FECTION, DISTURBED MITOPHAGY CONTRIBUTES TO DAMAGED MITOCHONDRIA ACCUMULATION IN EFFECTOR CD4 T CELLS LEADING TO APOPTOSIS

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Chagas disease is characterized by inefficient host immune response during acute phase of infection, enabling the establishment of chronic disease. We have recently demonstrated that acute infection triggers mitochondrial ROS (mROS) production and mitochondrial alterations in effector CD4 T cells leading to functional alterations and apoptosis. The aim of our work was to evaluate the mechanism involved in the accumulation of damaged mitochondria, and if this could be prevented by the antioxidant N-acetyl cysteine (NAC) or the mitophagy inducer Nicotinamide Riboside (NR). To achieve this, CD4 T cells were isolated from spleen of non-infected (NI), acute (AP) and chronic phase (CP) infected BALB/c mice, with 500 trypomastigotes. Mitophagy was evaluated using mitochondrial potential independent probe (MTgreen) and antibodies for LC3 and LAMP1. Cells were cultured with or without chloroquine and colocalization was evaluated by confocal microscopy. CD4 T cells from AP cultured with chloroquine did not show significant increase in MTgreen and LAMP1 colocalization compared to CCCP-treated NI CD4 T cells used as positive control (*p<0,05) suggesting a defect in mitophagy. Then, we aimed to evaluate by flow cytometry, mROS production, frequency of cells with damaged mitochondria and apoptosis in effector CD4 T cells from AP infected mice treated with NAC, NR and vehicle as control. We did not found differences between NAC and control group. In contrast, NR treatment reduced the percentage of CD4 T cells with damaged mitochondria (*p<0.05), although we did not observed difference in mROS production. Moreover, apoptosis frequency was also diminished (**p<0.01). Depolarized mitochondria accumulation, probably due to a defect in mitophagy, could be restored by NR, and thus prevent apoptosis. Taken together, this evidence stablishes association between accumulated damaged mitochondria, and impaired mitophagy leading to apoptosis in CD4 T cells during acute *T. cruzi* infection.

192. (208) **ROLE OF B LYMPHOCYTES IN THE IMMUNE RESPONSE TO SHIGA TOXIN- PRODUCING ESCHERICHIA COLI INFECTION**

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We previously demonstrated a better outcome in weaned BALB/c mice (BALB) compared to C57BL/6 (C57) after Shiga toxin (Stx)-producing *E. coli* (STEC) infection. The main difference found was the early appearance of specific anti-STEC (aSTEC) and anti-Stx antibodies (aStx) in BALB. The aStx developed by infected BALB not only protected them against an intravenous (i.v.) Stx challenge, but also passive immunization with their sera protected C57 after STEC infection. The aim of this work is to determine if B cell-dependent response triggered after infection is necessary to guarantee the survival of infected BALB.
We administered a single i.v. dose of anti-B220 antibody (aB220; 4 mg/mouse) to BALB to deplete B cells. We analyzed the percentage of CD19 positive cells (%CD19$^+$) in mesenteric lymph node (MLN) and spleen at different times (4, 24 and 48 h) by flow cytometry. This treatment induced a significant B cell depletion in MLN at 4, 24 and 48 h vs controls (control vs B-depleted: 4, 24 and 48 h, p<0.001; ANOVA). %CD19$^+$ cells in spleen at 4, 24 and 48 h were significantly lower than controls; however, at 48 h the %CD19$^+$ cells in B-depleted mice started to increase (control vs B-depleted: 4, 24 and 48 h, p<0.0001; B-depleted at 48 h vs B-depleted at 4 and 24 h, p<0.05; ANOVA).
To study the role of specific B-dependent response, i.v. aB220 or PBS were administered to BALB 1 h before and 3 h post infection. To guarantee B-cell depletion, aB220 injection was repeated twice a day till the third day of infection intraperitoneally. B-depleted mice showed increased mortality rates (p<0.05, Log-Rank test), higher urea levels (p<0.05, t test) and a significant weight loss on day 3 p.i. (p<0.0001, ANOVA). Also, they didn't develop significant levels of aSTEC IgA at day 4 p.i., assayed as IgA coated bacteria by flow cytometry (p<0.01, t test).
We concluded that B cell stimulation and the consequent antibody response play a key role in protection against STEC infections.

193. (217) **CASE REPORT: VIRAL SHEDDING FOR 120 DAYS IN AN ALLERGIC CHILD WITH COVID 19**

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Since it was first detected in Dec 2019, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has spread through the planet causing the novel coronavirus disease, Covid-19. Here, we report the case of a 9-year-old girl with persistent viral shedding based on RT-PCR detection. Her first positive (Ct=36) dated from March 4 2021, came in a preoperative physical examination (scheduled tonsillectomy), with no apparent symptoms. Her father fell ill and tested positive soon after that casual finding. Surgery was postponed. The patient came positive again on July 1 2021 (Ct=33) and on July 29 2021 (Ct=29). She had a persistent cough, which was also compatible with her allergic condition. Her surgery could not be postponed any longer and was operated on July 30 2021. Excised adenoids and tonsils were extensively rubbed with a swab to test whether the material detected resulted infectious or not, on Vero E6 cell cultures. Based on the absence of any cytopathic effect, we found it was not infective, even upon an intended amplification by a second passage. RT-PCR was negative when performed on the last supernatant. The histological pattern of her tonsillar and adenoid tissue was analyzed through H&E staining and immune cell populations were examined by FACS. Both aspects were compatible with her hyperplastic condition and also with a viral infection. We tested the anti-Spike specific response by ELISA on serum samples taken on Aug 6 2021 (IgM=1.34, cut off=0.584 and IgG=3.27, cut off=0.364). Finally, we determined the neutralizing antibodies titer on the same serum, using the wild type SARS-CoV-2 (titre=32, the mean of infected adults is 64). We concluded that, albeit the long period the genetic material of the virus was detected on her swabs, the patient does not seem to have a major immunological deficiency and could mount an appropriate immune response against the virus. Importantly, we demonstrated she was not able to transmit virus at the time of the surgery.

194. (244) **ADENOSINE REGULATES CYTOTOXIC CD4 T LYM-**