BIO_02 - Production of a monoclonal antibody against SARS-CoV-2 and determination of viral neutralization capacity

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Introduction: The emergence of the new coronavirus (SARS-CoV-2) in Wuhan, China, caused a worldwide epidemic of respiratory disease (COVID-19). SARS-CoV-2 belongs to the genus Betacoronavirus. It has structural proteins that include the spike protein (S), the envelope protein (E), the membrane protein (M) and the nucleocapsid protein (N). Various reports were published relating the antibody responses generated against protein S, as it is the most exposed protein of SARS-CoV-2. Monoclonal antibodies (mAbs) have many applications in diagnosis, treatment and can contribute to study the COVID-19.

Objective: Production of a monoclonal antibodies against SARS-CoV-2 spike protein and determination of the neutralization of SARS-CoV-2 infection.

Methodology: 1. Mouse Immunizations.SARS-CoV-2 spike protein (GenBank: MN908947) was mixed with an equivalent volume of Vaccine Self-Assembling Immune Matrix (VacSIM) adjuvant and injected subcutaneously into BALB/c mice. 40 µg of protein was used in the first injection and 20 µg at 9, 23, 30, 36, 52, 58, 64 days after the first injection. Spike protein without adjuvant was injected intraperitoneally 3 days prior to removal of the mouse spleen and cell fusion. 2. Determination of antibody titer against Spike protein.To determine the antibody titer against the SARS-CoV-2 spike protein, at 6 days after each immunization, the serum from mice was tested by ELISA. 3. Cell fusion, hybridoma selection and screening. For production of mAbs against SARS-CoV-2 spike protein, spleen cells of the most immune mouse were fused with SP2/0 (myeloma cells) using Poly Ethylene Glycol (PEG). The cells were then plated onto 96-well plates containing hypoxanthine-aminopterin-thymidine (HAT) medium. Seven days after cell fusion, 100 µL/well of Hypoxanthine-Thymidine (HT) medium was added. Supernatant of hybridoma cells was screened for detection of antibody by ELISA and the suitable clones were selected. 4. Preparation of human monoclonal antibody.Peripheral blood mononuclear cells (PBMCs) will be isolate from peripheral blood samples and fused with SPYMEG cells. After HAT selection, the hybridoma cells will be screening for detection of antibody and the suitable clones will be were selected.

Results: ELISA assay detected antibodies against SARS-CoV-2 spike protein (Pre immune serum: OD = 0.01 and 72 days after the first imunnization: OD = 0.95). Monoclonal antibody production requires the immunization with an immunogenic protein and test sampling of serum. Future analysis will further investigate these clones. The mAbs will be characterized to determine their affinity (dissociation constant, Kd) by ELISA and the specificity (by Western blot). Further, the mAbs will be tested to assess the neutralization capacity of SARS-CoV-2 in viral culture.

Conclusion: We believe these mAbs against SARS-CoV-2 will have potential therapeutic applications and can contribute to controlling and decreasing the number of cases the COVID-19.

Keywords: Monoclonal antibody; SARS-CoV-2; Spike protein