Rabies virus glycoprotein (RVGP) expression in Drosophila S2 cells and by Semliki Forest Virus. Synthesis and protection studies.

Astray RM¹, Jorge SAC¹, Lemos MAN¹, Ventini DC¹, Benmaamar R², Martorelli LFA¹, Kataoka APAG¹, Tonso A², Wagner R², Pereira CA²,²

1) Laboratório de Imunologia viral, Instituto Butantan, São Paulo, Brasil; 2) Laboratório de Células Animais, Dept de Engenharia Química, USP, São Paulo, Brasil; 3) Département récepteurs et protéines membrane, CNRS Strasbourg, France; 4) Laboratório de zoonoses e doenças transmitidas por vetores, São Paulo, Brasil.

**SFV-RVGP construction**

**Theoretical basis of viral antigen immunization for vaccine design**

- **Drosophila melanogaster** S2 cells system
  - Exogenous antigens - peptides within endosomes with MHC I presentation to CD4⁺ helper T cells
  - Inactivated viral vaccines
  - DNA vaccines
  - Natural infectious attenuated viral vaccines

- **Semliki Forest Virus system (SFV)**
  - Glycoadhesin antigens (Ag synthesized in vivo) - peptides into ER associate with MHC I and at cell surface xCD8 T cells

**R&D objectives**

DNA + protein → VLP or SFV + packaging recombinant RNA

**RVGP synthesis by S2MirRVGP-H cells in a bioreactor run**

**Mouse challenge with rabies virus**

Mouse protection against the challenge with rabies virus. Immune mice were challenged with rabies virus CVS (0.2 mL) for 30 days beginning at the 21st day after the first immunization. S2aRVGP-2x present in vitro synthesized RVGP for immunologic system. SFV-RVGP promotes RVGP in vivo production in the same organism cells.

**RVGP expression by S2 a BHK/SFV cells**

Cell activation after immunization. Splenocytes from immunized naive mice were stimulated with RVGP for cell proliferation, recognized with anti-CD8 antibodies labeled with fluorescence and measured by flow cytometry. Figure shows a higher yield of activated lymphocytes in SFV-RVGP immunized mice in vaccinated mice.

**Cellular immune response**

**Humoral immune response**

Antibody production after immunization. Balb/C mice (n = 14/group) were immunized 3 times (days 0, 7, 14) with S2aTRVGP-H (0.2 μg RVGP), with SFV-RVGP (0.3 x 10⁹ SFV-RNA) and rabies vaccine. The titers of anti-rabies antibodies were determined at days 14, 21 and 28 after the first immunization. Titers above 0.5 IU/mL are considered sufficient seroconversion levels.

Financial support: FAPESP/CNRS