Autophagy Reports

ISSN: (Print) (Online) Journal homepage: www.tandfonline.com/journals/kauo20

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To cite this article: Magda L. Atilano, Alexander J. Hull & Kerri J. Kinghorn (2024) Autophagic dysregulation triggers innate immune activation in glucocerebrosidase deficiency, Autophagy Reports, 3:1, 2372997, DOI: 10.1080/27694127.2024.2372997

To link to this article: https://doi.org/10.1080/27694127.2024.2372997

Published online: 08 Jul 2024.

AUTOPHAGY REPORTS
2024, VOL. 3, NO. 1, 2372997
https://doi.org/10.1080/27694127.2024.2372997

Autophagic dysregulation triggers innate immune activation in glucocerebrosidase deficiency

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ABSTRACT

Mutations in the *GBA1* (*glucosylceramidase beta 1*) gene cause the most common lysosomal storage disorder, Gaucher disease (GD), characterized by the lysosomal accumulation of glucosylceramide and lysosomal dysfunction. Downstream of defects in lysosomal-autophagosome fusion, GD cells display autophagic dysfunction. Immune activation and inflammation are also known features of GD pathogenesis. However, the precise link between autophagy and immune activation, and the tissue-specific nature of these pathologies, are yet to be determined. Here we summarize our recent manuscript, which probes the role of autophagy in stimulating a chronic innate immune response in a *Drosophila* GD model. The gut-brain axis is increasingly being implicated in disease pathology, and accordingly, we demonstrated gastrointestinal dysfunction and gut microbiome dysbiosis in GD flies. Moreover, intestinal cells display lysosomal-autophagic defects like those seen in the GD fly brain. Stimulation of autophagy with rapamycin treatment is sufficient to lower NF-κB signaling in the gut. Our research suggests that autophagic impairment in GD flies drives microbiome dysbiosis and chronic immune activation, with deleterious consequences on organismal health. We highlight pharmacological activation of autophagy, targeting tissues such as the gut, as a potential therapeutic strategy in GD.

Abbreviations

AMP, antimicrobial peptide; DAMP, damage associated molecular pattern; *GBA1*, glucosylceramidase beta 1; LC3, microtubule-associated protein 1 light chain 3; MEGF10, multiple EGF like domains 10; mTOR, mammalian target of rapamycin; PGRP, peptidoglycan recognition protein receptor; TRIF, Toll/IL-1R domain-containing adaptor-inducing IFN-β.

ARTICLE HISTORY Received: 16 May 2024; Revised: 17 Jun 2024; Accepted: 17 Jun 2024

KEYWORDS Gaucher disease; autophagy; lysosomal; Parkinson's disease; *GBA1*; glucocerebrosidase; innate immunity

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Gaucher disease (GD) is a rare inherited disorder of metabolism presenting with multiorgan involvement, infrequently with neurological manifestations. It is caused by bi-allelic mutations in the *GBA1* (*glucosylceramidase beta 1*) gene, encoding the lysosomal hydrolase glucocerebrosidase (GCase). This enzyme hydrolyses glucosylceramide (GluCer) to ceramide and glucose. *GBA1* mutations lead to lysosomal GluCer accumulation and consequent lysosomal dysfunction. Lipid-engorged cells of the reticuloendothelial system become deposited within various organs, commonly in the liver, spleen and bone marrow, to cause organ dysfunction and inflammation. Mutations in *GBA1* are also the most frequent genetic risk factor for Parkinson's disease (PD).

Downstream of the lysosomal dysfunction in GD, there is a block in autophagosome-lysosomal fusion, resulting in autophagic defects and loss of cellular protein homeostasis. An additional feature of GD is increased immune activation in diseased tissues. However, precisely how mutations in *GBA1* and associated cellular pathologies trigger immune activation, and whether the block in autophagy plays a role in these responses, is yet to be elucidated. Moreover, pathological communication between the gut and brain, the so-called gut-brain axis, is increasingly being implicated in neurodegenerative disorders such as PD. In this context, it has been hypothesized that local inflammation within the gut, as well as systemic immune activation, may trigger neuroinflammation in the brain via direct and indirect communication between these two organs. Nevertheless, the role of the gut, and specifically intestinal autophagy, has not been examined in GD and PD associated with *GBA1* mutations (*GBA1*-PD).

To address these knowledge gaps, we used a GD *Drosophila* model lacking the main fly orthologue of *GBA1, Gba1b* [1]. Transcriptomic analysis of GD fly heads revealed significant enrichment for genes mapping to immune processes and pathways. Further investigation revealed up-regulation of NF-κB innate immune signaling, namely of the Toll like/Toll and immune deficiency (IMD)/TRIF pathways, in GD fly heads, gut and fat body (the functional equivalent of the mammalian liver and adipose tissue). This alteration was associated with increased glial activation in the GD fly brain, as evidenced by elevated levels of the glial phagocytic receptor Draper/MEGF10.

The increased NF-κB signaling in the gut of GD flies led us to probe whether gut-brain axis communication plays a role in the disease pathogenesis. To this end, we demonstrated a constipation-like phenotype in GD flies compared to control healthy flies, with delayed early egestion following food consumption. We also established that there was increased intestinal barrier permeability to an ingested non-permeable dye. We next turned our attention to the gut microbial load and composition. Interestingly, the levels of gut bacteria were greater in the intestine of GD flies compared to controls. 16S ribosomal RNA-sequencing also indicated that there were significant

alterations in the microbial composition of GD fly guts. Taken together, we demonstrated that GD flies display gastrointestinal abnormalities and an altered intestinal microbiome, mirroring those seen in patients with PD.

To further interrogate the role of the gut and the altered microbiota in GD flies, we eradicated the intestinal microbiome by raising flies under germ-free conditions. The latter was achieved by feeding adult flies a cocktail of antibiotics throughout their lifespan. This led to an increase in the survival of GD flies, with improved climbing ability. Antibiotic treatment also returned NF-κB innate immune signaling to control levels in all studied tissues, including the head, fat body and gut. Glial activation in the brain was also reduced, consistent with the notion that there is gut microbiome-brain axis modulation. Together, these findings demonstrate that the intestinal gut microbiome is sufficient to trigger widespread systemic innate immune activation in GD flies.

The cellular defects underlying the observed gastrointestinal dysfunction were further investigated in GD flies by probing lysosomal-autophagic pathways in the gut wall. In keeping with the lysosomal-autophagic dysfunction previously reported in the GD fly brain, we observed an increase in acidified vesicles in the GD fly gut tissue. Moreover, the levels of the ubiquitin-binding selective autophagy receptor, Ref(2)P/p62, polyubiquitylation and Atg8a-II/LC3-II were all increased compared to control guts, indicative of a possible block in the autophagic flux. To determine to what extent the autophagy defects were driving gut pathology, we treated GD and control flies with the mTOR inhibitor rapamycin from young adulthood. In accordance with our previous work, we showed that rapamycin was able to significantly extend the longevity of GD flies. We also observed lowering of the innate immune response in the intestinal tissue independent of any effect on microbial load.

To substantiate the association between autophagy defects in the gut and innate immune activation, we next treated healthy flies with chloroquine. This compound has multiple effects, including blocking autophagosome-lysosome fusion, thus recapitulating the defects observed in *GBA1* deplete cells. Chronic chloroquine treatment led to an apparent block in autophagy in the fly gut, with increased Ref(2)P/p62 and Atg8/LC3-I and II levels. Elevated NF-κB signaling was also observed in the gut tissue of chloroquine-treated healthy flies, similar to that seen in GD flies. Thus, these findings suggest that autophagy impairment may be sufficient to stimulate an immune response in the gut. Interestingly, we established that rapamycin treatment extends lifespan and reduces gut immune activation to a similar degree as eradicating the gut microbiome. However, there was no additive or synergistic effects of combining these two interventions, alluding to the fact that these two therapeutic treatments act in the same pathogenic pathway. Our research thus demonstrates that autophagy impairment in GD flies is likely sufficient to deregulate the microbiome and induce chronic immune activation, with

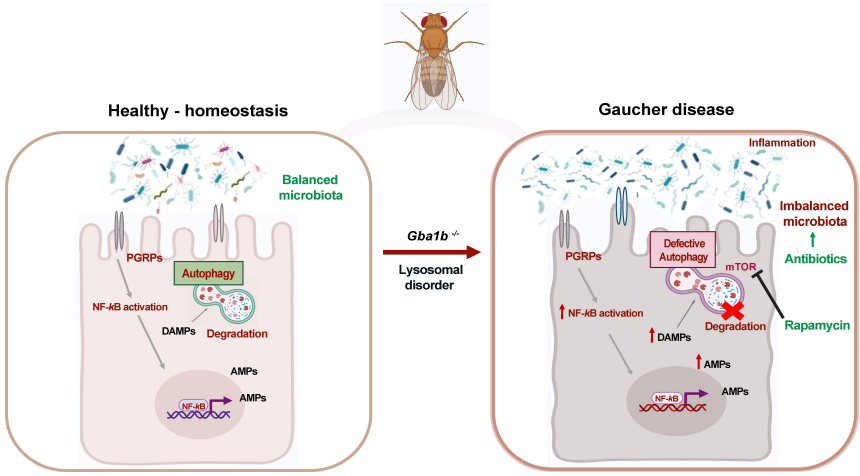

Figure 1. Gaucher disease (GD) flies, lacking *Gba1b*, the main fly orthologue of *GBA1*, display widespread lysosomal and autophagy defects, including in the gut wall. In response to pathogenic stimulation by the microbiome, NF-*k*B immune signaling is activated, leading to increased damage associated molecular patterns (DAMPs) and secreted immune mediators called antimicrobial peptides (AMPs). This is locally associated with intestinal microbiome dysbiosis and gut dysfunction. Eradicating the gut microbiome with antibiotic treatment or stimulating autophagy, and possibly other pathways, by treating GD flies with rapamycin, lowers intestinal NF-*k*B signaling, promoting organismal health. Created using Biorender.

subsequent negative consequences for lifespan and gut health (Figure 1). Our findings indicate that modulation of the gut microbiome, and stimulation of autophagy, in non-neuronal tissues such as the gut, offer potential therapeutic strategies for patients with *GBA1*-associated disorders such as GD and PD. Rapamycin is already a licensed drug, and is commonly used for immunosuppression in transplant patients. The strong immunomodulatory and metabolic effects associated with its use increase the risk of toxicity and side effects. Thus, targeting autophagy using more selective mTORC1 rapamycin analogues, or other effectors of the mTOR pathway, may offer a more effective therapeutic strategy. Further investigation is now needed to better understand the temporal and tissue-specific nature of the autophagy defects in *GBA1*-associated disease. This will guide the development of therapeutic avenues targeting autophagy and associated processes during critical, early pathogenic stages of the disease.

In addition, our work highlights an important role of the gut-brain axis in stimulating neuroinflammation in our GD model, with eradication of the gut microbiome lowering glial activation. Consistent with previous work in the field, we propose that a block in intestinal autophagy leads to chronic activation of NF-κB/IMD signaling due to failure

of the basal autophagic clearance of pathway intermediates. This subsequently triggers chronic immune activation in the gut, and other tissues lacking GCase activity, in response to the presence of the immune-stimulating microbiome. Taken together, our study suggests that therapeutically targeting autophagy systemically in tissues outside of the brain may prove to be beneficial for the treatment of *GBA1*-associated disorders.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This work was supported by the Wellcome Trust [Wellcome Clinical Career Development Fellowship, 214589/Z/18/Z to KJK] and funding from the Rosetrees Trust [M701 and M701A to KJK].

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