

ANTI-CPSMV IGY: A NOVEL TOOL FOR EXPLORING ANTI-TUMOR IMMUNE RESPONSE

Beatriz Modesta Moreira^{1*}, Louise Sousa de Souza¹, Dayanne Wysllate Araújo Ribeiro¹, Susanne Suely Santos Fonseca¹, Renata Kelly de Freitas Mano², Myrth Soares do Nascimento Remígio¹, Fernando Barbosa Tavares³, Paulo Pimentel de Assumpção¹, Maria Izabel Florindo Guedes², Livia Erika Carlos Marques¹

¹Oncology Research Center (NPO), Federal University of Pará (UFPA), Brazil

²Laboratory of Biotechnology and Molecular Biology (LBBM), State University of Ceará (UECE), Brazil

³Poultry farming, Federal Rural University of the Amazon (UFRA), Brazil

Introduction: Among plant viruses, the Cowpea Mosaic Virus (CPMV), of the genus Comovirus, stands out for being studied in an in situ antitumor vaccination modality, in which pattern recognition receptors are activated and lead to a cellular immune response that is effective against various tumors, while another Comovirus, the Cowpea Severe Mosaic Virus (CPSMV), remains little studied. In this context, it is feasible to produce anti-CPSMV antibodies by immunizing birds producing IgY immunoglobulin, an antibody with a high concentration in egg yolk, chemically stable, low cost, non-invasive for the animal and which does not induce human complement activation, showing high biotherapeutic and diagnostic potential. **Objectives:** To evaluate the immune response induced by CPSMV in laying hens and to obtain IgY antibodies to use as research input. **Methods:** Opinion number (CEUA-UFPA): 1326191222. The CPSMV was purified from *Vigna unguiculata* leaves, characterized by polyacrylamide gel (SDS-PAGE) and UV-Vis spectrometry. Two hens were given three doses of immunizations containing 50 µg of CPSMV with montanide adjuvant intramuscularly, with an interval of two and one week, respectively, between the second and third dose. Subsequently, the yolks were purified to obtain the IgY antibody by precipitation with ammonium sulfate. To examine the titration of antibodies at different dilutions, an Enzyme-Linked Immunosorbent Assay (ELISA) analysis was performed. **Results:** The virus was immunogenic, since it was possible to observe the presence of anti-CPSMV IgY antibodies in the egg yolks of immunized chickens. The IgY anti-CPSMV antibodies recognized the virus in an immunoblotting assay. The increase in specific antibodies for CPSMV occurred from the 7th day of the primary response, after immunization. The serial dilution of anti-CPSMV IgY antibodies showed detectable absorbance values even at lower antibody concentrations (1:12,800), with the highest absorbances observed at dilutions of 1:100 (0.857) and 1:200 (0.844). Furthermore, the anti-CPSMV IgY antibody was able to recognize CPSMV in metastatic

gastric cancer cell line, Pará gastric ascites cell line (AGP01), in the immunofluorescence assay, in which it was possible to observe the internalization of the virus with dispersion in the cytoplasm. **Conclusion:** These results reinforce the potential of CPSMV to induce an immune response and highlight the IgY platform as a rapid and accurate tool for the production of specific antibodies to Comoviruses.

Keywords: Plant virus; egg yolk immunoglobulin; immunotherapy.