EPSTEIN BARR VIRUS´ ROLE IN THE INTERPLAY OF TONSIL MACROPHAGES POLARIZATION AND PERIPHERY CYTOKINE EXPRESSION.

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Epstein Barr Virus (EBV) was the first tumor-associated virus described. Immune response against primary infection widely differs between pediatric and adult patients. Interestingly, our group described a high prevalence of pediatric lymphomas associated with the virus in children younger than 10 years old [1]. Since little is known about macrophage´s roll in the context of EBV infection in pediatrics, in a previous study, we characterized macrophages polarization at tonsils [2]. In this work, our aim was to evaluate the behavior of peripheral polarization cytokines and its relation with tissue findings to further comprehend the roll of macrophages in EBV infection and its contribution to lymphomagenesis.

We studied 59 patients undergoing tonsillectomy, aged between 1 and 15 years (median 5 years); formalin fixed paraffin embedded (FFPE), fresh tonsillar tissue and peripheral blood samples were obtained for the study. The cohort included 20 Primary Infected (PI), 23 Healthy Carriers (HC), 11 undergoing Reactivation (R) and 5 Not Infected (NI) patients. Immunohistochemistry (IHC) was performed in FFPE for IL-10, IFN-ɣ and TGF-ß cytokines; LMP-1, EBNA-2 and BMRF-1 viral proteins and CD68 (M1) and CD163 (M2) macrophages polarization markers. M1 profile was defined as CD68/CD163 >1.5 and M2 as CD163/CD68 >1.5. Cell count was made for each staining and expressed as +cells/area. IL-1β and TNF-α cytokines were assessed by qPCR. Finally, a bead-based multiplex assay panel, using fluorescence-encoded beads was performed in patient's serum for IL-12p70, TNF-α, IL-6, IL-10, IL-1β, TARC, IL-1Ra, IL-12p40, IL-23 and IP-10 Human M1/M2 Macrophage Panel (10-plex) (LEGENDplex, BioLegend).

No significant differences were observed in serum cytokine expression between EBV+ and EBV- patients ($p>0.05$ T test and MW) except for TARC, which was higher in NI patients ($p=0.0267$, MW). Among infection status (PI, HC and R), only IL-1β ($p=0.0384$, T.test), IL-12p40 ($p=0.0272$, T.test) and IL-23 ($p=0.0319$, MW) presented significant differences between R and HC, being higher in R. Comparing peripheral and tissue cytokine expression, only IL-10 showed a significant negative correlation ($r=-0.6598$; $p=0.0272$, Pearson). Regarding viral protein expression, patients were clustered in two groups, on one hand those who expressed Latency 0 and Latency I (EBERs+) as (L0-I) and on the other hand patients expressing Latency II (EBERs+ and LMP-1+) and Latency III (EBERs+, LMP-1+ and EBNA-2+) (LII-III). Both IL-1Ra ($p=0.0242$, MW) and TNF-α ($p=0.016$, MW) presented higher expression in L0-I patients. Continuing, we compared serum cytokines quantification between lytic BMRF-1 positive and negative patients, being TNF-α higher in absence of lytic antigen ($p=0.0174$, MW). Finally, we compare cytokine behavior between M1 and M2 patients, and TARC revealed a broader presence in M2 ($p=0.0123$, MW).

TARC, an important attractant of T-regulatory cells, secreted by anti-inflammatory macrophages was higher expressed in patients that presented M2 profile at the site of viral infection, suggesting a connection between tonsils and periphery scenario.

What's more, not only several cytokines displayed a differential behavior between R and HC patients, but also in patients with higher viral latent and lytic protein expression, TNF- α and IL-1Ra expressions were lower, suggesting the virus might influence in the peripheral compartment.

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