



## Autophagy Inducing Capacities of a Commercial Preparation Based on of *Bacillus thuringiensis* Cry1A-Cr2A Autophagy-Inducing Properties Toxins in Human Macrophages: Implication Against Mycobacterial Infections

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### Abstract

Autophagy is a highly conserved degradative and recycling program to maintain homeostasis. In particular, it plays an important role in the innate immune response against intracellular pathogens. Several studies have shown that BCG and/or *M. tuberculosis* block autophagosome formation, inhibiting thus, activation of the autophagy machinery, and survival of mycobacteria. Human macrophages preparation from Peripheral blood mononuclear cells (buffy coats of the healthy donor) (blood bank of INER. MX). Monocytes were further isolated by CD14 positive selection and differentiated into monocyte-derived macrophages. Infection of macrophages with *M. bovis* BCG vaccine (ATCC, Manassas, VA, US) at MOI 1:5. Stimulation with Cry1A-Cr2A (5 µg/ml Rapamycin (250 µg/ml) and/or Wortmannin (100 nM). Autophagy detection and fluorescence microscopy were made in the uninfected well and the infected-stimulated cells were stained with rabbit anti-LC3B coupled to Alexa Fluor 488. The percentage of cells with more than 5 LC3+ puncta (autophagosomes) was calculated as well as the percentage of bacteria co-localizing with LC3. The percentage of mycobacteria BCG co-localizing with light chain 3 (LC3-II) in human macrophages is greater in infected and stimulated human macrophages with the commercial preparation based on Cry1A-Cry2A Bt proteins versus rapamycin and/or Wortmannin. The commercial preparation of Cry1A-Cry2A in combination with the BCG vaccine represents a potential alternative to enhance the autophagy-mediated elimination of intracellular pathogens such as *M. tuberculosis*.

**Keywords:** *Bacillus thuringiensis*; Cry1A Toxins; Autophagy; Macrophages; Microtubule Light Chain 3 (LC3); Tuberculosis

### Introduction

Tuberculosis is still a major health problem worldwide and specially in developed countries [1,2]. One serious problem that are facing public health systems is the increased appearance of multidrug resistance strains (MDR) and excessive use of medication against intracellular pathogens [1-3] like *Mycobacterium tuberculosis* (*Mtb*) the causal agent of Tuberculosis [1,2]. The actual BCG vaccine (*Mycobacterium bovis* *Bacillus Calmette Guérin*) (BCG) [3-8] is protective against some forms of Tuberculosis in children and variable immunoprotection or lack of immunological memory in young and adult people [9-13].

Autophagy is highly conserved process to maintain homeostasis at molecular and cellular level [14-17]. Any organelle damage, misfolded proteins or pathogens trigger and activate the machinery of autophagy [18-20]. In this sense it has been reported that autophagy can be used for therapeutic intervention against intracellular pathogens as *M. tuberculosis* [21-26]. Therefore, one promising alternative against Tuberculosis is autophagy inducers are a potential alternative to enhance BCG immunity. Indeed, a recent paper have shown that a recombinant BCG vaccine with a peptide, enhance BCG autophagy inducing properties. BCG vaccine

and *M. tuberculosis* interfere with phagosome maturation is through the secretion of sap M phosphatase, which dephosphorylates phosphatidyl-inositol-3-kinase (PI-3K), considered a key initial trigger for phagosome maturation [24,26,31,32]. BCG or *M. tuberculosis* block the fusion of the autophagosome and lysosome and therefore autophagy induction, hampering thus activation of the innate and cellular immune response [31-34]. How this can be overcome?. Autophagy, defined as the self control eating process (from the greek word "self-eating"), is a highly conserved intracytoplasmic process for degradation and recycling of components for cellular homeostasis [35-38]. It is characterized by the formation of double-membrane bound vesicles, "autophagosomes", able to engulf different types of cargo: protein aggregates or damaged, defective organelles (selective autophagy), or non-specific cellular material (bulk autophagy), transport it to the vacuole/lysosome for digestion and recycling for energy production [32,36,39]. Other types of autophagy is the Chaperone-mediate autophagy (CMA) for selective degradation of single proteins. Microautophagy or endosomal microautophagy (eMI), at basal or induced levels, is a direct engulfment of proteins into lysosomes. On referring specifically to Macroautophagy this mechanism during starvation or treatment with rapamycin or amino acid leads to the inhibition of mTOR (target of rapamycin) [33,40]. The target of rapamycin (mTOR), known as a conserved Ser/Th kinase that sense energy inputs and nutritional demands [33,40]. Furthermore, activation of mTOR stimulates anabolic processes and biomass production, while its inhibition enhances catabolic processes, including autophagy [33,40].

Autophagy, and autophagosome biogenesis is mediated by the action of the associated proteins, the ATG protein, in particular ATG9 and ATG16 (or the yeast orthologue), proteins that can be associated from different membrane sources [39,41-45]. Several studies from the literature suggest that autophagy represents an opportunity of host immune response to tackle and overcome resistant intracellular pathogens [25,46-48]. Indeed, autophagy inducing drugs has been recently described as one of the strategies that can circumvent *MTb* infection disease [49-52]. Pharmacological activation of autophagy is achieved by blocking the kinase activity of mammalian target of rapamycin (mTOR) enzymatic complex 1 (mTORC1) leading to remove the autophagy inhibition state of the autophagy under physiological conditions [39,40,53].

In a previous work *in vivo* and *in vitro* assays either in the mouse model and/or in human cell lines (A549/THP-1 cell line) have shown that a commercial preparation of Cry1A-Cry2A Bt proteins enhanced BCG immunity. Thus, A549 or THP1 cells, infected with BCG vaccine and/or co-administered with Cry1A-Cry2A induced protective humoral (IgG antibodies) and cellular immune responses (IL-17 cytokine) even at low doses [54]. Interestingly at high concentrations of this commercial Cry1A preparation did not

affect the A549 viability nor of THP-1 cells [54]. The mechanism of immunogenicity and adjuvanticity using protoxina CryAc has been proposed to be mediated by receptor like molecules [55,56]. In the present study, we are reporting that a commercial preparation of *Bacillus thuringiensis*, Cry1A-Cry2A combined with BCG vaccine enhanced autophagy induction, determined by surface co-localization of the microtubule associated light chain-3 (LC3-II) protein determined by contrast and immune fluorescence microscopy in human macrophages (derived from PBMCs of healthy donors).

## Methods

### Human macrophages preparation

Peripheral blood mononuclear cells were obtained from buffy coats of healthy donor to the blood bank of the Instituto Nacional de Enfermedades Respiratorias (INER, CDAD of MX) Ismael Cosío Villegas. Monocytes were further isolated by CD14 positive selection using magnetic beads from Miltenyi (Miltenyi Biotech, Auburn, CA, US). Cells' viability was 100% by tripan blue exclusion. Monocytes were seeded in 8-well chamber slides ( $3 \times 10^5$  cells/well) and were allowed to differentiate into monocyte derived macrophages by culturing in complete RPMI-1640 (Lonza, Walkersville, MD, US) supplemented with 200mM L-glutamine and 10% heat-inactivated human serum (Valley Biomedicals, Winchester, VA, US) for 7 days. Medium was refreshed at day 4.

### Macrophage stimulation and infection

Cells were stimulated with 5  $\mu$ g/ml of Cry for 24 h. For infection experiments macrophages were infected with *M. bovis* BCG (ATCC, Manassas, VA, US) at MOI 5 during 1 h in RPMI with 30% nonheat-inactivated human serum and without antibiotics. Cells were extensively washed to discard non phagocitized bacteria and further stimulated with 5  $\mu$ g/ml of Cry and cultivation for additional 24 h. Medium was used as negative control and 250  $\mu$ g/ml of rapamycin (Sigma Aldrich, St. Louis, MO, US) were used as positive control. The autophagy inhibitor wortmannin (Fluka, Seelze, DE) was used at 100 nM 30 minutes prior stimulation when indicated. After the incubation period the medium was discarded, and cells were fixated with 4% paraformaldehyde and stored at 4°C until fluorescence microscopy staining. In selected experiments, the supernatants were cryopreserved at -20°C cells for further use.

### Autophagy detection by fluorescence microscopy

The uninfected cells were stained with rabbit anti-LC3B coupled to Alexa Fluor 488 (Novus Biologicals, CA, US). Nuclei were stained with Hoechst (Enzo Life Sciences, NY, US) following the manufacturer's instructions. The slides were mounted with Prolong Gold antifade reagent (Molecular probes, Carlsbad, CA, US). The cells were visualized with a fluorescence Axio Scope A1 microscope (Carl Zeiss, Oberkochen, DE), and the images were

acquired and analyzed with ZEN Pro software (Carl Zeiss). At least 100 cells were counted for each condition. We considered 5 puncta as basal expression, and the percentage of cells with more than 5 LC3+ puncta (autophagosomes) was calculated.

When analyzing infected cells, and in-house developed antibody was used to stain the bacteria that was revealed using a secondary antibody coupled to Alexa Fluor 594. The percentage of cells with more than 5 LC3+ puncta (autophagosomes) was calculated as well as the percentage of bacteria colocalizing with LC3 determined by the merging of green and red fluorescence that produces yellow images.

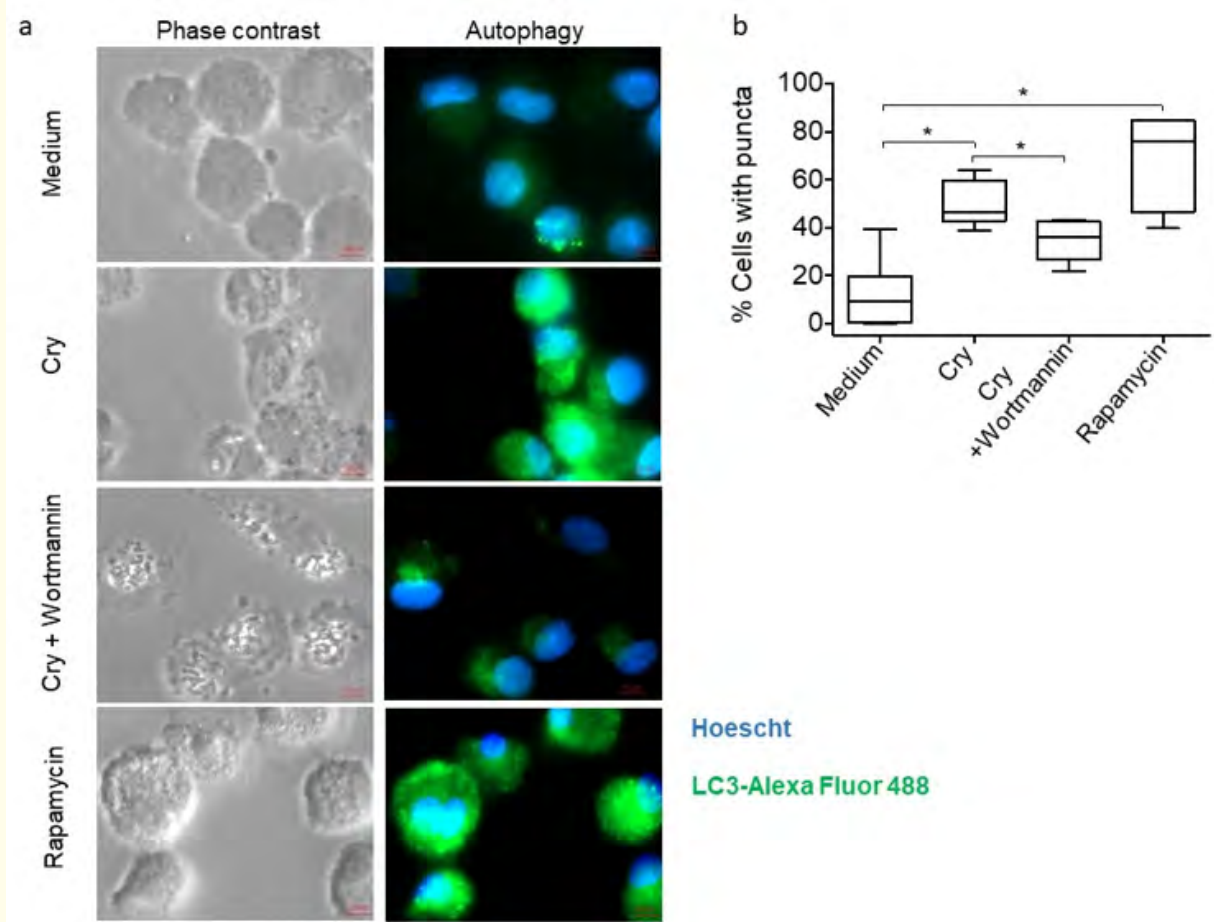
### Statistical analyses

Statistical analyses were performed using GraphPad Prism 6.0 (CA, US) using a Friedman’s non-parametric analysis of variance (ANOVA) followed by Dunn’s multiple comparison post-test. A  $p \leq 0.05$  was considered significant

## Results

### A commercial preparation of *Bacillus thuringiensis* Cry1A-2A proteins induce autophagy in human macrophages

Autophagy is induced as an immunological host response to intracellular pathogens as *Mycobacterium tuberculosis*. The commercial preparation based on Bt Cry1A-Cry2A proteins is able to enhance BCG immunity [54]. Thus, we tested whether in human macrophage stimulated with this commercial preparation, autophagy was induced. The effect was evaluated as described in material and methods. The percent of autophagic puncta induced in human macrophages stimulated by Cry1A-Cry2A, Rapamycin and/or Wortmannin stimulation were calculated and plotted ( $p < 0.05$ ). Macrophages activated with Cry1A-Cry2A show an increase in autophagic puncta labeled with microtubule light chain 3 LC versus medium, or Cry-Wortmannin. Rapamycin is the positive control of autophagy induction. Wortmannin is the autophagy inhibitor (Figure 1a) ( $P < 0.05$ ).



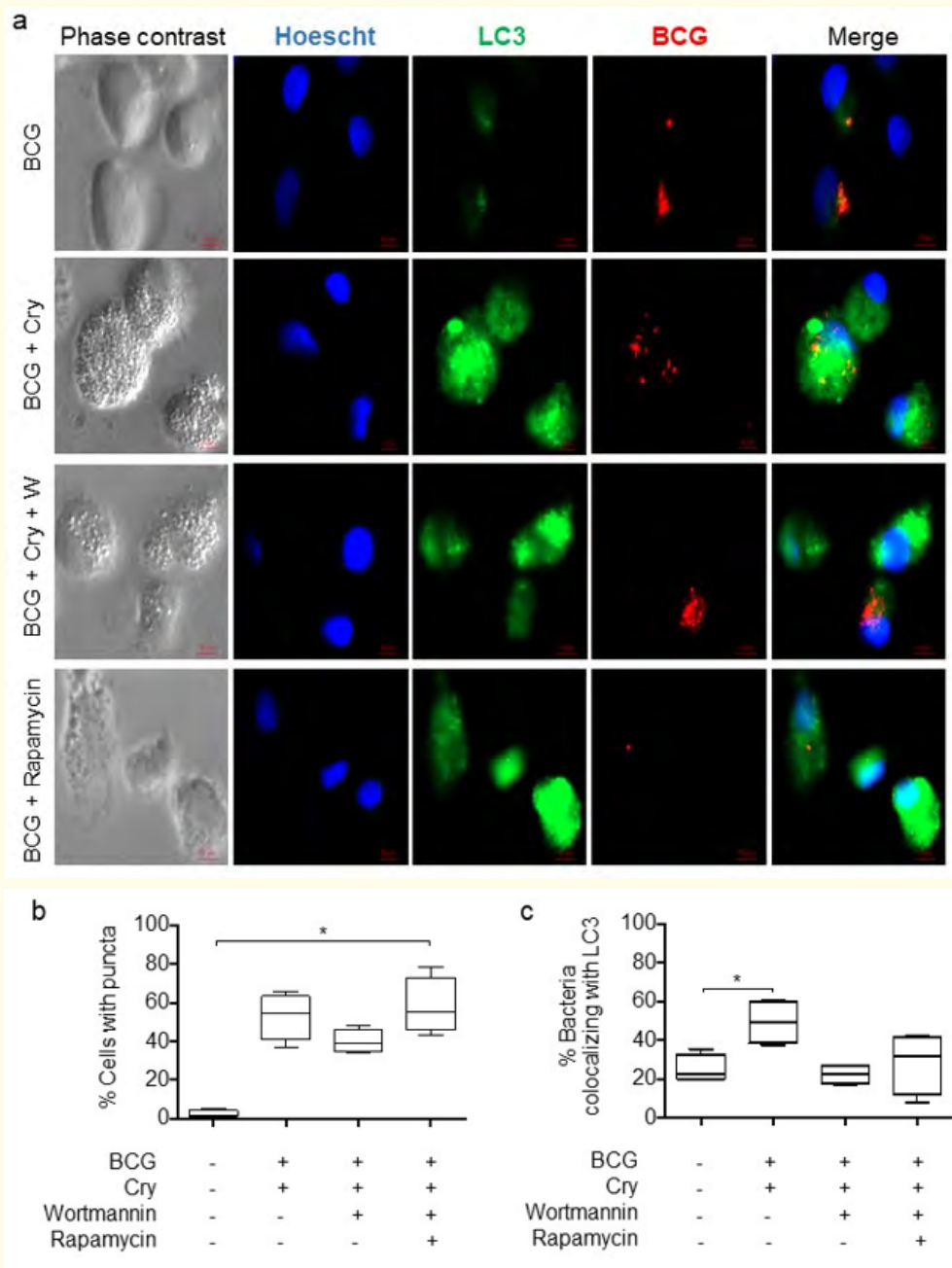
**Figure 1:** Autophagy –inducing of a commercial preparation based on a commercial preparation of *Bacillus thuringiensis* Cry1A-Cry2A proteins in human macrophages.

Macrophages were stimulated with 5 µg/ml of Cry in presence or absence of the autophagy inhibitor wortmannin (100nM) during 24 h. Medium and 250 µ g/ml of rapamycin were used as controls. Cell preparations were stained with anti-human LC3 antibody coupled to Alexa Fluor 488 and nuclei were stained with Hoescht. a) Cells were observed at 100X. Size bars indicate 10 µm. Depicted are representative images. b) Cells with LC3 puncta were counted and the percentage of cells with puncta were determined. Depicted are box plots with medians and quartiles, n = 8, \*p < 0.05.

**Commercial preparation of Cry1A-Cry2A of *Bacillus thuringiensis* combined with BCG vaccine enhance autophagy inducing in human macrophages**

Next, since we observed a mild autophagy induction induced by the commercial preparation of Cry1A-Cry2A, we measured the possibility that the stimulation of BCG infected human

macrophages showed an increase in the autophagy induction. From figure 2a-b), we found that the percentage of mycobacteria BCG co-localizing with light chain 3 (LC3-II) in human macrophages is greater in infected and stimulated human macrophages with the commercial preparation based on Cry1A-Cry2A BT proteins than with Rapamycin and/or Wortmanin. (Figure 2a-b) ( $P < 0.05$ ).



**Figure 2:** A commercial preparation based on *Bacillus thuringiensis* Cry1A-Cry2A proteins enhance BCG vaccine autophagy inducing properties in human macrophages.

Macrophages were infected with *M. bovis* BCG at MOI 5 during 2 h after extensive washing of non phagocitized bacteria. Further, the cells were stimulated with 5 µg/ml of Cry in presence or absence of the autophagy inhibitor wortmannin (20 nM) and were incubated for additional 24 h. Medium and 250 µg/ml of rapamycin were used as controls. Cell preparations were stained with anti-human LC3, BCG were stained with the proper antibodies and nuclei were stained with Hoescht. a) Cells were observed at 100X.

Size bars indicate 10 µm. Depicted are

representative images. b) Cells with LC3 puncta were counted and the percentage of cells with puncta were determined. Depicted are box plots with medians and quartiles,  $n = 4$ ,  $*p < 0.05$ . c) Bacteria were counted and the percentage of bacteria colocalizing with LC3 were determined. Depicted are box plots with medians and quartiles,  $n = 4$ ,  $*p < 0.05$ .

## Discussion and Conclusion

In the present work, we are reporting that a commercial preparation of *Bacillus thuringiensis* Cry1A-Cry2A co-administered with *Mycobacterium bovis* *Bacillus Calmette Guerin* (BCG) vaccine enhance surface co-localization of mycobacteria and the microtubule light chain (LC3II) in human macrophages.

Autophagy is a highly conserved eukaryotic catabolic process that is fundamental to maintain homeostasis at cellular and physiological levels [14-17]. Autophagy activation leads to degradation and recycling of the excess of cytoplasmic cargo, damaged organelles, misfolded proteins, microbes [14-17,36]. The molecular machinery of the autophagy is under the control of the target of rapamycin kinase (mTOR) a conserved Serine/Threonine kinase, the activated protein kinase (AMPK), and the set of proteins associated to autophagy, ATGs proteins [39,40,53]. In mammals around seven to eight proteins associated to autophagy has been described. Among the most common, are Atg8c (mAtgs), and the microtubule-associated light chain-3, -LC3A, LC3B, LC3B2 and LC3C [34,57-60]. LC3B usually is used as autophagosomal marker [20,21,27,28]. However, other members of this set of proteins can also be used as markers of membrane association or autophagosome formation and the lysosomes markers LAMP1 and CD68 [39,59-61].

How autophagy becomes pivotal for cellular homeostasis?, How is triggered?. It is known that autophagy is triggered in the presence of nutrients (i.e. aminoacids), starvation), and/or growth factors. In the absence of any of these components, mTOR1 is activated and autophagy is inhibited. MTOR and the transcription factor EB (TFEB) works together through collaboration of GABARAP members (mAtg8 family) and other LC3-positive membranes to maintain cellular function, lysosomal biogenesis, and the homeostatic mechanism to avoid any deleterious effects in the body tissues [26,40,62-64]. Furthermore, under starvation conditions, or during cellular stress as *Mtb* infection, immunity related GTPase family M (IRGM) is activated [64,65]. IRGM is inhibitor of mTOR kinase activity, but it also promotes PPP3/calcineurin's protein phosphatase activity, which collective translates into effective TFEB activation. Thus, IRGM becomes the master regulation of the mTOR inhibition and autophagy induction [34,48,63,64]. Moreover, IRGM control phosphatase activity of PPP3CB, the translocation of TFEB to the nucleus, and therefore, have a role in the lysosomal biogenesis [40].

Of relevance is that the connection of immunity and autophagy has been suggested to occur through TLR signalization and engagement linking with the lysosomal pathways. Particulate antigens as for example bacteria can trigger on a human derived macrophages the autophagosome marker LC-3 to be rapidly

recruited to the phagosome in a manner that depends on the autophagy pathway proteins ATG5 and ATG7 [14,26,37,61,65,66]. This process is preceded by recruitment of Beclin 1 and phosphoinositide-3-OH kinase activity, translocation of Beclin 1 and LC-3 to the phagosome, association with phagosome fusion with lysosome [14,26,37,65,66]. This will favor targeting the cargo or the intracellular pathogens to the proteasome lysosomal degradation, peptide presentation through MHC-II or MHC-I to CD4+ or CD8+ T cellular activation [14,35,40,62].

Under the settings of BCG vaccination, autophagy is induced as a defense mechanism of the host to the infection. Briefly, BCG vaccine as antigen is phagocytose by the antigen presenting cells i.e. (macrophages), endocytosed, released by the ESX-1 secretion system to the cytoplasm. Mycobacterial DNA is recognized by the STING-dependent pathway, and the autophagic receptors p62 and NDSP52 [20,46,61,65,66]. Then, mycobacteria is ubiquitinated by the ubiquitin ligase Perkin, TRIM16, Galectin-3, ATG16L-1 and the Ubiquitin ligase Smurf1, activating autophagy, the lysosomal pathway for processing, and presented loaded on MHC-II to CD4+T cells or peptide-loaded on MHC-I loaded to CD8+ T cells [14,37,65,66]. However, the highly variability in the BCG vaccine immune protection can be due to defects in APCs presentation and T cell activation [7,29]. BCG vaccine sequestered in phagosomes inhibits digestion of immunodominant antigens (i.e Ag85B) leading to the impairment of antigen presentation, T cell activation, and the induction of long lasting immunological memory [14,26]. On the contrary BCG vaccine and/or *Mtb* are capable to block or sequester autophagosome formation, maturation, and fusion with the lysosome pathways, leading to an incomplete or delay in the host immune response [14,26-28].

To take advantage of the autophagy machinery as an innate host response is that can be harnessing as a mechanism of delivering vaccines and/or cargo (microbes) that links lysosomal pathways with antigen presentation and T cellular immune response [14,37,65,66]. At this point, is noteworthy recent on the effect of the combination of BCG vaccine and rapamycin. BCG-autophagy induction was enhanced gainst *M. tuberculosis* infection. Rapamycin enhanced BCG vaccine delivery to lysosomes, and thereby increased BCG antigen presentation to CD4+ and/or CD8+ T cell activation [14,26,37,65,66]. Furthermore, a recombinant BCG vaccine that over express Ag85B and mixed with the C5 peptide derived from *Mtb* CFP-10 protein elicited a strong Th1-type cellular response represented by the IL-12, IL1- $\beta$ , and TNF-alpha, restoring antigen MHC-II presentation via TLR-2 and MyD88. phosphorylation of p38MAPK and ERK against *M. tuberculosis* (Kahn., et al. 2019) [29]. In a previous work, we have shown that a commercial preparation of Cry1A-Cry2A Bt proteins, behaves as immunogen and adjuvant of BCG vaccine [54]. In the present study, human macrophages

derived from healthy patients PBMCs upon BCG and Cry1A-Cry2A, enhanced autophagy induction. Bt Cry proteins showed an increase in the colocalization of LC3 versus rapamycin and/or wortmannin (Figure 1A-B) In the combination of BCG vaccine, at low concentration it is increased the co-localization of LC3 in human derived PBMC macrophages (Figure 2A-B). From the data reported here, we think that this potential alternative can endowed to the actual BCG vaccine with autophagy inducing properties that can harnessed against either infectious diseases (i.e. mycobacteria of the complex of *M. tuberculosis*) or other chronic diseases (i.e. cancer).

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### Conflict of Interest Statement

"I, as the corresponding author, declare, on behalf of all authors of the paper, that no financial conflict of interest exists in relation to the work described. "I, as the corresponding author, declare that the results/data/figures in this manuscript have not been published, nor are they under consideration for publication elsewhere".

### Author Contribution Statement

G.G.G.M. conceptualization, data analysis, writing of the manuscript. A.D.R.H and E.J. Methodology, Data analysis, Research. Y.G. Research, Data analysis. JMFV. Research, "I certify that the above information is true and correct. All the authors contributed to the study and the manuscript.

### Bibliography

1. WHO. "Guidelines for the treatment of drug-susceptible tuberculosis and patient care". 2017 update. WHO (2017).
2. WHO. "The top 10 causes of death". WHO (2019).
3. Zumla A and Maeurer M. "Host-Directed therapies for tackling multi-drug resistant tuberculosis: learning from the Pasteur-Bechamp debates". *Clinical Infectious Diseases* 61 (2015): 1432-1438.
4. Maher D and Raviglione M. "Global epidemiology of Tuberculosis". *Clinics in Chest Medicine* 26 (2005): 167-182.
5. Young DB., et al. "Confronting the scientific obstacle to global control of Tuberculosis". *Journal of Clinical Investigation* 118 (2008): 1255-1260.
6. Trunz BB., et al. "Effect of BCG vaccination on childhood tuberculous meningitis and miliary tuberculosis worldwide: a meta-analysis and assessment of cost-effectiveness". *Lancet* 367 (2006): 1173-1180.
7. da Costa A C., et al. "Recombinant BCG: innovations on an old vaccine. Scope of BCG strains and strategies to improve long-lasting memory". *Frontiers in Immunology* 5 (2014): 152.
8. Wilkie M., et al. "Functional in-vitro evaluation of the non-specific effects of BCG vaccination in a randomised controlled clinical study". *Scientific Report* 12 (2022): 7808.
9. Abebe F. "Is Interferon-gamma the right marker for Bacille Calmette-Güerin-induced immune protection? The missing link in our understanding of Tuberculosis". *Clinical and Experimental Immunology* 169 (2012): 213-219.
10. Koul A., et al. "Interplay between mycobacteria and host signaling pathways". *Nature Reviews Microbiology* 2 (2004): 189-202.
11. Pulendran B and Ahmed R. "Translating innate immunity into immunological memory: implications for vaccine development". *Cell* 124 (2006): 849-863.
12. Mohr I and Sonenberg N. "Host translation of the nexus of infection and immunity". *Cell Host Microbe* 12 (2012): 470-483.
13. Kolloli A and Subbian S. "Host-directed therapeutic strategies for tuberculosis". *Frontiers in Medicine (Lausanne)*. 4 (2017): 171.
14. Deretic V. "Autophagy in inflammation, infection, and immunometabolism". *Immunity* 54 (2021): 437-453.
15. Benbrook DM and Long A. "Integration of autophagy, proteasome degradation, unfolded protein response and apoptosis". *Experimental Oncology* 34 (2012): 286-297.
16. Kimmy JM and Stallings CL. "Bacterial pathogens versus autophagy: implications for therapeutic interventions". *Trends in Molecular Medicine* 22 (2016): 1060-1076.
17. Klionsky DJ and Emr SD. "Autophagy as a regulated pathway of cellular degradation". *Science* 290 (2000): 1717-1721.
18. Pareja ME and Colombo MI. "Autophagic clearance of bacterial pathogens: molecular recognition of intracellular microorganisms". *Frontiers in Cellular and Infection Microbiology* 3 (2013): 54.
19. Bah A and Vergne I. "Macrophage autophagy and bacterial infections". *Frontiers in Immunology* 8 (2017): 1483.

20. Levine B and Kroemer G. "Biological Functions of Autophagy Genes: A Disease Perspective". *Cell* 176 (2019): 11-42.
21. Castillo EF, et al. "Autophagy protects against active tuberculosis by suppressing bacterial burden and inflammation". *Proceedings of the National Academy of Sciences of the United States of America* 109 (2012): E3168-76.
22. Goletti D, et al. "Autophagy in Mycobacterium tuberculosis infection: a passe partout to flush the intruder out?" *Cytokine Growth Factor Review* 24 (2013): 335-343.
23. Romagnoli A, et al. "ESX-1 dependent impairment of autophagic flux by Mycobacterium tuberculosis in human dendritic cells". *Autophagy* 8 (2012): 1357-1370.
24. Kaufmann SHE, et al. "Host- directed therapies for bacterial and viral infections". *Nature Reviews Drug Discovery* 17 (2018): 35-56.
25. Gutierrez MG, et al. "Autophagy is a defense mechanism inhibiting BCG and Mycobacterium tuberculosis survival in infected macrophages". *Cell* 119 (2004): 753-766.
26. Palk S, et al. "Autophagy: A new strategy for host-directed therapy of tuberculosis". *Virulence* 10 (2019): 448-459.
27. Pellegrini JM, et al. "Shedding Light on Autophagy During Human Tuberculosis. A Long Way to Go". *Frontiers in Cellular and Infection Microbiology* 11 (2022): 820095.
28. Pellegrini JM, et al. "Neutrophil autophagy during human active tuberculosis is modulated by SLAMF1". *Autophagy* 17 (2021): 2629-2638.
29. Khan A, et al. "An autophagy-inducing and TLR-2 activating BCG vaccine induces a robust protection against Tuberculosis in mice". *NPJ Vaccines* 4 (2019): 34.
30. Gupta A, et al. "Targeted pulmonary delivery of inducers of host macrophage autophagy as a potential host-directed chemotherapy of tuberculosis". *Advanced Drug Delivery Reviews* 1 (2016): 10-20.
31. Rovetta AI, et al. "IFN-g-mediated immune responses enhance autophagy against Mycobacterium tuberculosis antigens in patients with active tuberculosis". *Autophagy* 10 (2014): 2109-2121.
32. Krakauer T. "Inflammasomes, Autophagy, and Cell Death: The Trinity of Innate Host Defense against Intracellular Bacteria". *Nutrition and Inflammation* (2019).
33. Kuma A, et al. "Autophagy-monitoring and autophagy-deficiente mice". *Autophagy* 12 (2017): 1619-1628.
34. Lerena MC, et al. "Mycobacterium marinum induces a marked LC3 recruitment to its containing phagosome that depends on a functional ESX-1 secretion system". *Cell Microbiology* 13 (2011): 814-835.
35. Mahrpour M, et al. "Autophagy in Health and Disease. 1. Regulation and significance of autophagy; an overview". *American Journal of Physiology-Cell Physiology* 298 (2010): C776-C785.
36. Mizushima N, et al. "Autophagy fights disease through cellular self-digestion". *Nature* 451 (2008): 1069-1075.
37. Delgado MA and Deretic V. "Toll-like receptors in control of immunological autophagy". *Cell Death and Differentiation* 16 (2009): 976-983.
38. Yuting M, et al. "Autophagy and cellular immune responses". *Immunity* 39 (2013): 211-227.
39. Vincent O, et al. "The WIPI Gene Family and Neurodegenerative Diseases: Insights From Yeast and Dictyostelium Models". *Frontiers in Cell and Developmental Biology* 9 (2021): 737071.
40. Kumar S, et al. "Mammalian Atg8-family proteins are upstream regulators of the lysosomal system by controlling MTOR and TFEB". *Autophagy* 16 (2020): 2305-2306.
41. Ragus M, et al. "Architecture. Architecture of the Atg17 complex as a Scaffold for Autophagosome Biogenesis". *Cell* 151 (2012): 1501-1512.
42. Alers S, et al. "ATG13. Just a companion, or an executor of the autophagic program. ATG13". *Autophagy* 6 (2014): 944-956.
43. Soto-Burgos J, et al. "Dynamics of Autophagosome Formation". *Plant Physiology* 126 (2018): 219-229.
44. Stanley RE, et al. "The beginning of the end: How scaffolds nucleate autophagosome biogenesis". *Trends in Cell Biology* 24 (2014): 1-19.
45. Zavodszky E, et al. "Biology and trafficking of ATG9 and ATG16l two proteins that regulate autophagosome formation". *FEBS Letter* 587 (2013): 1988-1996.
46. Harris J, et al. "Th1-Th2 polarisation and autophagy in the control of intracellular mycobacteria by macrophages". *Veterinary Immunology and Immunopathology* 128 (2009): 37-43.
47. Songane M, et al. "The role of autophagy in host defence against Mycobacterium tuberculosis infection". *Tuberculosis (Edinb)* 92 (2012): 388-396.

48. Qin C., *et al.* "The molecular regulation of autophagy in antimicrobial immunity". *Journal of Molecular Cell Biology* 14 (2022): mjac015.
49. Siregar TAP, *et al.* "The autophagy-resistant Mycobacterium tuberculosis Beijing strain upregulates KatG to evade starvation-induced autophagic restriction". *Pathogens and Disease* 80 (2022): ftac004.
50. Jurado JO, *et al.* "IL-17 and IFN-gamma expression in lymphocytes from patients with active tuberculosis correlates with the severity of the disease". *Journal of Leukocyte Biology* 91 (2012): 991-1002.
51. Laopanupong T, *et al.* "Lysosome repositioning as an autophagy escape mechanism by Mycobacterium tuberculosis Beijing strain". *Scientific Report* 11 (2021): 4342.
52. Intemann CD, *et al.* "Autophagy gene variant IRGM -261T contributes to protection from tuberculosis caused by Mycobacterium tuberculosis but not by *M. africanum* strains". *PLoS Pathogen* 5 (2009): e1000577.
53. Thellung S, *et al.* "Autophagy activator drugs: A new Opportunity in Neuroprotection from Misfolded Protein Toxicity". *International Journal of Molecular Sciences* 20 (2019): 901-934.
54. Favela-Hernández JM, *et al.* "The potential of a commercial product based on *Bacillus thuringiensis* Cry1A-Cry2A as a immunogen and adjuvant". *Madridge Journal of Immunology* 2 (2018): 58-64.
55. Verdin-Teran SL, *et al.* "Immunization with Cry1Ac from *Bacillus thuringiensis* Increases Intestinal IgG Response and Induces the Expression of FcRn in the Intestinal Epithelium of Adult Mice". *Scand. Journal of Immunology* 70 (2009): 596-607.
56. Rubio-Infante N, *et al.* "The Macrophage Activation Induced by *Bacillus thuringiensis* Cry1Ac Protoxin Involves ERK1/2 and p38 Pathways and the Interaction with Cell-Surface-HSP70". *Journal of Cellular Biochemistry* 119 (2018): 580-598.
57. Mehta P, *et al.* "Noncanonical autophagy: one small step for LC3, one giant leap for immunity". *Current Opinion in Immunology* 26 (2014): 69-75.
58. Ma J, *et al.* "Dectin-1-triggered recruitment of light chain 3 protein to phagosomes facilitates major histocompatibility complex class II presentation of fungal-derived antigens". *Journal of Biological Chemistry* 287 (2012): 34149-34156.
59. Sprenkeler EG, *et al.* "LC3-associated phagocytosis: a crucial mechanism for antifungal host defence against *Aspergillus fumigatus*". *Cell Microbiology* 18 (2016): 1208-1216.
60. Martinez J, *et al.* "Molecular characterization of LC3-associated phagocytosis reveals distinct roles for Rubicon, NOX2 and autophagy proteins". *Nature Cell Biology* 17 (2015): 893-906.
61. Gubas A and Dikic I. "A guide to the regulation of selective autophagy receptors". *FEBS Journal* 289 (2022): 75-89.
62. Park HJ, *et al.* "IL-10 inhibits the starvation induced autophagy in macrophages via class I phosphatidylinositol 3-kinase (PI3K) pathway". *Molecular Immunology* 48 (2011): 720-727.
63. Feng CG, *et al.* "Interferon inducible immunity-related GTPase Irgm1 regulates IFN gamma-dependent host defense, lymphocyte survival and autophagy". *Autophagy* 5 (2009): 232-234.
64. Kim BH, *et al.* "A family of IFN-gamma-inducible 65-kD GTPases protects against bacterial infection". *Science* 332 (2011): 717-721.
65. Sanjuan MA, *et al.* "Toll-like receptor signalling in macrophages links the autophagy pathway to phagocytosis". *Nature* 450 (2007): 1253-1257.
66. Sanjuan MA, *et al.* "Toll-like receptor signaling in the lysosomal pathways". *Immunology Review* 227 (2009): 203-220.