



[Vaccine](#)

[Volume 27, Issue 46](#), 30 October 2009, Pages 6415-6419

Advances in Vaccine Technology II 2008

[doi:10.1016/j.vaccine.2009.06.019](https://doi.org/10.1016/j.vaccine.2009.06.019)

Respiratory syncytial virus subunit vaccine based on a recombinant fusion protein expressed transiently in mammalian cells

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Available online 24 June 2009.

Abstract

Although respiratory syncytial virus (RSV) causes severe lower respiratory tract infection in infants and adults at risk, no RSV vaccine is currently available. In this report, efforts toward the generation of an RSV subunit vaccine using recombinant RSV fusion protein (rRSV-F) are described. The recombinant protein was produced by transient gene expression (TGE) in suspension-adapted human embryonic kidney cells (HEK-293E) in 4 L orbitally shaken bioreactors. It was then purified and formulated in immunostimulating reconstituted influenza virosomes (IRIVs). The candidate vaccine induced anti-RSV-F neutralizing antibodies in mice, and challenge studies in cotton rats are ongoing. If successful in preclinical and clinical trials, this will be the first recombinant subunit vaccine produced by large-scale TGE in mammalian cells.

Keywords: Transient transfection; Recombinant protein; RSV vaccine; Virosomes

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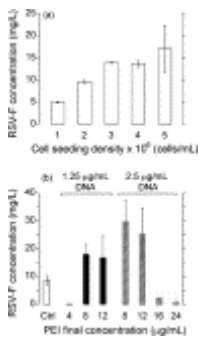


Fig. 1. Optimization of transfection parameters for expression of rRSV-F in HEK-293E cells. (a) Cells were transfected in “CultiFlask 50” tubes at a cell density of 20×10^6 cell/mL with 1.25 μ g pcDFsyn and 4 μ g PEI per 1×10^6 cells. The cells were diluted in Ex-Cell293 medium to different cell densities as indicated, to 10 mL of final volume. rRSV-F was measured by ELISA at day 2 post-transfection. (b) Cells were transfected with different amounts of pcDFsyn and PEI as indicated. After transfection, the cells were diluted to 3×10^6 cells/mL in Ex-Cell293 medium. The control is from HEK-293E cells transfected with 1.25 μ g/mL pcDFsyn and 4 μ g/mL PEI and diluted to 1×10^6 cells/mL after 3 h.

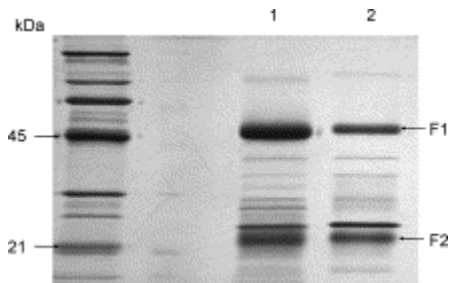


Fig. 2. SDS-PAGE analysis of purified RSV-F. rRSV-F expressed in HEK-293E cells in a 4-L transfection was purified by chromatography (lane 2) and compared to vRSV-F purified in a similar way from infected Vero cells (lane 1). Protein samples were

electrophoresed on a reducing 12% polyacrylamide gel. The positions of the F1 and F2 subunits of RSV-F are indicated.

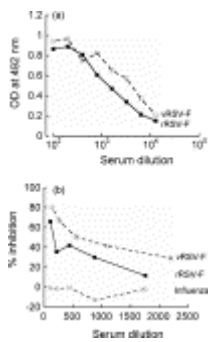



Fig. 3. Analysis of humoral immune response of BALB/c mice immunized with different RSV-F preparations. (a) Relative anti-RSV-F antibody concentrations in serum of mice immunized with IRIVs containing either vRSV-F (□) or rRSV-F (■) were measured as described in Section 2. (b) RSV neutralization assay with serum of mice immunized with IRIVs containing vRSV-F (□) or rRSV-F (■) was performed as described in Section 2. Serum of mice infected with influenza virus served as a negative control (○).

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