UNIVERSIDADE PAULISTA – UNIP
Programa de Pós-Graduação *Stricto Sensu* em Patologia Ambiental e Experimental

FAPESP: Project number 2019/20710-9

Crotoxin modulates the M1 profile of macrophages infected with *Encephalitozoon cuniculi*

C.G. Nascimento de Oliveira[*], J.G. Xavier[*], A.M. Alvares-Saraiva[*], E.C. Pérez[*], D.D. Spadacci-Morena[†], S.C. Sampaio[†] and M.A. Lallo[*]

[*]Patologia Ambiental e Experimental, Universidade Paulista-UNIP and [†]Departamento de Fisiopatologia, Instituto Butantan, São Paulo, BR

INTRODUCTION

Crotoxin (CTX), a bioactive extract from the snake *Crotalus durissus terrificus*, demonstrated the ability to modulate the profile of macrophages infected with *Leishmania amazonensis*, with increased phagocytic capacity and elimination of intracellular parasites. Microsporidia are opportunistic, obligate intracellular fungi that infect vertebrates and invertebrates, having demonstrated the ability to modulate the macrophage profile.

OBJECTIVE

The aim of this study was to evaluate the effects of crotoxin on the viability of spores of the microsporidian *Encephalitozoon cuniculi*, as well as on the microbicidal activity of macrophages in vitro.

Materials and Methods

Peritoneal adherent cells (APerC), obtained from peritoneal washings of BALB/c mice, were infected with spores of *E. cuniculi* and treated with CTX (2.4 and 4.8 for 3 h. The profile and viability of macrophages, cytokine production and microbicidal activity were assessed as parameters.

RESULTS

Macrophages infected with *E. cuniculi* and treated with CTX showed an increase in the M1 profile (Fig.1), more necrosis (Fig.2) and increased production of cytokines TNF-alpha and IL-6 (Fig.3). Also, the spores obtained from these macrophages had a reduced proliferative capacity.

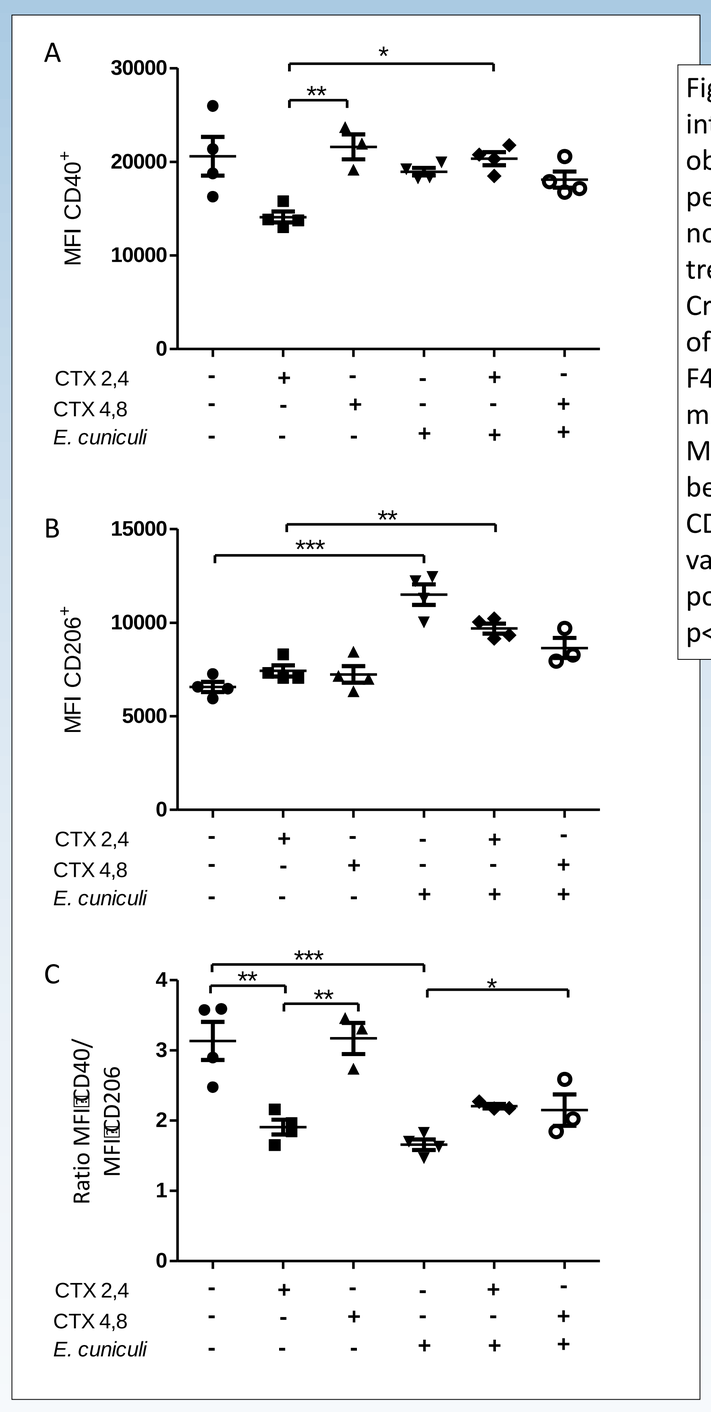

Figure 1. Mean fluorescence intensity (MFI) of macrophages obtained from adherent peritoneal cells challenged (+) or not (-) with *E. cuniculi* and treated (+) or not (-) with Crotoxin (CTX) at concentrations of 2.4 µg/mL or 4.8 µg/mL. A) F4/80+CD40+ MFI in macrophages. B) F4/80+CD206+ MFI in macrophages. C) Ratio between MFI CD40+/ MFI CD206+. One-way analysis of variance (ANOVA) with Tukey post-test revealed p<0.05*,p<0.01**, p<0.001***.

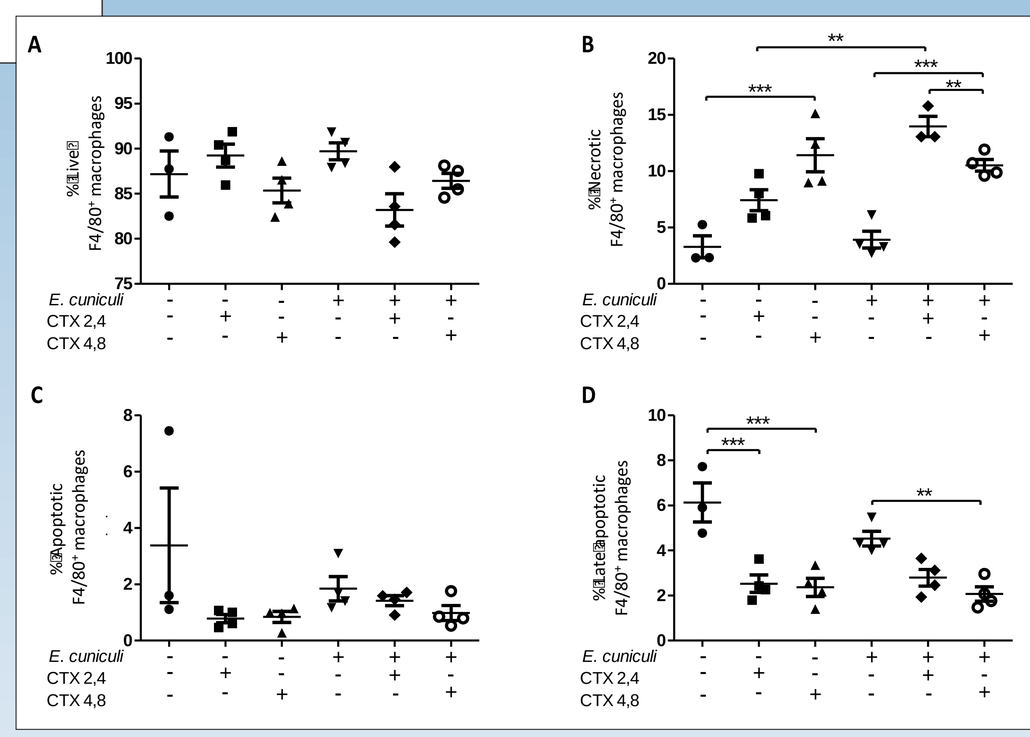

Figure 2. Comparison of macrophage viability against (+) or not (-) *E. cuniculi* infection and (+) or not (-) treatment with crotoxin (CTX) at concentrations of 2.4 µg/mL or 4.8 µg/ mL. A) Percentage of live macrophages. B) Percentage of deaths. C) Percentage of apoptosis. D) Percentage of late apoptosis. One-way analysis of variance (ANOVA) with Tukey post-test revealed p<0.01**, p<0.001***.

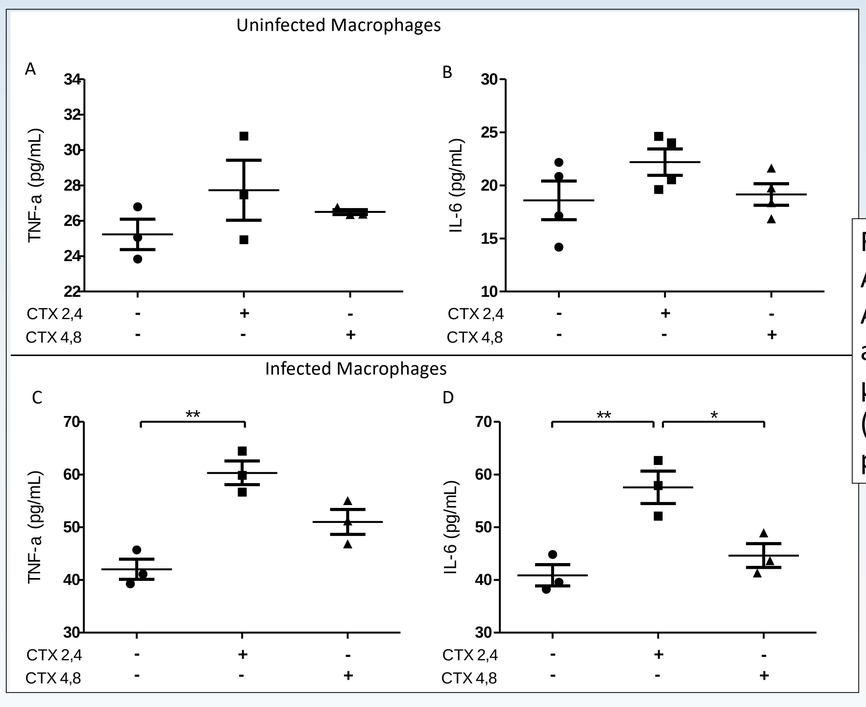

Figure 3. Quantification of inflammatory cytokines released by APerCs. Cytokines TNF-α and IL-6 produced and released by APerCs cells infected (+) or not (-) with *Encephalitozoon cuniculi* and treated (+) or not (-) with crotoxin (CTX) 2.4 µg/mL and 4, 8 µg/mL in 3 hours of observation. One-way analysis of variance (ANOVA) with Tukey's post test revealed Tukey revealed, p<0.01*, p<0.001***.

CONCLUSION

The results allowed us to conclude that CTX modulated macrophages infected with *E. cuniculi* to the M1 profile with increased production of pro-inflammatory cytokines and greater microbicidal activity evidenced potential to modulate the fungistatic and/or fungicidal activity of macrophages against *E. cuniculi.*

References

Han, B., Pan, G., & Weiss, L. M. (2021). Microsporidiosis in humans. *Clin Microbiol Ver. 34*(4), e00010-20.

Pereira A, Alvares-Saraiva AM, Konno FTC, Spadacci-Morena DD, Perez EC, Mariano M, Lallo MA. B-1 cell-mediated modulation of M1 macrophage profile ameliorates microbicidal functions and disrupt the evasion mechanisms of *Encephalitozoon cuniculi*. PLoS Negl Trop Dis. Sep 19;13(9):e0007674. doi: 10.1371/journal.pntd.0007674.

Farias, L. H. S., Rodrigues, A. P. D., Coêlho, E. C., Santos, M. F. D., Sampaio, S. C., & Silva, E. O. (2017). Crotoxin stimulates an M1 activation profile in murine macrophages during *Leishmania amazonensis* infection. Parasitol, *144*(11), 1458-1467.