Extended Abstract Virology Research Journal 2018
Vol.2 No.2

Euro Virology 2018: Role of Herpesviruses dUTPases in the immune dysregulation associated with myalgic encephalomyelitis/chronic fatigue syndrome and Gulf war illness- Maria Eugenia Ariza- The Ohio State University

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Myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) and Gulf war illness (GWI) are debilitating diseases presenting with complex immune, endocrine and neurological symptoms. Annual health care costs are estimated at $24 billion. Diagnosis is based on exclusion and there are currently no validated tests for definitive diagnosis of either syndrome. While there is accumulating evidence supporting the premise that some herpesviruses may act as possible triggers in ME/CFS, the mechanism by which they contribute to the pathogenesis of ME/CFS remains unclear. Our studies are the first to demonstrate that the deoxyuridine triphosphate nucleotidohydrolase (dUTPase) encoded by the human herpesviruses represents a new class of pathogen-associated molecular pattern (PAMP) proteins, which alter immune and neurocognitive functions. In this study, we demonstrate that ME/CFS and GWI patients' sera exhibit reactivation of multiple herpesviruses, differential antibody expression patterns to the herpesviruses-encoded dUTPase early protein as well as increased autoantibodies to the human nuclear dUTPase. A significant increase in IL-21 levels was also observed in a cohort of ME/CFS patients. Interestingly, IL-21 is produced at high levels by CD4+ follicular helper T cell (TFH) and regulates germinal center (GC) B cell survival and plasmacell differentiation. Further in vitro studies in primary human cells demonstrated that the EBV-dUTPase induced activin A secretion by dendritic cells, which lead to the increased formation of CD4+ follicular helper T cells (TFH) and subsequent production of IL-21 and CXCL13 by TFH. Our data suggest a role for the herpesviruses dUTPase proteins in the immune dysregulation and pathophysiology observed in these patients possibly by altering the GC reaction and antibody responses as well as inducing the production of pleiotropic cytokines. Thus, screening for the presence of anti-herpesvirus dUTPase antibodies in these patients may serve as useful diagnostic biomarkers for the selection of appropriate treatments.

In over half of cases, ME/CFS onset is associated with acute "flu-like" symptoms. There are multiple reports in the literature suggesting a role for viruses, particularly human herpesvirus-6 (HHV-6) and Epstein-Barr virus (EBV) in ME/CFS. However, the data concerning a causal relationship between a virus and ME/CFS has not been conclusively demonstrated and remains a challenge because of the heterogeneity of the patient population and the realization of possibly multiple etiologies.

We have previously demonstrated that the deoxyuridine triphosphate nucleotidohydrolase (dUTPase) encoded by EBV, HHV-6 and VZV possess novel immunoregulatory functions by triggering the activation of toll-like receptor (TLR) 2, which leads to the activation of NF-κB and subsequent modulation of downstream genes involved in chronic inflammation, effector T-cell function and neurotransmitter function. We have also shown that the EBV-encoded dUTPase is secreted in exosomes, which function as intracellular messengers, induces the secretion of the pro-inflammatory TH1/TH17 cytokines, alters T- and NK cell function in vitro, and induces anxiety and sickness behavior in mice. While the HHV-6A U45 gene was cloned from the HHV-6A strain GS, the recombinant protein exhibits greater than 94% identity to the HHV-6B strain Z29 U45 gene. Therefore, it is unlikely that the U45 recombinant protein can distinguish between these strains.

ELISAs were performed using 96-microtiter well plates (Nunc-Immuno Plate MaxiSorp Surface) were coated overnight at 4 °C with recombinant dUTPase protein at 2.5 μg/ml in phosphate buffered saline (PBS). Plates were washed on a Biotek ELx50 plate washer three times with PBS/0.05 % Tween 20 (200 μl) and blocked with blocking buffer (PBS/2.5 % BSA; 100 μl) at room temperature (RT). All serum samples were used at a 1:800 dilution in blocking buffer and incubated for 2 h at RT in duplicate. Plates were washed three times with PBS/0.05% Tween 20 (200 μl) followed by incubation with anti-human-IgG horseradish peroxidase (HRP)-conjugated secondary antibody (Sigma Chemical Co. St. Louis MO) at 1:1000 dilution in blocking buffer at RT for 1 h. Plates were washed six times with PBS/0.05 % Tween 20 and incubated for 15 minutes with 100 μl of tetramethylbenzidine (Invitrogen, Carlsbad, CA). A volume of 50 μl H2SO4 (2 M) was added to stop the reaction and plates were read at 490 and 690 nm for background on a Lab Systems Multiskan MCC/340 plate reader using the Genesis v3.05 Life Sciences Ltd software. The background from the 490 nm uncoated wells and PBS-BSA (negative controls) were subtracted from the mean absorbance of the coated wells. ROC curves were constructed to determine optimal threshold points for each antigen using serum obtained from a healthy control donor. Intra-assay variation was determined to be less than 0.02 while inter-assay variation ranged from 0.005 for human to 0.049 for VZV.

This work is partly presented at 10th International Virology Summit & 4th International Conference on Influenza & Zoonotic Diseases July on 02-04, 2018 held at Vienna, Austria

A positive reaction was defined as a serum sample that led to a signal 3-times over the background OD of the control serum (HHV-6: 0.124 ± 0.045; EBV: 0.104 ± 0.025; VZV: 0.256 ± 0.016; human dUTPase: 0.017 ± 0.005). For the studies involving sera from the Good Day Bad Day study, if any of the four samples from a patient were positive for an anti-dUTPase antibody that individual was considered positive for that particular dUTPase.

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