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12-8-2005

# Orally Delivered, Plant-Produced Tat Protein Primes Mice For a Challenge DNA Vaccine Expressing Tat

#### A V. Karasev

Biotechnology Foundation Laboratories at Thomas Jefferson University, Department of Microbiology and Immunology, Doylestown, PA 18901, alexander.karasev@jefferson.edu

#### S Foulke

Institute of Human Virology, University of Maryland Biotechnology Institute, Baltimore, MD 21202

#### C Wellens

Biotechnology Foundation Laboratories at Thomas Jefferson University, Department of Microbiology and Immunology, Doylestown, PA 18901

#### I Zwierzynski

Biotechnology Foundation Laboratories at Thomas Jefferson University, Department of Microbiology and Immunology, Doylestown, PA 18901

#### R Baldwin

Biotechnology Foundation Laboratories at Thomas Jefferson University, Department of Microbiology and Immunology, Doylestown, PA 18901

#### **Recommended** Citation

Karasev, A V.; Foulke, S; Wellens, C; Zwierzynski, I; Baldwin, R; Koprowski, H; and Reitz, M S. Jr, "Orally Delivered, Plant-Produced Tat Protein Primes Mice For a Challenge DNA Vaccine Expressing Tat" (2005). *Department of Microbiology and Immunology Faculty Papers*. Paper 11. http://jdc.jefferson.edu/mifp/11

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#### Authors

A V. Karasev, S Foulke, C Wellens, I Zwierzynski, R Baldwin, H Koprowski, and M S. Reitz Jr

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## Orally Delivered, Plant-Produced Tat Protein Primes Mice For a Challenge DNA Vaccine Expressing Tat

A V Karasev<sup>\*‡1</sup>, S Foulke<sup>2</sup>, C Wellens<sup>1</sup>, I Zwierzynski<sup>1</sup>, R Baldwin<sup>1</sup>, H Koprowski<sup>1</sup> and MS Reitz Jr<sup>2</sup>

Address: <sup>1</sup>Biotechnology Foundation Laboratories at Thomas Jefferson University, Department of Microbiology and Immunology, Doylestown, PA 18901 and <sup>2</sup>Institute of Human Virology, University of Maryland Biotechnology Institute, Baltimore, MD 21202

Email: A V Karasev\* - alexander.karasev@jefferson.edu

\* Corresponding author #Presenting author

from 2005 International Meeting of The Institute of Human Virology Baltimore, USA, 29 August – 2 September 2005

Published: 8 December 2005 Retrovirology 2005, **2**(Suppl 1):S67 doi:10.1186/1742-4690-2-S1-S67

The Tat protein has been recently explored as a prospective vaccine candidate against HIV-1 with broad, subtype non-specific action. A truncated version of Tat()Tat) with the basic loop, involved in immunosuppression, removed has been previously demonstrated as efficacious as the full-size Tat protein. We produced both full-size Tat and truncated \_Tat in plants, including one edible species spinach, thus simultaneously addressing problems of an inexpensive Tat production and a direct delivery through the mucosal route. We tested this oral delivery route in a mouse model. Tat and )Tat genes were assembled from a set of synthetic overlapping oligonucleotides, and subsequently cloned into a plant virus-based expression vector. Codon optimization allows production of up to 300-500 mg of Tat or )Tat antigen per 1 g of leaf tissue in spinach. Spinach plants inoculated with the Tat-producing constructs were collected and fed to mice 7-14 days post inoculation with or without mucosal adjuvants. Mice were fed with the Tat-producing or control vector-inoculated spinach. After 3 voluntary feedings, 1 week apart, 1 g per mice, no differences were detected in the growth rate or behavior of the animals fed with these two types of spinach. None of the animals developed measurable Tat antibodies. Challenge DNA vaccination with a homologous Tatexpressing construct was performed using a gene gun. Following DNA vaccination, however, mice previously receiving oral Tat with cholera toxin as an adjuvant, developed higher antibody titers to Tat than did the controls, with the titers peaking at four weeks post-vaccination. Thus, our data suggested that oral Tat primed for the development of Tat antibodies when mice were challengevaccinated with plasmid DNA for expression of Tat.