includes release of serotonin and neuropeptides (Kuraishi et al., 2015). It should therefore be possible to study nerve-immune cross talk in the *Drosophila* system with relevance for human physiology.

4.2 The Importance of the Gut Commensal Microbiota in Health and Disease

Humans contain rich and diverse microbial communities in their intestines, and our understanding of their importance in health and disease has increased vastly during the last decade. The commensal gut microbes are crucial partners in absorption of nutrients and as suppliers of essential nutrients, and their roles in shaping an organism's metabolic and immune status have become increasingly evident (Wu et al., 2015). In addition, we are just starting to understand their influence on development, and physiology, with direct effects on general health, aging, and lifetime expectancy (Sommer & Backhed, 2013). In fact, many of these processes can be mimicked in the *Drosophila* model (Buchon, Broderick, & Lemaitre, 2013). The human gut contains many orders of magnitude more bacteria than the fly gut,

distributed over more than 500 taxa per individual. Interestingly, the bacterial composition in the *Drosophila* gut seems to be more diverse than the previously reported 5–30 taxa (Broderick & Lemaitre, 2012) and may in fact show more overlap with the human gut microbiome than anticipated (Dantoft et al., 2016). The human gut is dominated by Firmicutes and Bacteroidetes and also contains Proteobacteria, Actinobacteria, Fusobacteria, Verrucomicrobia, and Cyanobacteria (Lozupone et al., 2012), while the fly gut is dominated by Proteobacteria and Firmicutes and also contains Actinobacteria and Bacteroidetes (Broderick & Lemaitre, 2012). The ease with which *Drosophila* can be cultivated in germ-free conditions has stimulated a wealth of studies reporting on the impact of both pathogens as well as of commensals in regulating local and systemic immunity, gut epithelium regeneration, metabolism, physiology, and age-related tissue dysfunction. We will discuss some of these topics in the following sections, but refer to recent reviews for a comprehensive coverage of this intense research area in *Drosophila* (Buchon, Broderick, et al., 2013; Erkosar & Leulier, 2014; Lee & Lee, 2014; Li & Jasper, 2016).

4.2.1 Microbial Metabolites and Regulation of the Immune Responses

The role of microbial metabolites in regulation of human physiology is a large and very active area of research. For a detailed review about microbial metabolites and their role in human metabolism and immunity, see Donia and Fischbach (2015). Gut microbiota produce many important metabolites, including short-chain fatty acids (SCFAs) such as butyrate, acetate, and propionate. SCFAs are known to serve as nutrients for human colonic cells and also to suppress inflammation, proliferation, and the development of cancer in the human colon (Louis et al., 2014). In fact, reduction of butyrate-producing bacteria by antibiotics or in germ-free systems inevitably leads to more inflammation. The mechanisms for butyrate-mediated effects have partly been delineated and involve G-protein-coupled receptors on the surface of both epithelial and immune cells. Via binding to G-protein-coupled receptors, GPR43 and GPR109A, butyrate inhibits inflammation, reduces oxidative stress, and promotes mucosal defenses (Macia et al., 2015). In addition, butyrate induces AMP production in colonic epithelial cells (Schauber et al., 2003) and restores mucosal defenses during *Shigella* infections (Raqib et al., 2006).

The presence of *Lactobacillus plantarum* and *Acetobacter pomorum* in the *Drosophila* gut was shown to confer positive effects on metabolism and growth, by activating the insulin pathway (Shin et al., 2011; Storelli

et al., 2011). Some of the effects could be restored by food supplementation with acetic acid, but additional, unknown metabolites are likely to also be important. When searching for virulence factors in *Vibrio cholera* in a *Drosophila* model, it was observed that the bacterial two-component system CrbRS played a major role on host organism symptoms and survival (Hang et al., 2014). The CrbRS regulates an acetate switch that activates acetate consumption, leading to downregulation of host insulin signaling and host lethality. Similar effects of SCFA on human metabolism and insulin sensitivity are likely to occur, but this is an area which not yet has been fully explored (Canfora et al., 2015).

4.3 Recognition of Microbes in Human and *Drosophila* Gut

As mentioned earlier, several of the key processes of microbial recognition, signaling, and responses are well conserved between human and fly. For example, both recognize microbes via PRRs and the different host receptor systems show conserved and nonconserved features. In human gut, such PRRs include TLRs at the cell membrane, the NLRs, CLRs, and RIG-I-like receptors inside the cell (Cao, 2015). The expression levels of these receptor systems vary along the gastrointestinal tract and are hardwired to specific response programs, including expression of cytokines, chemokines, and AMPs (Sperandio et al., 2015). Similarly, the large *Drosophila* family of different PGRPs, which include extracellular, membrane-bound, and intracellular members are expressed differently along the fly gut (Bosco-Drayon et al., 2012; Marianes & Spradling, 2013) and confer both activation and negative feedback regulation on expression of AMPs (Royet & Charroux, 2013; Royet et al., 2011).

An outstanding question in the field of intestinal immunity is how the host can differentiate between the innocuous normal flora and potentially pathogenic microbes. Several explanations have been proposed. First, in the human gut, an intact mucus layer keeps bacteria at a distance and pathogens may penetrate the mucus layer and cause inflammation and disease. Similarly, the *Drosophila* midgut is lined by a chitinous peritrophic matrix, which separates the bacteria within the gut lumen from the gut epithelium (Lemaitre & Miguel-Aliaga, 2013).

Second, TLRs have been exclusively found inside the epithelial cells of the mammalian gut (Hornef et al., 2003), which would create a tolerant extracellular environment for the microbiota and only respond to invading pathogenic bacteria. In the *Drosophila* gut, several layers of negative

regulation of the IMD pathway fulfill a similar purpose of increasing the tolerance to the commensal flora (Buchon, Broderick, et al., 2013). For example, one of the predominant sensors of Gram-negative bacteria in the *Drosophila* midgut, PGRP-LE, is an intracellular PRR (just as mammalian gut TLRs) (Bosco-Drayon et al., 2012). Other PGRP family members, like PGRP-SCs and PGRP-LB, are amidases that degrade the immune elicitor peptidoglycan (Mellroth et al., 2003; Zaidman-Remy et al., 2006) so that under normal conditions the peptidoglycan concentration is kept low. The genes for negative regulators of the IMD pathway are activated upon infection and create a negative feedback loop (Buchon, Broderick, et al., 2013). The transcriptional repressors Caudal (homologous to human Cdx2/Cdx4) and Pdm1/Nub (homologous to human Oct1/Oct2) bind AMP gene promoters and prevent expression in different parts of the midgut in healthy conditions (Dantoft et al., 2013; Ryu et al., 2004). An important layer of recognition of pathogens vs commensals is prevalent in the *Drosophila* gut, where it was found that many pathogens release uracil (Lee et al., 2013). Uracil is a strong inducer of ROS secretion and other immune responses, as described further in Section 4.5.

Finally, it has been proposed that the normal situation in the human gut is dominated by immunoregulatory cells, sustaining an immunosuppressive environment and thus controlling inflammation. This hypothesis is supported by the fact that deletion of IL-10, an immunosuppressive cytokine, leads to spontaneous colitis. Also in humans with a mutation in IL-10, colitis is a common symptom (Glocker et al., 2009). Although experimental evidence for an immunosuppressive role of *Drosophila* hemocytes in gut immunity is missing so far, hemocytes are adhering to the gut epithelium and were found to stimulate phagocytosis, ISC activity, and to contribute to intestinal dysplasia in aging flies (Ayyaz et al., 2015; Zaidman-Remy et al., 2012). Thus, this indicates the possibility to use flies to study mechanisms of recruitment and adhesion of circulating immune cells to the gut epithelium, and of interactions that may influence gut pathologies in humans.

4.4 Innate Immune Responses in the Human and *Drosophila* Gut—Effector Molecules

In order to keep the microbiota in check, the intestinal epithelium is equipped with a plethora of responses downstream of microbial recognition. AMPs and proteins constitute families with similar bactericidal and bacteriostatic activities, as well as antifungal properties.

In humans, the Paneth cells of the small intestine produce the human AMPs defensin 5 and 6 (HD-5 and HD-6) with specific functions. HD-5 belongs to the α-defensin family and has broad antimicrobial activity against a range of bacteria. In particular, it is active against *Salmonella*. To test the functional importance of HD-5, a mouse model for overexpression of HD-5 was created. Notably, oral inoculation of these mice with *S. typhimurium*, which normally would have killed the mice, resulted in complete protection from disease. In contrast, a systemic challenge resulted in 100% mortality of both wild type and transgenic mice, clearly showing that HD5 protected the mucosa from *Salmonella* invasion. A follow-up study could also show that the normal flora of HD-5 expressing mice was fundamentally changed compared to wild-type mice. Thus, one single AMP has profound effects on mucosal immunity and determines the composition of the normal flora (Salzman et al., 2010). In contrast to HD-5, HD-6 has no antimicrobial activity, and its role in intestinal immunity has remained elusive. However, a recent study could show that HD-6 forms amyloid structures, which entangle bacteria and remove them from the mucosal wall, thereby preventing invasion without direct killing (Chu et al., 2012).

Human colonic epithelial cells produce the AMPs LL-37 and β-defensins. The role of LL-37 is illustrated by the fact that *Shigella* spp., a common human pathogen causing dysenteriae, downregulates LL-37 expression as a part of its invasion program (Islam et al., 2001; Sperandio et al., 2008). Notably, the SCFA butyrate can counteract this effect and upregulates LL-37 expression, which restores colonic immunity and improves symptoms in a rabbit model (Raqib et al., 2006), as well as in humans (Sayem et al., 2011). In addition to AMPs, colonic epithelial cells produce antimicrobial proteins, such as the lectin REGIIIgamma (Cash et al., 2006). Deletion of this protein from mouse intestine results in loss of bacterial-mucosal segregation and leads to mucosal inflammation caused by the microbiota (Loonen et al., 2014; Vaishnava et al., 2011).

In *Drosophila*, several AMP genes are constitutively expressed in different parts of the intestinal epithelium in a highly regionalized manner (Dutta et al., 2015; Marianes & Spradling, 2013; Tzou et al., 2000). Most AMPs are also strongly upregulated upon infection, primarily in an IMD pathway-dependent manner (Ryu et al., 2006). While the *Drosophila* Toll pathway is not active in the gut, the JAK/STAT pathway regulates some AMPs in response to epithelial damage (Buchon, Broderick, Poidevin, Pradervand, & Lemaitre, 2009). Flies mutant in the IMD pathway are more sensitive to oral infection by pathogenic bacteria, such as *Pseudomonas*

entomophila (Liehl et al., 2006) and *S. marcescens* (Nehme et al., 2007), while wild-type flies are very resistant to the presence of bacteria in their food, except for dedicated insect pathogens that have evolved features to effectively evade the innate immune system.

4.5 Dual Roles for ROS in the Intestinal Epithelium

Another key response system in the gut is the production of ROS. Intestinal epithelial cells in both mice and flies produce ROS, and it has been suggested that during enteric infections, high levels of ROS act as bactericidal effectors, while during homeostatic conditions, the presence of commensals stimulates low levels of ROS that act as signaling molecules to promote ISC proliferation (Lambeth & Neish, 2014). ROS can be produced by two independent, but evolutionarily conserved pathways, one involving the NAPDH oxidase (NOX) and the other by dual oxidase (DUOX). The importance of ROS for an intact intestinal barrier in mammals is evident from studies in the mouse where NOX-deficient mice develop colitis in response to avirulent *Salmonella* (Felmy et al., 2013; Rodrigues-Sousa et al., 2014). Patients with chronic granulomatous disease (CGD) lack a functional NOX and suffer from frequent bacterial and fungal infections. In addition, CGD patients develop severe colitis, possibly due to lack of control of the normal microbiota, but the exact mechanism is still unknown (Broides et al., 2016; Leiding & Holland, 1993). Interestingly, it appears that impaired ROS production leads to deficient autophagy and an excess of IL1-beta, which presumably could drive inflammation and cause colitis (de Luca et al., 2014; van de Veerdonk & Dinarello, 2014). Most interestingly, and in correlation with the examples described earlier, Jones et al. (2015) reported that commensal *Lactobacillus* bacteria promoted NOX-dependent ROS production and subsequent ISC proliferation in both the *Drosophila* and murine gut, suggesting that NOX is important in regulating gut homeostasis in normal conditions, and that this requires the presence of commensal microbiota such as *Lactobacillus*. In contrast, activation of DUOX, which has been studied extensively in *Drosophila*, requires the presence of pathogens (Kim & Lee, 2014). In fact, DUOX acts in parallel with NF-κB-dependent AMP production in the *Drosophila* gut, and it was shown that either pathway protects against enteric infection and only when both pathways are impaired, the flies will succumb due to infection (Ryu et al., 2006). Both the ROS-producing enzyme activity of DUOX and its gene expression are activated by the presence of microorganisms (Ha, Lee, Park, et al., 2009; Ha, Lee, Seo, et al.,

2009). As mentioned earlier, this has been linked to pathogen-produced uracil, which was found to be a strong elicitor of immune responses in the *Drosophila* gut (Lee et al., 2013). The receptor for uracil is unknown, but is likely to be a G-protein-coupled receptor. The uracil-induced DUOX activation was recently shown to be modulated by Hedgehog signaling (Lee et al., 2015).

DUOX enzymes have so far not been studied extensively in mammals, but recent work suggests that DUOX2, which is expressed in gut epithelium and upregulated in patients with inflammatory bowel diseases, regulates interactions between the intestinal microbiota and the mucosa to maintain immune homeostasis in mice. Mucosal dysbiosis leads to increased expression of DUOX2, which might be a marker of perturbed mucosal homeostasis in patients with early-stage inflammatory bowel disease (Grasberger et al., 2015). Taken together, intact ROS production is crucial for an intact intestinal barrier, regulation of ISC proliferation and of pathogen-induced immune responses both in humans and in *Drosophila*.

4.6 Autophagy as an Effector Mechanism

Intracellular bacteria are killed and degraded by the autophagic system in both human and *Drosophila* intestinal cells. An intact autophagic system promotes *Drosophila* survival after *Listeria* infection (Yano et al., 2008) and is important for control of the *Drosophila* symbiont *Wolbachia* (Voronin et al., 2012). In humans, autophagy has been shown to be essential for mucosal protection against invasive *Salmonella* (Benjamin et al., 2013) and is also associated with Crohn's disease (Salem et al., 2015). Autophagy also acts as an antiviral response process, which is conserved between flies and mammals (Lamiable & Imler, 2014; Moy et al., 2014). The main autophagic pathways are well conserved between fly and human, and pharmacological modulation of autophagy has been analyzed in *Drosophila* models of neurodegenerative disease (Jaiswal et al., 2012).

The activation of autophagy can occur by starvation or via activation of the mTOR system in both the fly and human systems. In addition, activation of surface-associated PRRs as well as of intracellular recognition systems leads to increased autophagy in both species. In the fly, intracellular PGRP-LE activates autophagy and the mammalian counterparts NOD1/2 bind to ATG16L in human cells. Likewise, the transcription factors FoxO and TFEB (*Drosophila* homologue Mitf) increase transcription of several autophagy-related genes.

The *Drosophila* model has been very useful in defining key regulatory genes for autophagy activation, with importance for bacterial infection. One such study found the gene MORN2 to be essential for LC3-associated phagocytosis and to be conserved between fly and humans (Abnave et al., 2014). Another example is the gene CAP-D3, which was found to be important for the innate immune response in *Drosophila* by regulating the expression of AMP genes (Longworth et al., 2012). The same group set out to study the role of CAP-D3 in ulcerative colitis (UC) in humans. Interestingly, in colonic biopsies from UC patients, the level of this protein was significantly lower than in healthy controls. Moreover, CAP-D3 was found to regulate autophagy in human cells and decreased expression levels of CAP-D3 impaired clearance of *Salmonella*, suggesting a conserved role for this protein in intestinal immunity (Schuster et al., 2015). Finally, large genome-wide screens in Crohn's patients have revealed mutations in the pattern recognition receptor, NOD2 (Liu et al., 2015), and in the autophagy-related genes ATG16L1 and IRGM (Salem et al., 2015). Combined, these findings underscore the existence of defects in both recognition and autophagy effector mechanisms in Crohn's pathogenesis.

4.7 The Intestinal Barrier and Aging—Examples from Human and *Drosophila*

The intestinal barrier keeps microbial products away from the circulation. When this barrier fails in diseases such as gastrointestinal inflammation, HIV, or hepatitis, bacterial products leak from the intestine into the circulation, causing a "leaky gut syndrome." This process has been named "microbial translocation" and is considered to drive systemic inflammation and subsequent increase of cardiovascular disease and premature death.

There are several aspects of the leaky gut concept with relation to the *Drosophila* model system, and especially the links between aging and a leaky gut phenotype have been addressed in the fly. It has been shown that the intestinal barrier function correlates well with the expected life span of the fly. Markers of the aging fly include increased expression of AMPs in the intestine and impaired insulin-signaling pathways (Rera et al., 2012). Recently, these effects in the aging fly could be coupled to changes in the microbiota. In fact, the changes in the bacterial flora preceded and could predict the subsequent impairment of the intestinal barrier function (Clark et al., 2015). Aging has also been shown to lead to chronic activation of FoxO in the *Drosophila* intestine, which caused a reduced expression of PGRP-SC2, a negative regulator of IMD/Relish innate immune signaling.

This caused commensal dysbiosis, stem cell hyperproliferation, epithelial dysplasia, and reduced life span of flies (Guo et al., 2014). Also in humans, the intestinal barrier appears to be reduced during aging (Mabbott, 2015), but the link to the microbiota is less established than in the *Drosophila* system. Based on the many overlapping aspects of intestinal barrier function and aging between humans and *Drosophila*, the *Drosophila* model has been proposed to serve as a platform for further studies in this field (Jasper, 2015).

4.8 Gut Regeneration and Microbiota Interactions in Inflammation and Cancer

The cells of the gut epithelium of both human and flies are short lived and replaced constantly. The rate of shedding of old/dead epithelial cells has to be kept in balance with the renewal from a pool of long-lived ISCs. The balance is especially critical during damage or infection, when the acute need for cell replenishment leads to an increase in ISC proliferation and differentiation, with the risk of overproliferation unless it is well controlled. This regenerative homeostasis is controlled by a large number of signaling pathways that are evolutionarily conserved between human and *Drosophila*, such as JAK/STAT, RTK/Ras/MAPK, Hippo, JNK, Notch/Delta, wnt/wg, BMP, and insulin-signaling pathways (for a comprehensive review, see Jiang & Edgar, 2012). Mutations in components of some of these pathways lead to hyperproliferation both in flies and in mice, while others cause premature differentiation and loss of the ISC pool. Many also act as tumor suppressor pathways in humans and have been linked to the development of colorectal cancer (CRC). This process involves transformation of healthy epithelial cells into premalignant adenomas and sometimes malignant cancer. It is well accepted that recurrent damage caused by chronic inflammation, microbial dysbiosis, or presence of certain bacteria, such as *Helicobacter pylori* and—more recently—*Fusobacterium nucleatum*, is a risk factor for development of premalignant conditions in the stomach and colon, respectively (Gur et al., 2015; Lee et al., 2016).

When the *Drosophila* gut epithelium is damaged by wounding or pathogenic infection, the JNK pathway is activated in the stressed, dying ECs, which then secrete IL6-like cytokines, Upds, which subsequently activate JAK/STAT signaling in neighboring ISCs. Together with activation of receptor tyrosine kinase (RTK) signaling by ligands secreted from the visceral musculature, this leads to proliferation of ISCs. The Hippo signaling pathway, a central regulator of organ size in flies and man, is also activated in ECs and ISCs, leading to autocrine cytokine signaling, which further

stimulates proliferation. Later in the process, the same Hippo pathway acts negatively to turn off cytokine production and to block the activity of the Yorkie transcription factor so that the system can return to homeostasis (Jiang & Edgar, 2012). Aberrant regulation of the Hippo pathway, as well as of the pathways feeding into it, is prone to uncontrolled hyperproliferation and tumor-like phenotypes, particularly when combined with microbial dysbiosis, such as in the aged *Drosophila* gut. In fact, deregulation of Hippo signaling has been linked to many human tumors including CRC development, underscoring the importance of the discoveries made in *Drosophila*.

The microbiota is also an important factor in CRC and increasing evidence point out a protective role of bacteria producing SCFAs, including butyrate, propionate, and acetate. The mechanism whereby SCFAs protect against CRC is not fully elucidated but probably involves inhibition of inflammatory cells, reducing oxidative stress and promoting a healthy microbiota. Reciprocally, it is clear that some bacterial species produce metabolites with direct or indirect toxic effects on the epithelial cell, possibly also involving DNA damage, with direct consequences for malignant transformation. Such compounds include secondary bile acids, ROS, and *N*-nitrosamines (for a detailed review on the topic, see Louis et al., 2014).

Given the far-reaching similarities between human and fly with regard to microbiota-mediated effects on epithelial cell proliferation and differentiation, with links to adenoma and CRC development in humans, we suggest that this is an area where *Drosophila* should continue to provide fundamental insight into common processes with implications for human disease.

5. *DROSOPHILA* AS A MODEL FOR HUMAN RESPIRATORY ORGAN DISEASES LINKED TO INFECTION AND INFLAMMATION

Respiratory tract infections (RTIs) are very common in clinical practice and lead to significant morbidity and mortality worldwide. RTIs can further be classified according to viral and bacterial causes. Viral RTIs include the most common RTI viruses, i.e., influenza (adults and children) and RSV (children) but will not be further discussed here. Bacterial RTIs comprise pneumococcal pneumonia, which is the most common single etiology to community-acquired pneumonia. In contrast, hospital-associated pneumonia is mostly caused by other bacterial species, including the opportunistic pathogens *S. aureus*, *Klebsiella pneumoniae*, and *P. aeruginosa*, which

often are multidrug resistant and difficult to treat. The number of patients with immunosuppression is increasing in modern medicine, due to more advanced surgery, organ transplantation, and use of antiinflammatory drugs, blocking important immune pathways. The result is that the bacteriological cause to severe RTI in patients is increasingly diverse. Moreover, the emerging epidemic of multidrug-resistant bacteria requires a deeper understanding of host–microbial interactions, processes that could be further studied in *Drosophila* as a model.

Rodents are the most commonly used animal models for lung diseases such as pneumonia, asthma, and lung cancer. Although the rodent models mimic human lungs well in terms of chemical and physical conditions and also provide the possibility to carry out in vivo lung infections and studies of other lung pathologies, the results cannot be extrapolated directly from these models to humans. In addition, experimental lung infections in mammals are controversial from an ethical standpoint, and development of complementary models is desirable. The *Drosophila* model is one of the most interesting ones, as it has an airway system that can be regarded as a lung equivalent. As described earlier, *Drosophila* is a cost-effective model that can be infected by human pathogens and screened in different genetic background to pinpoint important host factors both for immunity and for pathogen virulence, and used in drug screens for chemical compounds that can inhibit disease progression.

On a superficial level the respiratory organs of flies and humans may seem very different. However, there are in fact far-reaching similarities in the development, physiology, function, and in responses to microbes between insect trachea and the lungs of mammals. Although these are not homologous organs, both airway systems consist of epithelial tubular organs that supply the whole organism with oxygen. The insect trachea is, just as the human lungs, a gas-filled branched tubular organ consisting of primary, secondary, and terminal branches. The exchange surface area increases with branching; hence, most gas exchange occurs in the distal parts. The organogenesis of these branched tubular networks has been found to share many fundamental principles between the fly and mammalian airway systems, especially in genetic components and the signaling pathways that control their branching (Horowitz & Simons, 2008; Samakovlis et al., 1996). In this aspect, the *Drosophila* embryo has been an excellent model for manipulating gene activities and subsequent analyses of the consequences for development and maturation of the trachea. However, it has been much less utilized for functional assays of airway performance in the larval stages or in response to

environmental factors. In contrast, the prenatal and early postnatal development of mammalian lungs, as well as exposure to environmental risk factors, have got much attention in recent years in being predictive for the development of chronic lung diseases later in life (Krauss-Etschmann et al., 2013). Further development of the fly model, with more physiological readouts of airway function, such as oxygen consumption, would improve its usefulness.

5.1 Human Lung Responses to Infection

The human lung depends on epithelial cells for keeping the barrier intact against the external environment. Just as other epithelia, lung epithelial cells provide both physical and chemical protection against microbes. Airway epithelia express most of the known PRRs including TLRs, NLRs, and CLRs, thus enabling recognition of bacteria, virus, and fungi. In analogy with the gut, the human lung contains a microbiome, which differs significantly between healthy individuals and those with an inflammatory lung disorder, such as asthma and cystic fibrosis (CF). It is clear that this component of the human lung has to be taken into account for a full understanding of any disease process affecting the lung. Most of the effector systems present in other epithelia are also present in the lung. For example, mucins, which constitute an important part of mucociliary clearance, are important for containing and removing pathogenic bacteria. Defect mucin production has been shown in CF, for example, and a lack of mucus transport, like in the cilia-deficient Kartagener's syndrome, is associated with chronic bacterial RTIs in these patients, thus lending support for a key role of mucins in lung immunity. In addition, airway epithelia produce AMPs of both the cathelicidin and defensin families during infections. The importance of LL-37 in lung immunity has been shown by using a mouse knockout model (Kovach et al., 2012). It is, however, important to remember that an important source of LL-37 in the lung is from incoming neutrophils. Neutrophils also deliver human α-defensins to the site of infection. In contrast, human β-defensins are exclusively produced by epithelial cells. Finally, the ROS-based effector system is necessary for an intact lung defense against microbes, as shown in the CF-lung where ROS levels are decreased (Hiemstra et al., 2015).

5.2 *Drosophila* Tracheal Responses to Infection

The lumen of *Drosophila* trachea is covered by a cuticular lining that serves as a physical barrier against dehydration, and also against microbes that may

enter through the tracheal openings. When *Drosophila* larvae are forced to crawl in food infected with pathogenic bacteria or fungus, the immune-competent tracheal epithelial cells respond by expressing AMPs and other immune and stress response genes (Ferrandon et al., 1998; Tzou et al., 2000; Wagner et al., 2008). The IMD pathway is the major immuno-responsive pathway activated in trachea upon bacterial infection. It requires PGRP-LC or PGRP-LE, while PGRP-LF seems to act as a negative regulator (Maillet et al., 2008; Persson et al., 2007; Takehana et al., 2004). The subsequent transcriptional activation by NF-κB/Relish in *Drosophila* trachea elicits expression of a smaller set of immune-induced genes compared to other immunoresponsive tissues (Gendrin et al., 2013). This may be due to the presence of negative regulation, as suggested for different regions of the gut epithelium, or due to the requirement of trachea-specific positive regulators, or a combination of these.

The activation of the IMD pathway was found to not be strictly cell autonomous in tracheal epithelial cells as it could spread to neighboring cells (Akhouayri et al., 2011; Takehana et al., 2004). This nonautonomous spreading was enhanced in mutants of Toll-8/Tollo, its putative ligand Spätzle-2, and intracellular mediator ECT-4 (a TIR domain protein homologous to mammalian SARM), indicating a role of this Toll-8/Tollo pathway in negative regulation. In a study by Wagner et al. (2009), it was reported that prolonged infection of the *Drosophila* tracheal system initiates remodeling processes of epithelial structure, primarily as thickening of the epithelial cell layer. Microarray analysis indicated changes in expression of genes involved in tracheal development and cell cycle progression, and of genes known to modulate Hedgehog-, JNK-, JAK/STAT-, MAP/ERK kinase-, and Ecdysone-dependent signaling (Wagner et al., 2009). This is highly interesting in the light of the chronic inflammatory diseases of the human lung. However, more in-depth mechanistic studies will be required to understand the nature of the observed remodeling of the airway epithelium, and its usefulness as a model for human diseases that lead to lung epithelium remodeling and metaplasia.

5.3 *Drosophila* as a Model of Specific Lung Infections and Diseases

5.3.1 Asthma and Chronic Obstructive Pulmonary Diseases (COPD)

Asthma and chronic obstructive pulmonary disease (COPD) are the most prevalent chronic inflammatory diseases of human lungs. They share that structural alterations in the lung tissue lead to variable impairment of airflow.

In addition, a varying degree of inflammation is present, leading to a vicious circle of viral and bacterial infections followed by more inflammation and airway remodeling. The first line therapy is inhaled corticosteroids, which significantly improve the clinical condition, but also further suppress local immune responses, and thus may contribute to prolonged infectious susceptibility.

The cause of asthma is not known but the prevailing idea is that a genetic susceptibility interacts with environmental factors. A number of genes have been shown to be associated with asthma, including IL-33, PCHD1, and orosomucoid 1-like 3 (ORMDL3). Interestingly, these genes are expressed in lung epithelial cells, which suggest that the innate part of the immune system is more important than previously thought. The immunological profile in asthma is dominated by a strong Th2 dominance and release of IL-4 and IL-13. These cytokines impair the epithelial barrier, downregulate AMP expression, and provide a niche for respiratory viruses.

Although asthma previously has been regarded as a disease with strong links to adaptive immune responses, recent findings suggest that innate immune signaling within airway epithelial cells plays a primary role. In both asthma and COPD, NF-κB signaling is a central player in inflammatory gene expression, regulating cytokine activity, and airway pathology (recently reviewed in Schuliga, 2015). Asthma susceptibility genes have been identified by genome-wide association studies and good models are needed to clarify the roles of these genes in normal and diseased airway tissues. *Drosophila* has been suggested as a favorable model for elucidation of the physiological and pathophysiological significance of asthma susceptibility genes (Roeder et al., 2012). A recent report addressed the role of one of these predicted human asthma susceptibility genes in *Drosophila* (Kallsen et al., 2015). Polymorphisms in the human gene for an endoplasmic reticulum transmembrane protein, *ORMDL3*, have been highly associated with childhood asthma (Moffatt et al., 2007). *ORMDL3* was first studied in a mouse model, and its overexpression led to increased airway remodeling and airway responses typical of asthma (Miller et al., 2015). The *Drosophila* study corroborates these results and serves as an example to how the functional role of human asthma-linked candidate genes can be tackled in the fly model (Kallsen et al., 2015).

5.3.2 Hypercapnia

Hypercapnia is a condition of elevated blood and tissue concentrations of CO_2, which is common in patients with severe COPD. It has also been

linked to exacerbations of bacterial and viral infections in patients with other lung diseases, such as pneumonia, adenoviral lung infections, and CF. It is known that hypercapnia blocks NF-κB activation and normal expression of a number of immunoregulatory factors, and that it suppresses phagocytosis, ROS activation, and autophagy. The mechanisms underlying the immunosuppressive conditions of hypercapnia have been highly obscure. Recent work in *Drosophila*, in which many of the immune suppressive characteristics of hypercapnia are conserved, led to the identification of the zink finger homeodomain 2 (Zfh2) as a mediator of the hypercapnic immune suppression (Helenius, Haake, et al., 2016). The mammalian orthologs of Zfh2 are ZFHX3/ATBF1 and ZFHX4. By using a genome-wide RNAi screen in *Drosophila* S2 cells, followed by functional assays in vivo, it was shown that mutation in *zfh2* enable flies to mount a stronger immune response and survive infection better after exposure to hypercapnia. Thus, Zfh2 suppresses immune responses after CO_2 exposure, but not in normal air conditions. In a follow-up chemical drug screen with a CO_2-responsive luciferase reporter in *Drosophila* S2 cells, the same group identified a plant alkaloid, evoxine, as an inhibitor of some of the hypercapnia-induced immune defects (Helenius, Nair, et al., 2016). Most importantly, evoxine did rescue immune response capacity not only in *Drosophila* cells but also in human THP-1 macrophages. This indicates a strong evolutionary conservation of the pathway(s) regulating hypercapnia-induced immune suppression and also demonstrates that pharmacological drug screening for CO_2 effects can be addressed in *Drosophila* cells.

5.3.3 Cystic Fibrosis

Cystic fibrosis (CF) patients are frequently infected with *P. aeruginosa*. Chronic infections are linked to biofilm formation, and there is a need for simple infection models in which biofilm formation and its consequences can be followed. *P. aeruginosa* was shown already in 1972 to be a virulent pathogen of *Drosophila* (Boman et al., 1972), while less virulent *P. aeruginosa* mutants have been characterized subsequently using this host (D'Argenio et al., 2001). Mulcahy et al. (2011) developed a *Drosophila* in vivo model of *P. aeruginosa* biofilm formation and could show that biofilm infections were less virulent than nonbiofilm infections. The *Burkholderia cepacia* complex is a group of related bacterial species that are especially problematic for CF patients. *Drosophila* has been used in several studies as a host to study the virulence of different *B. cepacia* strains and mutants, and to isolate

specific virulence factors (Castonguay-Vanier et al., 2010; Schwager et al., 2013).

5.3.4 Tuberculosis

Tuberculosis is a serious human lung infectious disease, caused by *M. tuberculosis*. The mouse has been the dominating infection model used; however, mice are not a natural host for *M. tuberculosis* and disease progression including latency and reactivation has not been possible to study in this model. Instead, lethal infections of zebrafish and *Drosophila* with *M. marinum* have emerged as powerful models not only for acute infection stages but also for disease progression and immunopathology (Dionne et al., 2003, 2006). *Drosophila* has also been developed as a suitable host for testing new drugs against serious *M. abscessus* infections (Oh et al., 2013).

5.3.5 Fungal Lung Infections

The lungs are prone to fungal infections as they are exposed to airborne spores of common molds such as *Aspergillus* and *Fusarium*, which then can disseminate and lead to invasive aspergillosis in immune-compromised humans and also in flies (Lemaitre et al., 1996). As described earlier, *Drosophila* has been used to study virulence of a number of human fungal pathogens. The fly model has further been used for combinatorial drug tests and revealed synergistic effects of, for example, voriconazole and terbinafine against *Aspergillus* infection (Lionakis & Kontoyiannis, 2005).

5.4 The Role of Intestinal Microbiota in Lung Diseases

An increasing literature describes how the commensal gut microbiota affects lung immune responses in mammals. It has been reported that the microbiota composition of the gastrointestinal tract can affect allergy and asthma development, immune responses to lung infectious diseases, and trigger systemic inflammatory responses (reviewed in Samuelson et al., 2015).

When *Drosophila* larvae creep in the food and contaminate it with its excrements, gut microbiota will come in direct contact with the tracheal openings, the spiracles. It is likely that the composition of the gut microbiota will affect tracheal immune responses to pathogens. Although this seems as an interesting model to study the direct effects of gut microbiota on tracheal immunity and airway functions, as well as the response to other environmental factors, the possibility of using *Drosophila* has not yet been evaluated.

6. *DROSOPHILA* AS A MODEL OF HUMAN SKIN INFECTIONS AND WOUND HEALING

Although there are important structural differences between human and *Drosophila* skin (Harden, 2005), *Drosophila* can serve as a good model for skin development, barrier immunity, and wound healing, and as a screening tool for novel therapeutic targets and drug discovery (Munoz-Soriano et al., 2014).

The physical structure and relatively impermeable nature of the outer layers of skin and epidermis, like hair and nails in humans and the cuticle of insects, serve as efficient protection against many types of physical and chemical types of stress. However, the underlying skin and epidermis must be flexible and allow for exchange of gases, fluids, and molecules, which makes them vulnerable to insult. In addition, some microorganisms have evolved ways to breach the physical barrier. To further protect the underlying tissues, barrier epithelia are also equipped with chemical and immunological barriers, which creates unfavorable conditions for microorganisms, such as high salt concentration, low pH, production of lipid-rich sebum, ROS, and AMPs.

6.1 Expression and Regulation of AMPs in Skin/Epidermis

The most prominent innate immune effector molecules in the skin/epidermis of both humans and flies are AMPs. There is constitutive expression of some AMPs in the absence of microbial stimuli, while expression of other AMPs requires the presence of microbial products.

The dominating families of AMPs in human skin are the cathelicidins and β-defensins. Keratinocytes are the primary cells in the skin to produce AMPs under normal conditions, but resident mast cells also contribute, and upon infection AMP-producing neutrophils are recruited (Gallo & Hooper, 2012). In *Drosophila*, relatively few studies have addressed expression of AMPs in the epidermis. It was shown using reporter assays that the gene for *CecA1* is activated in infected wounds in larvae (Önfelt Tingvall et al., 2001), and that expression of *CecA1* and Diptericin (*Dipt*) can be induced by bacteria-derived molecules in the epidermis of embryos (Esfahani & Engstrom, 2010; Tingvall et al., 2001). This epidermal expression was dependent on the IMD pathway and on the downstream NF-κB transcription factor Relish.

The human cathelicidin gene is upregulated in skin in response to injury and infection, by the vitamin D receptor (VDR) and its ligand 1,25 dihydroxyvitamin D3 (Gombart et al., 2005; Liu et al., 2006; Wang et al., 2004). Interestingly, *Drosophila* AMP expression is also regulated by nuclear hormone receptors, such as the ecdysone receptor (EcR; Rus et al., 2013). The ligand 20-hydroxyecdysone (20E) has been shown to be involved in pathogen-induced AMP expression in flies and in cell lines (Dimarcq et al., 1997; Meister & Richards, 1996), but a role in epidermal AMP gene expression has so far not been reported. In contrast to the regulation of human immune genes by the VDR, which directly targets the AMP gene regulatory regions, *Drosophila* EcR regulation seems to be indirect, via regulation of the pattern recognition receptor PGRP-LC and of other IMD pathway components (Rus et al., 2013). However, the role of VDR in human innate immunity also plays many other roles, both direct and indirect. The roles of nuclear hormone receptor signaling in innate immunity are likely much larger than our knowledge of today. The use of *Drosophila* should enable systematic analysis of individual hormone receptors and their functions.

6.2 Skin Microbiota

The skin of humans and the cuticle of insects are habitats of huge and diverse populations of microbiota. Many of these are probably just transient "guests," but many species can be recognized as human skin commensals. The composition of resident microbes in the skin may play important roles in both causing and preventing noninfectious skin diseases, such as psoriasis, atopic dermatitis, rosacea, and acne. As in other epithelia, commensal and symbiotic bacteria can serve as beneficial constituents by directly competing with and protect against growth of more pathogenic species. They also stimulate innate and adaptive immunity in the host, thereby strengthening both barrier functions and responses that prevent infections. However, genetic predisposition, injuries, and other causes of altered barrier integrity may promote pathogenic growth of normally nonpathogenic species or drive skin microbiota to initiate or amplify human skin disorders (Belkaid & Segre, 2014).

Analysis of bacterial composition of human skin has revealed four dominating phyla: *Actinobacteria*, *Firmicutes*, *Bacteroidetes*, and *Proteobacteria* (Grice et al., 2009). These phyla also dominate the human inner mucosal surfaces, but the relative proportions differ considerably. Depending on local

differences in skin physiology (moist, dry, or sebaceous), certain species are dominating in different areas, such as *Propionibacterium* spp. at sebaceous sites, *Staphylococcus* spp. and *Corynebacterium* spp. in moist areas, while *Malassezia* fungal species dominate dry areas of the body (Findley et al., 2013).

Analysis of the *Drosophila* microbiota has to a large extent focused on the analysis of the gut (Broderick & Lemaitre, 2012). *Drosophila* laboratory strains were also found to carry several bacterial species in their guts that can be considered as human skin commensals, but that also can cause severe skin infections, such as *Staphylococcus* spp., *Streptococcus* spp., *Corynebacterium* spp., and *Neisseria* spp. (Dantoft et al., 2016). It suggests that *Drosophila* may harbor a gut flora that includes a broad range of human commensals and opportunistic species. Although this may primarily reflect the close physical interaction between humans and flies in laboratory settings, it also suggests that a broader range of host–microbe interaction studies may be conducted in flies than previously anticipated.

6.3 Wound Healing and Immunity

Wound healing and related problems are very common in medical clinics. Much research is focused on human cell cultures and mouse models. But tissue damage is a process that involves the entire organism, and whole-animal models are needed for a comprehensive understanding. Wound healing, tissue repair, and regeneration are intimately coupled to activation of immune responses. This will prevent infection and fight invading pathogens, but is also involved in local and systemic signals that induce tissue repair or replacement. *Drosophila* is a good model for many aspects of wound repair including local immune responses that have been shown to be evolutionarily conserved.

An important part of the wound healing process is the reepithelialization and recreation of barrier functions. This process differs in an important aspect between humans and *Drosophila*, as human epidermis contains stem cells that proliferate and migrate to the wound site to heal the wounds, while *Drosophila* epidermis do not have proliferating cells (Harden, 2005) and reepithelialization has to occur by other mechanisms. In the *Drosophila* embryo, an actin–myosin cable closes the hole like a purse string, and the actin cytoskeleton is also important in larval wound healing (Razzell et al., 2011). In *Drosophila* adult skin, diploid epithelial cells undergo polyploidization and cell fusion to create large cells that can grow in size, spread, and heal the wound (Losick et al., 2013). Although the mechanisms

that replace the lost cell mass is different, considerable conservation in activation of signals and cellular activities during wound healing has been demonstrated between *Drosophila* and humans, as reviewed in Davis and Engstrom (2012), Lee and Miura (2014), Munoz-Soriano et al. (2014), Razzell et al. (2011), and Stramer and Dionne (2014), and only a few examples will be given here. In both *Drosophila* and mouse, transcription factors of the Grainy head (GRH) family have been shown to activate genes involved in cross-linking processes and in scab formation at the wound site (Harden, 2005) as well as in cuticle formation in flies and corneum stratum formation in humans (Mace et al., 2005; Ting et al., 2005; Wang & Samakovlis, 2012). The c-Jun N-terminal kinase (JNK) cascade is activated at the wound site and is necessary for tissue repair in both flies and human (Angel et al., 2001; Ramet, Lanot, et al., 2002). The damaged cells produce ROS locally, such as the release of hydrogen peroxide by calcium flashes and DUOX activation, which subsequently triggers recruitment of inflammatory cells both in zebra fish and in flies (Razzell et al., 2013). As mentioned earlier, immune responses are also triggered at the wound site, as revealed by local expression of AMPs in *Drosophila* (Önfelt Tingvall et al., 2001) and in humans (Mangoni et al., 2016). Finally, the tissue damage activates systemic responses where the *Drosophila* system provides a good model to follow the inter organ communication and its consequences at the whole organism level (Lee & Miura, 2014). These examples indicate the impact research in *Drosophila* has had for our general understanding of epithelial repair processes. The high level of conservation underscores the usefulness of this model in future studies of skin integrity, barrier functions, and wound healing.

7. CONCLUDING REMARKS

Here we have reviewed the innate immune system of *Drosophila* and human with a focus on the gut, the respiratory tract, and the skin. There is no doubt that many key components of innate immunity have been well conserved during evolution, including microbial recognition, intracellular signaling pathways, and effector mechanisms. Future studies of epithelial immunity using the *Drosophila* model are in fact likely to identify many more components and processes that are evolutionarily ancient. In addition to increasing our present knowledge, such findings may have great relevance for understanding the underlying mechanisms of human disease. A deepened collaboration between researchers active in the *Drosophila* field and medical

scientists should likely promote scientific breakthroughs of medical importance in this area.

The accessibility to next-generation sequencing in clinical practice has opened up a new avenue for molecular understanding of human disease with great clinical relevance. In fact, it is now possible to perform whole genome sequencing of a patient's DNA and to obtain a list of candidate genes in a few days. However, the functional validation of candidate genes and linking them to disease is still a huge undertaking. The *Drosophila* model with its versatile genetic toolbox provides excellent possibilities to unravel the specific roles of candidate genes emanating from such human diagnostic projects.

In human medical research of innate immunity the field is now ready to turn the detailed knowledge on innate immunity into therapeutic approaches. There are many attempts to boost impaired immune pathways or to block excessive inflammation by targeted approaches. Another approach is to induce effector mechanisms, such as AMP expression or activation of autophagy. The *Drosophila* model can provide a fast-track to screen for novel compounds directed toward specific receptors or pathways. In particular, this strategy could be very useful if coupled to large chemical libraries consisting of already approved drugs, which will shorten the time from experimental setup to clinical use by many years.

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