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The *Drosophila* cytokine, GBP: a model that illuminates the yin-yang of inflammation and longevity in humans?

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Abstract

Our laboratories have determined that the *Drosophila* cytokine, Growth-blocking peptide (GBP), mediates its biological effects through the Mthl10 G-protein coupled receptor. In this *Cytokine Stimulus*, we discuss the functional plasticity of the GBP/Mthl10 axis, and we propose that conserved components of this regulatory network may be relevant to human health.

We live in a dangerous world. Our survival relies upon the speed and efficacy with which the cells of our innate immune system can combat invading pathogens through both cellular and humoral pathways [1]. However, innate immune cells also participate in the management of responses to stress, general maintenance of organismal homeostasis, and they also play roles in growth and development [2,3]. These pleiotropic properties of immune cells are inherently hazardous; collateral tissue damage can be caused by the very inflammatory responses that are implemented to protect against environmental stress [4]. Thus, there is considerable interest in the idea that the expression of the various responses of innate immune cells is carefully measured through ‘functional plasticity’: a microenvironment-dependent, phenotypic flexibility which selects specific functions to serve for an appropriate time-frame, in distinct contexts [4]. Serious consequences arise out of the failure of this carefully orchestrated balancing-act: either a chronic, unresolved stress, versus an increased risk of inflammatory-based health problems such as arthritis, atherosclerosis, neurodegenerative disorders, metabolic maladaptation, and cancer. Clearly, functional plasticity is important, but little is known concerning the underlying molecular mechanisms [4].

Drosophila has long been considered a relevant, genetically tractable model for studying general mechanisms of innate immunity [5]. Thus, we have studied the molecular pathways that mediate the actions of one particular insect cytokine, Growth-blocking peptide (GBP).

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This cytokine was initially found in the hemolymph of growth-inhibited lepidopteran larvae that had been infected with a parasitic wasp [6]. *Drosophila* GBP also regulates humoral and cellular innate immune responses [7], it participates in metabolic homeostasis [8] and adaptation to low temperatures [9]. These multiple biological activities of this cytokine show it to be an appropriate model for studies into functional plasticity. Moreover, we [10] noted that *Drosophila* GBP exhibits sequence similarity to the human defensin BD2, a member of the immunomodulatory β -defensin family [11]; both human and fly cytokines are small, cationic peptides produced by protease action upon larger, precursor proteins [11,12]. Furthermore, the inositol phosphate (IP)/Ca²⁺ signaling cascade is recruited by both *Drosophila* GBP [13] and human BD2 [14] to serve their biological actions. Thus, we hypothesized that, by characterizing the molecular basis for GBP activities in the fly, we would gain insight into fundamental mechanisms of cytokine signaling in humans.

We [15] demonstrated that *Drosophila* GBP activates an IP/Ca²⁺ signaling cascade that dictates the timing and the intensity of the separate cellular and humoral components of the innate immune response to be reciprocally regulated, even within a single cell. Specifically, we discovered GBP initially prioritizes neutralization of an invading pathogen by activating cellular defense programs (phagocytosis and encapsulation), while inhibiting humoral pathways (release of inflammatory cytokines), thereby minimizing the risk of collateral damage. We have described how GBP achieves these effects through an IP/Ca²⁺ mediated activation of a receptor-regulated tyrosine kinase cascade (the PVR/ERK pathway) [15].

We next set out to identify the cell-surface protein that initiates these signaling events: the GBP receptor. We deployed a dsRNA library that targets *Drosophila* genes encoding membrane proteins. Using a line of *Drosophila* S3 cells that host a genetically encoded Ca²⁺-sensor, GCaMP3, we screened for gene knockdowns that attenuated GBP-dependent Ca²⁺ mobilization [10]. One of our top 'hits' was a *Drosophila* G-protein coupled receptor (GPCR), Mthl10, that previously had no known function - it had been an orphan receptor. To validate that Mthl10 is a GBP receptor, we prepared a recombinant version of the ectodomain and demonstrated it binds a fluorophore tagged version of GBP by fluorescence polarization [10].

Having identified the GBP receptor, we next probed its functionality through genetic approaches. Knockdown of *Mthl10* in the fly resulted in increased mortality upon bacterial infection, and impaired adaptation to an environmental stress (cold temperature). Yet, in a protected, unstressed laboratory environment, the lifespan of *Mthl10* knockdown flies increased by 25% [10]. Conversely, lifespan was reduced by up to 16% upon overexpression of GBP – i.e., induction of a pro-inflammatory state (Fig. 1). These data support a theory that aging is associated with a maladaptive induction of subclinical and low-grade inflammation, termed “inflammaging” [16]. Our results further indicate that a young organism's efforts to defend against repeated infections and other environmental stress may result in a Pyrrhic victory that impacts longevity. From a human-health perspective, avoiding infections is not a particularly viable anti-inflammatory strategy. However, there may be an alternative option: there is evidence humans can reduce their circulating levels of inflammatory cytokines, without detriment to immune responses, through a moderate

reduction in dietary calories [17]. Nevertheless, the mechanism that underlies this effect is not fully understood.

Dietary restriction, which is well-known to extend lifespan in animal models [17,18], is associated with reduced expression of the *GBP* cytokine gene in *Drosophila* [8]. Moreover, nutrient status sensing by *Drosophila* target of rapamycin (TOR) [19] stimulates the fat body to secrete GBP, which releases insulin-like peptides (ILPs) from the brain [8]. In our study, we demonstrated that *Mthl10* is expressed in the brain cells that produce and secrete the ILPs [10]. Moreover, knockdown of *Mthl10* expression inhibits ILP secretion [10]. That is, we demonstrated that metabolic homeostasis and immune responses are both co-ordinated by the GBP/*Mthl10* axis. This should be functionally significant because the mounting of an immune response is bioenergetically expensive; it takes a considerable energy investment by immune cells to synthesize and secrete a battery of cytokine and immunity-related effector molecules. Nevertheless, there may be a potential downside to this aspect of functional plasticity: TOR hyperactivation by excess nutrient intake [19] could recruit GBP/*Mthl10* to exacerbate metabolic inflammation (Fig. 1); this may help explain why nutrient excess in *Drosophila* can model human metabolic syndrome [19].

The *Mthl10* ectodomain is not conserved in humans [20]. Indeed, niche-specific selection pressures have caused many GPCR ectodomains and their ligands to co-diversify [21]. Nevertheless, there is considerable conservation of intracellular coupling between GPCRs and G-proteins; as a result, flies and mammals share many of the same downstream, GPCR-coupled signaling cascades [21]. In this respect, it is notable that the proposed human ortholog of GBP, i.e, the cytokine BD2 (see above), also activates IP/Ca²⁺ signaling through a GPCR, albeit one that has not yet been characterized at a molecular level [14]. Thus, we propose that studies into molecular aspects of GBP function have relevance to human biology. Characterization of the human BD2 GPCR may help rationalize why inflammation is reduced by calorie restriction in humans (see above), and may uncover a new drug target to combat persistent inflammation and extend healthy human lifespan [16].

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Figure 1.

Graphic depicting the impact upon longevity of the GBP/Mthl10 axis through its coordination of metabolic homeostasis with immune responses. AMP = anti-microbial peptides.