Multiple TGF-β Superfamily Signals Modulate the Adult Drosophila Immune Response

Rebecca I. Clark,1,2 Katie J. Woodcock,3 Frédéric Geissmann,1 Céline Trouillet,1 and Marc S. Dionne1,4

1Centre for the Molecular and Cellular Biology of Inflammation and Peter Gor ner Department of Immunobiology, King’s College London School of Medicine, London SE1 1UL, UK

Summary

TGF-β superfamily signals play complex roles in regulation of tissue repair and inflammation in mammals [1]. Drosophila melanogaster is a well-established model for the study of innate immune function [2, 3] and wound healing [4–7]. Here, we explore the role and regulation of two TGF-β superfamily members, dawdle and decapentaplegic (dpp), in response to wounding and infection in adult Drosophila. We find that both TGF-β signals exhibit complex regulation in response to wounding and infection, each is expressed in a subset of phagocytes, and each inhibits a specific arm of the immune response. dpp is rapidly activated by wounds and represses the production of antimicrobial peptides; flies lacking dpp function display persistent, strong antimicrobial peptide expression after even a small wound. dawdle, in contrast, is activated by Gram-positive bacterial infection but repressed by Gram-negative infection or wounding; its role is to limit infection-induced melanization. Flies lacking dawdle function exhibit melanization even when uninfected. Together, these data imply a model in which the bone morphogenetic protein (BMP) dpp is an important inhibitor of inflammation following sterile injury whereas the activin-like dawdle determines the nature of the induced immune response.

Results and Discussion

decapentaplegic and dawdle Are Regulated by Immune Challenge

The innate immune response and its underlying pathways are highly conserved between Drosophila and mammals [3, 8, 9]. Although much work in Drosophila has focused on the central pathways of pattern recognition [3], many other signals modulate innate immune mechanisms, and many of these are also evolutionarily conserved [8, 9]. Because TGF-β superfamily signals are critical regulators of mammalian immune responses [1], we examined the immune regulation of two of these signals in the fly: decapentaplegic (dpp), a bone morphogenetic protein (BMP)-type signal, and dawdle (daw), an activin/TGF-β-like signal.

dpp and dawdle expression were regulated by immune challenge. dawdle expression was initially repressed 1 hr after injection of either Micrococcus luteus or sterile phosphate-buffered saline (PBS) and subsequently induced 6 hr after M. luteus infection (Figure 1A). dpp was induced by either sterile wounding or infection, though with slightly different timing (Figure 1B). E. coli infection did not change dpp or dawdle expression beyond the effect of wounding alone (see Figure S1 available online).

To analyze the signaling underlying dawdle and dpp regulation by M. luteus infection, we first examined the role of NF-κB family members. In adult flies, the Toll pathway acts via the NF-κB-like factor Dif, while the imd pathway acts via the NF-κB-like factor Rel [3]. In Dif/Rel double mutants, baseline expression of dawdle was reduced and its induction by M. luteus infection was eliminated, but the early repression of dawdle was unaffected (Figure 1C). Untreated Dif/Rel mutants showed higher expression of dpp than controls, but, as with dawdle, induction of dpp by wounding or infection was lost (Figure 1D).

dpp and dawdle are therefore NF-κB-regulated. To assess the relative contributions of Toll and imd pathways, we assayed expression of dpp and dawdle in flies mutant only for Rel. Untreated Rel mutants showed increased baseline expression of dpp and dawdle, similar to Dif/Rel double mutants (Figure S1C; Figure 1B). Loss of Rel alone did not impair dawdle induction following infection (Figure S1C). However, the peak in dpp expression at 1 hr following M. luteus infection was lost in Rel mutants, though some dpp induction following wounding was retained (Figure 1E). To determine whether Toll signaling was sufficient to induce dpp/dawdle, we expressed activated Toll (UAS-T110b; [10]) with heat-shock Gal4 (Figure S1D). Both dpp and dawdle were induced by Toll activation (Figure 1F).

These data suggest that Rel drives dpp induction upon infection whereas Dif does so in response to wounding. In contrast, Dif drives dawdle expression upon infection.

Finally, we tested the role of the Jun N-terminal kinase (JNK) pathway in regulation of dpp and dawdle by wounding; this pathway is required for wound healing in Drosophila [4, 5]. Immune-induced JNK activation is mediated by Tak1 downstream of imd, and in larvae Tak1 is required for activation of JNK by sterile wounds [11, 12]. Therefore, we assayed dpp and dawdle in Tak1 mutants. Tak1 mutants showed a significant increase in baseline dpp expression relative to controls (Figure S1E). Loss of Tak1 did not significantly alter dpp induction following infection or wounding (Figure S1E), but dawdle repression 1 hr following injection with PBS or M. luteus was abolished in Tak1 mutants (data not shown; Figure 1G). Thus, Tak1 activation represses dawdle.

These data indicate that JNK and NF-κB regulate dpp and dawdle in different immune contexts. The differences in regulation of these ligands suggested that they might play distinct roles in the immune response.

dpp Suppresses the AMP Response to Wounding

We next examined the function of wound-induced Dpp. Dpp signals via a receptor complex containing the type I receptors Tkv and/or Sax and the type II receptor Punt [13]. Activated Tkv/Sax phosphorylates the transcription factor Mad. Phosphorylated Mad binds the co-Smad Medea to regulate target gene expression. Repressive activity is conferred by binding of the Mad-Medea complex to silencer elements, which allow recruitment of the corepressor Schnurri [14]. Independent of
the work described here, we carried out an in silico screen to identify transcription factors responsible for coordinated gene regulation following immune activation. This identified Mad-Med-shn silencer elements near many antimicrobial peptide (AMP) genes (Figure S2A). Notably, the silencer elements identified near Defensin are functional, repressing pentagonome magg, the surrounding gene [15]. This suggested that wound-induced Dpp might repress AMP expression via Mad.

To test whether Dpp is sufficient to repress AMP expression, we injected wild-type flies with human BMP-4, the homolog of dpp, or with vehicle only and assayed AMP responses to this injection. Each AMP assayed showed a lower transcript level in samples that had received BMP-4, relative to vehicle controls (Figure S2B). We then confirmed this result with the endogenous signal. Because BMPs often signal as heterodimers and in these cases, the heterodimer is generally more potent [16], we overexpressed both dpp and gbb (the second BMP in the adult fly) in wounded animals under the control of heat-shock Gal4 (Figure S2C) and assayed AMP expression 3 hr after wounding. BMP expression induced following wounding reduced expression of five of six assayed AMPs (Figure 2A). Finally, to test the in vivo role of the dpp-Mad signal, we assayed AMP expression in flies with Mad knocked down in the fat body, the tissue primarily responsible for AMP expression upon systemic immune challenge. Loss of fat body Mad increased AMP expression, particularly after sterile wounding (Figure 2B).

These data indicate that Dpp represses AMP expression following wounding, particularly in the absence of infection. The presence of Mad-Med-shn silencer elements near AMP genes suggests that this repression is in part direct. Dpp may thus be important following tissue damage in the absence of infection to avoid unnecessary AMP responses.

Dawdle Suppresses Melanization via the Activin Pathway
To identify daw’s immune role, we produced animals carrying a ubiquitous daw knockdown (Figure S3A). Over 50% of these flies had melanotic tumors (Figure 3A), suggesting that daw inhibits melanization, a key effector mechanism of arthropod immunity. The melanization cascade is tightly controlled, presumably to prevent immune-induced pathology [17, 18]. On assaying known regulators of melanization in these flies, we found increased expression of Serine protease 7 (Sp7), which is required specifically for infection-induced melanization [19] (Figure 3B).

daw signals primarily via the sole Drosophila type I activin receptor, Baboon (babo) [20]. To test whether daw-babo signaling is sufficient to inhibit Sp7 expression, we assayed Sp7 levels in flies overexpressing daw or activated babo (Figure S3B) in adult fat body using the Gal4 driver c564 with tubulin-Gal80ts. daw overexpression or activated babo expression dampened Sp7 induction by infection but did not affect Sp7 in untreated or PBS-injected animals (Figure 3C). Thus, endogenous daw inhibits Sp7 expression in the absence of infection, whereas daw overexpression can inhibit infection-induced expression. In the context of our data on daw regulation, this suggests that activated Dif drives daw expression, shutting down Sp7 to limit infection-induced melanization.

Sp7 is important for resistance to Listeria monocytogenes and Salmonella typhimurium infections [21]. We thus examined the role of daw during Listeria infection. Sp7 expression was induced early following Listeria infection (Figure S3D). The temperature shift involved in our infection protocol confounded interpretation of daw expression at early time points; however, daw was strongly induced on the fourth day postinfection, relative to untreated and PBS-injected controls.
The peak in daw expression 5 days postinfection (Figure 3D) correlated with a plateau in Sp7 levels (Figure S3D); after this time, daw levels fell but Sp7 did not change, implying the presence of other Sp7 regulators. Overexpressing daw or activated babo resulted in rapid death from Listeria infection (Figure 3E), suggesting that suppression of Sp7 by daw is detrimental to survival, much like the loss of Sp7 through mutation [21].

Unlike BMP manipulations, activated babo expression in the fat body gave no consistent effect on AMPs (Figure S3C), and Mad knockdown did not induce melanization (data not shown). This indicates distinct immune roles for daw and dpp.

Figure 3. Dawdle Signals via the Activin Pathway to Suppress Melanization  
(A) Melanotic tumors in daw knockdown flies (w;UAS-daw-IR/+;tubulin-Gal4/+). Two independent inverted repeat (IR) lines are shown (VDRC13420 and VDRC105309). Tumors are indicated by arrow. 
(B) Sp7 expression 5 days after eclosion in daw knockdowns and driver-only controls. 
(C) Sp7 expression in flies overexpressing daw or activated babo in adult fat body (w;UAS-daw/c564/tubulin-Gal80+/+ or c564/+;UAS-act.babo/tubulin-Gal80) relative to controls (c564/+;tubulin-Gal80+/+). Animals were untreated or collected 6 hr postinjection with PBS or mixed E. coli and M. luteus. Expression is normalized to untreated driver-only controls. 
(D) daw expression following Listeria infection of wild-type flies. Expression is normalized to day 0 untreated levels. 
(E) Survival of daw- and activated babo-expressing flies following Listeria infection, relative to driver-only controls. (Lines labeled “OE” correspond to overexpressors.) Survival of both misexpression lines is different from controls (p < 0.001).

For qRT-PCR assays in (B)-(D), expression was initially normalized to Rp11, and means are shown ±SEM. ***p < 0.001, **p < 0.01, *p < 0.05 by Mann-Whitney test. See also Figure S3.
abdominal regions or the legs (Figure S4C). dpp-Gal4 and daw-Gal4 were also expressed in pericardial nephrocytes, as was crq-Gal4 (Figure S4A), and in the adult salivary gland (Figure S4D). dpp-Gal4 was expressed in gut as previously described [25], whereas daw-Gal4 gave no gut expression (Figure S4E). These patterns were corroborated with different dpp-Gal4 and daw-Gal4 lines (data not shown). We detected no change in mRFP expression after infection or wounding with either driver (data not shown).

*Drosophila* hemocytes are macrophage-like cells that phagocytose bacteria and apoptotic cells and secrete extracellular matrix components and immune peptides [2]. Due to the hemocyte-like expression patterns of the dpp-Gal4 and daw-Gal4 lines, we assayed the phagocytic activity of dpp- and daw-expressing cells by injecting fluorescent dead *Staphylococcus aureus*, labeling phagocytes throughout the animal. All images

![Image of the document](image-url)
Neither injection changed the number of cells expressing dpp-Gal4 or daw-Gal4 (Table S1).

Finally, we confirmed that dpp and daw were expressed by Hml* cells. We used fluorescence-activated cell sorting (FACS) to isolate Hml* cells from adult Drosophila, an approach previously used in larvae [26] (Figure 4V). Quantitative RT-PCR on FACS-isolated hemocytes showed expression of Hml, as expected, as well as crq, dpp, and daw (Figure 4V; Figure S4F).

dpp and daw are therefore each expressed in a subset of hemocytes. Further work will be necessary to characterize the subsets of the adult hemocyte population, the extent to which these subsets overlap, and the implications of distinct gene expression profiles for hemocyte function.

Conclusions

We show that the TGF-β superfamily members daw and dpp are physiological regulators of Drosophila immunity. dpp is induced by wounding and infection and helps resolve the antimicrobial peptide response, whereas daw is repressed by wounding, is induced by the Toll pathway, and limits infection-induced melanization. The modulation of the downstream signaling pathways in the fat body is sufficient to produce significant changes in whole-animal levels of target gene transcripts. However, although the fat body is responsible for the majority of induced AMP expression, many other tissues respond to immune activation. dpp and daw, as secreted signals, may act systemically to regulate other target genes in other tissues throughout the animal.

dpp and daw are expressed in hemocytes but also in other tissues. The regulation of these signals in a given tissue may reflect a distinct function for that tissue in sensing infection or wounding. The expression of dpp and daw in a fraction of hemocytes is particularly intriguing in this context. The fact that hemocyte-specific overexpression of dpp is sufficient to repress AMP induction [27] supports a hemocyte origin for this signal. Several types of hemocyte have been characterized in Drosophila larvae [3, 28]; however, the hemocytes of the adult fly have been largely neglected and are widely believed to consist of a single cell type. To our knowledge, this is the first indication that the hemocyte population in the adult fly is comprised of distinct subsets of cells that can be defined through distinct gene expression profiles. We think it likely that expression of dpp and daw by a subset or subsets of phagocytes indicates distinct immunomodulatory functions for these cells.

Both dpp and daw inhibit immune responses. This aligns the fly with mammals, in which both activin/TGF-β-like and BMP-like signals are broadly anti-inflammatory [29–31], in contrast with C. elegans, where the TGF-β superfamily member dbi-1 promotes a variety of antimicrobial responses [32, 33]. Study of these signals in Drosophila will allow further characterization of individual signals and of mechanisms of signal integration in a way that is not currently possible in more complex systems.

Supplemental Information

Supplemental Information includes four figures, one table, and Supplemental Experimental Procedures and can be found with this article online at doi:10.1016/j.cub.2011.08.048.

Acknowledgments

We thank Robert Ray, Mike O’Connor, Julian Ng, Dominique Ferrandon, the Vienna Drosophila RNAi Center, and the Bloomington Drosophila Stock Center for providing flies. Karen Liu, Mimi Shirasu-Hiza, Miriam Baron, Rob Ray, Brian Stramer, and the other members of the Dionne and Stramer laboratories provided valuable discussion and/or comments on the manuscript. This work was supported by funding from the Wellcome Trust and the Biotechnology and Biological Sciences Research Council (BB/E02128X/1).

Received: April 20, 2011
Revised: August 2, 2011
Accepted: August 19, 2011
Published online: September 29, 2011

References


