IDENTIFICATION OF T CELL MIMOTOPES USING VARIABLE EPITOPE LIBRARY IN A MOUSE MODEL OF BREAST CANCER

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Introduction. Breast cancer is the leading cause of cancer-related death in women world-wide. [(1)] Advances in molecular immunology and biotechnology have created an opportunity for developing active vaccination strategies that engage the patient´s own immune system in the fight against breast cancer. [(2)] A mimotope is an epitope that functionally mimics natural protein epitopes and activates the immune system against the original antigen resulting in a specific immune response. [(3)] They might be ideal antigen surrogates in tumor immunology able to break tolerance toward self.
The objective of this work is to obtain mimotopes of epitopes recognized by T lymphocytes by the screening of variable epitope library (VEL) and to determine the immunogenic properties of mimotopes identified and their antitumoral effect in a mouse model of breast cancer.

Methods. In order to identify mimotopes of T cell we used VEL. The epitopes employed contain nine amino acids in length, five of which represent any of the 20 amino acids and three are representing the MHC-binding motifs; also we used an epitope bearing three MHC anchors amino acids and six Alanines (Ala) as a control. To construct the VEL, three partially overlapping oligonucleotides were used in a PCR assembly to generate a DNA fragment. The DNA was amplified by PCR and cloned into phagemid vector pG8SAET in order to express the epitope variants on M13 phage surface as fusions with the major phage coat protein (cpVIII) generating a phage-displayed library, as we have reported previously. [(4)] Groups of 5 female, 4-6-week-old BALB/c mice were used. Mice were inoculated with $1X10^4$ 4T1 cells and immunized with the VEL in the form of M13 phage particles. We performed proliferation assays with spleen cells obtained from mice on day 15 after immunization and phage particles corresponding to individual epitope variant. After 72h of incubation, cells were harvested and the cell proliferation analyzed by flow cytometry.

Results.

1. Variable epitope library induce increase in cell proliferation

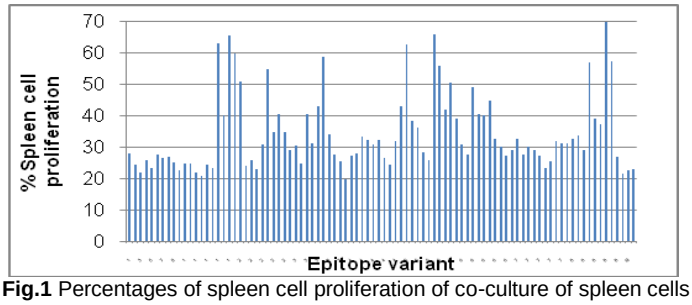

Fig.1 Percentages of spleen cell proliferation of co-culture of spleen cells from mice implanted with 4T1 cells and immunized with the variable epitope library (G5d) and phage particles corresponding to individual epitope variant.

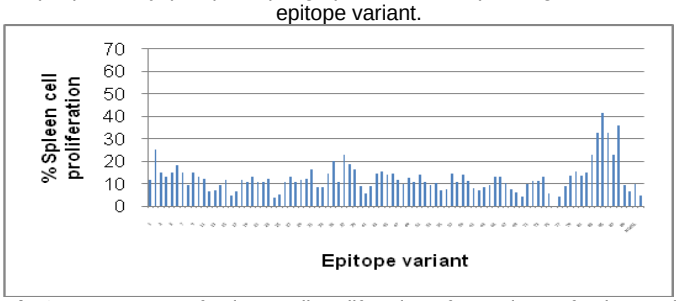

Fig.2 Percentages of spleen cell proliferation of co-culture of spleen cells from mice implanted with 4T1 cells and immunized with the epitope nominal (NGd) and phage particles corresponding to individual epitope variant.

Conclusions. By comparing the percentages of proliferation in Figures 1 with 2, we can observe that these percentages were higher in co-cultures where spleen cells from mice implanted with 4T1 cells and immunized with VEL were employed as compared to cells from control mice. This allows us to make a selection of epitopes with immunogenic properties that might be potential vaccine components. Currently, using this strategy, we are evaluating the antitumor effect of a group of selected mimotopes.
Due to the high antigenic variability present in tumor cells and to the tolerance to tumor-associated antigens, we believe that this strategy of selection of mimotopes related to the cellular responses against tumors will allow to generate immunogens capable of breaking immune tolerance and building a new generation of effective vaccines against cancer.

References.

1. Polyak K. (2007). *J Clin Invest*. 117(11):3155-3163.
2. Emens L, Reilly R, Jaffee E. (2005). *Endocr Relat Cancer*. 12(1):1-17.
3. Shanmugam A, Suriano R, Goswami N, Chaudhuri D, Ashok B, Rajoria S, George A, Mittelman A, Tiwari R. (2011). *Cell Stress Chaperones*. 16(2): 225-234.
4. Pedroza C, Charles C, Saavedra R, Govezensky T, Vaca L, Avaniss E, Gevorkian G, Manoutcharian K. (2009). *Mol Immunol*. 47 (2-3): 270-282.