Pseudotyped Influenza A Virus as a Vaccine for the Induction of Heterotypic Immunity

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Cross-immunity tests have shown that this swine influenza virus bears a close antigenic relationship to the virus strain of human origin which has been chiefly used in our work. Ferrets after recovery from disease caused by the swine virus proved to be solidly immune to the human strain of virus. Ferrets convalescent from the human virus disease were not completely immune to the pig strain of virus.
Properties Desired of a Pre-Pandemic Live Attenuated Influenza Vaccine

1) Can infect but not replicate

2) Is Immunologically complete

3) Cannot donate a functional Haemagglutinin gene to Seasonal Influenza Viruses

4) Given i.n. to promote local immune responses

5) Induces broad immunity at least within an HA subtype
Specific Residues of the Influenza A Virus Hemagglutinin Viral RNA Are Important for Efficient Packaging into Budding Virions

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S- HA Influenza

A del to create frame shift

ATG

GCGGCCGCCACCACCATG

NotI site-New Kozac

STOP

NotI

S-eGFP Influenza

Cleavage site inactivated

Arg AGA

CAG Gln

EcoRI

3' UTR 45bp
Production of Pseudotyped Influenza

MDCK Cells

No Viable Virus

MDCK + PR8 HA

PR8 HA Pseudotype Particles

MDCK + Eng195 HA

Eng195 HA Pseudotype Particles
S-FLU Nomenclature:

[HA/NA Genotype].Surface HA

[S-eGFP/N1(PR8)].H1(PR8)

[S-eGFP/N1(Eng195)].H1(Eng195)

[S-eGFP/N1(PR8)].H5*(VN1203)
Immune Responses to S-FLU

A) Spleen

- PR8 S-HA FLU
- PR8 S-eGFP FLU
- VGM

B) Antibody ELISA

- PR8 S-HA FLU 40
- PR8 S-eGFP FLU 640
- VGM <20
- VGM ip <20
- PR8 +ve control 10240

C) MN Assay

- PR8 S-HA FLU <20
- PR8 S-eGFP FLU <20
- VGM <20
- VGM ip <20
- PR8 +ve control 1280
Neutralising Antibody Response to
[S-eGFP/N1(Eng195)].H1(Eng195) v [S-eGFP/N1(PR8)].H1(PR8)
10x Dose (320 HAU) i.p
S-FLU virus is not pathogenic
Cross-Protection by S-FLU

A. 32 HAU PR8 Challenge

B. 32 HAU B/Lee Challenge

C. Challenge PR8 1 month

D. Challenge X31 1 month
Reduced viral replication on challenge
Post Challenge Responses

T Cells

Antibody

A

Spleen

Lungs

B

OD450 nm

Log Dilution

C

OD450 nm

Dilution Factor

PR8 S-HA FLU 1280
320 HAU PR8 ip 5120
PR8 S-eGFP FLU 1280
VGM <20

PR8 S-HA FLU 1280
320 HAU PR8 ip 2560
PR8 S-eGFP FLU 840
VGM <20

SFU / 10^6 Cells

SFU / 10^6 Cells

NP467
HA519
F18

NP467
HA519
F18
Class I restricted T cells from the LUNGS

Daniel Puleston

Control

Challenged

S-Flu Vaccine + Challenged

Young

CD8+

Old

NP 366-74/Db Tetramer
Importance of local administration - 1

[S-eGFP/NA(PR8)].H1(PR8)
Day 0 dose 1, Day 14 dose 2, Challenge d 47.
In 32 HAU
Ip 320 HAU
Intraperitoneal priming results in T cells in the spleen but less in the lungs.

**Intraperitoneal priming**

- **Spleen Cells**
  - Eng195 eGFP Flu
  - PR8 eGFP Flu
  - PR8 virus
  - VGM

- **Lung Cells**
  - Eng195 eGFP Flu
  - PR8 eGFP Flu
  - PR8 virus
  - VGM

**Local Priming**

- **Spleen**
  - PR8 S-HA FLU
  - PR8 S-eGFP FLU
  - VGM

- **Lungs**
  - PR8 S-HA FLU
  - PR8 S-eGFP FLU
  - VGM
Heterotypic Immunity induced by [S-eGFP/NA(Eng195)].H1(Eng195)

A/PR/8/34
0.32 HAU Challenge

32 HAU Challenge

X31 Challenge
Importance of local administration - 2

[S-eGFP/NA(Eng195)].H1(Eng195)
Day 0 dose 1, Day 14 dose 2, Challenge d 47.
In 32 HAU
Ip 320 HAU
Summary:

**Non-Replicating** Pseudotyped influenza (S-FLU) can provide cross-protective immunity to Influenza A viruses.

Strong local T cell response in lung

Moderate strain specific antibody response

Should be given locally to lung for full effect

Effective vaccine dose $10^4$ fold less than recombinant Adenovirus
Challenges:

Aerosol administration

Generality to other species

Safety

Yield

Regulatory issues