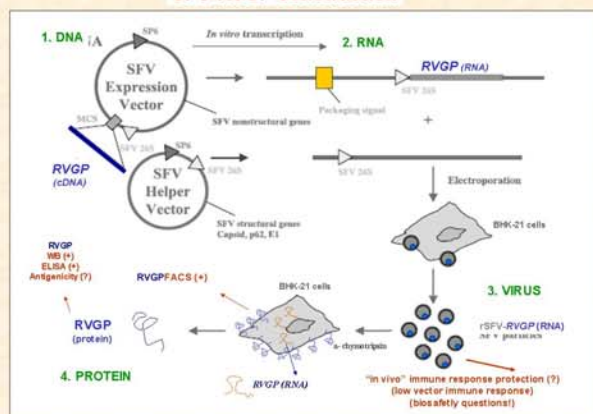


## Rabies virus glycoprotein (RVGP) expression in *Drosophila* S2 cells and by Semliki Forest Virus. Synthesis and protection studies.

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### SFV-RVGP construction



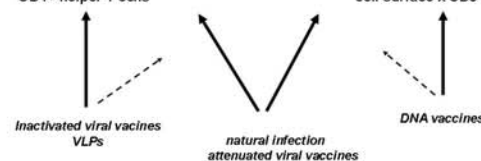
### Theoretical basis of viral antigen immunization for vaccine design

#### *Drosophila melanogaster* S2 cells system

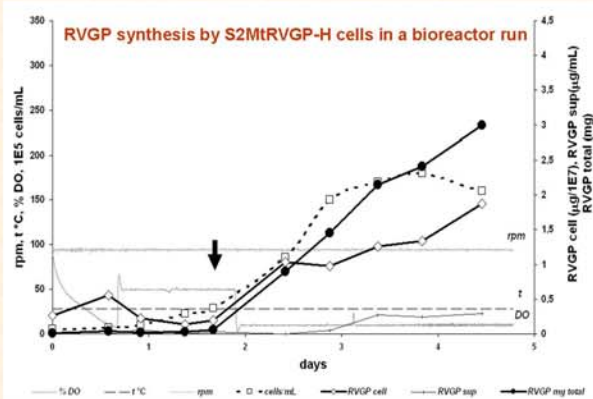
Exogenous antigens - peptides within endosomes with MHC II presentation to CD4+ helper T cells

#### Semliki Forest Virus system (SFV)

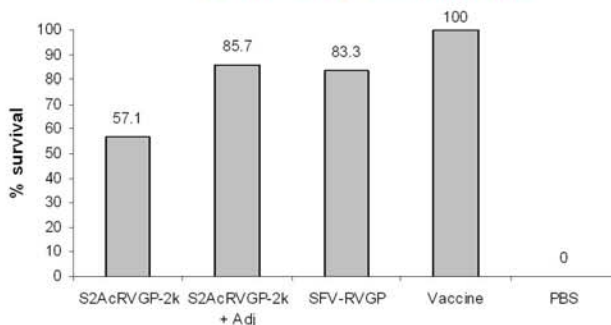
Cytosolic antigens (Ag synthesis "in vivo") - peptides into ER associate with MHC I and at cell surface x CD8+ CTLs.



R&D objectives → DNA + protein VLP or SFV "packaging recombinant RNA"

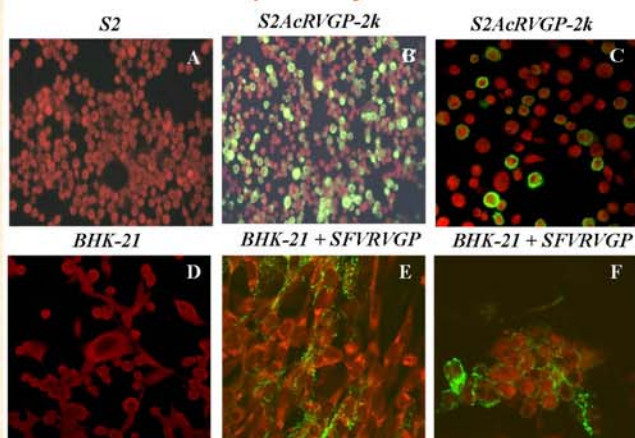


### Mouse challenge with rabies virus



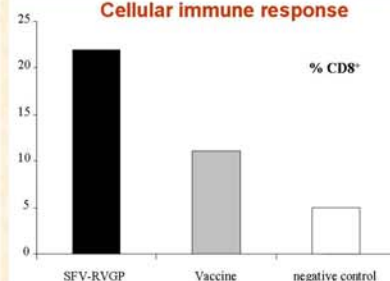
Mouse protection against the challenge with rabies virus. Immunized mice were challenged with rabies virus CVS (30 DL<sub>50</sub>) for 30 days beginning at the 21<sup>st</sup> day after the first immunization. S2AcRVGP-2k present *in vitro* synthesized RVGP for immunologic system, SFV-RVGP promotes RVGP *in vivo* production in the own organism cells.

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



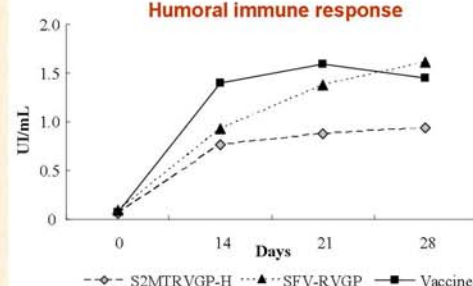
Immunofluorescence of S2MTRVGP-H and BHK-21 cells infected with SFV carrying RVGP genetic information (SFV-RVGP). Labeled with monoclonal antibodies IgG anti-RVGP conjugated with fluorescein. Evans blue contrast (red). Visualization and digital photography in fluorescence microscope (A - B) or confocal (C - F). Wild-type S2 cells (A), S2MTRVGP-H (B) and (C), BHK-21 (D), BHK-21 + SFV-RVGP (E) and (F).

### Cellular immune response



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

### Humoral immune response



Antibody production after immunization. Balb/c mice (n = 14 / group) were immunized 3 times (days 0, 7, 14) with S2MTRVGP-H (0.2 μg RVGP), with SFV-RVGP (8.3 x 10<sup>6</sup> SFV-RNA) and rabies vaccine. The titres of anti-rabies virus antibodies were determined at days 14, 21 and 28 after the first immunization. Titres above 0.5 UI/mL are considered sufficient seroconversion levels.

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