ROLE OF FUCOIDANS IN THE RESTORATION OF THE ANTI-TUMOR T-CELL RESPONSE INHIBITED BY EBV LATENCY III B CELLS *VIA* THE PD-L1 IMMUNE CHECKPOINT

Saliba J.[1], Manseur C[2]. Gressein C[1]., Groult H[2], Akil H.[1], Tannoury M.[3], Troutaud D.[1], Feuillard J.[1], Arnaudin I.[2] ,Jayat-Vignoles C[1].

[1] CRIBL Laboratory, Limoges University, UMR CNRS 7276 U1262, France; [2] LIENSs Laboratory, La Rochelle University, UMR CNRS 7266, France; [3] Lebanese University, Faculty of Sciences II, Lebanon.

jennifer.saliba@unilim.fr

The Epstein Barr virus (EBV) infects more than 95% of the world's population and persists latently in the body. It has the ability to immortalize B cells and is associated with many lymphomas. We have shown that EBV latency III B cells inhibit the anti-tumor T cell response by mimicking regulatory B cells (B-regs). They secrete immunosuppressive cytokines (IL-10, IL-35, TGFβ) and overexpress the immune checkpoint PD-L1 (Programmed Death-Ligand 1) responsible for anergic regulatory T cell (T-regs) expansion and effector T cell inhibition, through the PD-L1/PD-1 interaction [1]. This could explain emergence and development of EBV-associated B-cell lymphomas in immunocompetent persons. Actually, we are interested in the restoration of the T cell response inhibited by EBV latency III B cells.

The work was carried out on three newly established Lymphoblastoid Cell Lines (LCLs, EBV latency III B cells). We were interested by fucoidans, which are sulfated polysaccharides extracted from brown seaweed that gained attention in the last years because of their *in vitro* and *in vivo* anticancer activities depending on their polymerization degree. Very few data have been reported on lymphoma cells and on immune checkpoints. Cells were treated with native fucoidans of *Fucus vesiculosus* or with two original depolymerized fractions.

We observed a decrease in proliferation and an induction of apoptosis with the two depolymerized fractions. We also highlighted a PD-L1 decrease at both transcriptional and cell surface levels of apoptotic and viable cells, while PD-L1 total expression remained high and unchanged. In addition, preliminary results emphasized alteration of the actin network. This agrees with our previous results on EBV latency III B cells, showing that PD-L1 is stored in secretory lysosomes and that an increase of the actin network density promotes their fusion with cell membrane and surface overexpression [2]. We did not observe any toxic effect on PBMC (peripheral blood mononuclear cells) and isolated T cells (activated or not). Fucoidans could contribute to restore anti-tumoral immune response, alone or as an adjuvant of anti-PD-L1 immunotherapy, due to its proapoptotic effects combined with its ability to decrease membrane expression of PD-L1. We plan to conduct functional experiments thanks to our autologous EBV latency III B cells / T cells co-culture model after treatments with fucoidans, alone or with anti-PD-L1 monoclonal antibodies. It is expected a potentiation of apoptosis for the combined treatment.

Complementary results have shown that, in addition to PD-L1, EBV latency III B cells overexpress other immune checkpoints involved in the anti-tumoral response and stored in secretory lysosomes for unconventional secretion. Fucoidan could also contribute to decrease their release.

Key words: EBV, latency III, B lymphomas, T lymphocytes, fucoidans, inhibitory checkpoints, immunosurveillance.

[1] Auclair, H., Ouk-Martin, C., Roland, L., Santa, P., Al Mohamad, H., Faumont, N., Feuillard, J., and Jayat-Vignoles, C. (2019). EBV Latency III-Transformed B Cells Are Inducers of Conventional and Unconventional Regulatory T Cells in a PD-L1-Dependent Manner. J. Immunol. Baltim. Md 1950 *203*, 1665–1674.

[2] Durand-Panteix, S., Farhat, M., Youlyouz-Marfak, I., Rouaud, P., Ouk-Martin, C., David, A., Faumont, N., Feuillard, J., and Jayat-Vignoles, C. (2012). B7-H1, which represses EBV-immortalized B cell killing by autologous T and NK cells, is oppositely regulated by c-Myc and EBV latency III program at both mRNA and secretory lysosome levels. J. Immunol. Baltim. Md 1950 *189*, 181–190.