Production fo a safe EMCF-FMDV recombinant vaccine against FMD
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Production of a safe EMCV-FMDV recombinant vaccine against FMD

Margot Carocci

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Plan

Introduction

Why a recombinant vaccine EMCV-FMDV?

Results

I. production and characterization of EMCVΔ2A
II. Virulence attenuation on human primary astrocytes
III. Virulence attenuation on mice
IV. Production EMCV-FMDV

Conclusion
Why a recombinant vaccine EMCV-FMDV?

- A live attenuated vaccine: fast and good protection
- Easy to produce less confinement, less expensive
- Distinguish easily vaccinated and infected animals
Why a recombinant vaccine EMCV-FMDV?

**FMDV / EMCV**

- *Picornaviridae*:
  - positive ssRNA, ~8kb
  - Structural and genomic organization similar
  - Encode a single polyprotein,
  - Similar viral cycle

They are the 2 Picornaviridae, of different genus (*Aphtovirus / Cardiovirus*), showing more similarity
Why a recombinant vaccine EMCV-FMDV?

Genomic organization

Clivage sites
- 3C<sub>pro</sub>
- L<sub>pro</sub>
- 2A
Why a recombinant vaccine EMCV-FMDV?

Development of a chimera EMCV-FMDV virus:

Production of an hybrid genome:
Towards a safe vaccine

**EMCV**: Encephalomyocarditis virus

Can infect many different animal species

Depending on the animal species and the viral strain, EMCV can cause
- Myocarditis
- Reproduction disorders
- Diabetes
- Encephalitis

=> Attenuation of EMCV virulence is needed
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I. **EMCVΔ2A production and characterization**

B279 strain 210 passed on BHK-21: non-virulent for pigs

(P.Denis and F. Koenen Arch Virol 2003)

Test virulence on mice C57Bl/6

C9 clone => a deletion in the 2A protein sequence.
I. EMCVΔ2A production and characterization

In vitro transcription

Transfection

EMCVΔ2A production

Incorporation of a deletion in 2A, using a reverse genetic previously developed in our lab (Bakkali et al., 2002).
I. **EMCVΔ2A production and characterization**

One-step growth cycle

![Graph showing virus titer over time for EMCVΔ2A and EMCV](graph.png)

- Virus titer in log TCID50/mL
- hpi (hours post-infection)
- EMCV Δ2A
- EMCV
I. EMCVΔ2A production and characterization

Plaque assay on BHK-21

EMCV

EMCV Δ2A
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II. Virulence att. on human primary astrocytes

EMCV

Able to infect a wide range of animal species.

Has been described to possibly infect and cause of head ache, vomiting, malaise, fever... In Human.
(Oberste et al, Emerg Inf Dis 2009)

Test infectivity of Human primary astrocytes by EMCV, and the attenuation of EMCVΔ2A.
II. Virulence att. on human primary astrocytes

**EMCV** can infect and replicate on human primary astrocytes

**EMCVΔ2A** is able to infect and replicate on human primary astrocytes but less efficiently.
EMCVΔ2A virus is less virulent than the wild type on Human primary Astrocytes.
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II. Virulence attenuation on mice

Intraperitoneally inoculation

4x10^5 pfu/ mice

C57BL/6

Clinical signs, death?
III. Virulence attenuation on mice

EMCV Δ2A does not induce any clinical sign in C57BL/6 mice
III. Virulence attenuation on mice

Number of viral RNA copy:

Detection of EMCV Δ2A in mice heart only at 3dpi 1 out of 5 mice heart & at 9dpi in 3 out of 5. No more at 22dpi.

=> EMCV Δ2A is able to replicate in vivo.

EMCV Δ2A does not reach the mice CNS.
Virulence attenuation of EMCVΔ2A:

- on BHK-21
- on human primary astrocytes
(With first demonstration of human primary astrocytes sensitivity to EMCV.)
- on mice

The EMCVΔ2A virus is an attenuated virus,

Which should be a safe base for a recombinant EMCV-FMDV vaccine.
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IV. Production of **EMCVΔ2A-P1FMDV**

1. **PCRs from cDNA of EMCV.**
2. Shuttle plasmid with partial cDNA of EMCV deleted in 2A protein.
3. Mutagenesis insertion restrictions sites.
4. Substitution of EMCV P1 by FMDV-P1 in the shuttle plasmid.
5. Substitution du fragment genomique EMCV (P1-2A) par corresponding fragment from the shuttle vector (P1FA-2AΔ).
7. Production of chimera virus?
IV. Production of EMCVΔ2A-P1FMDV

[Image of gel electrophoresis with lanes labeled P1FMDV 3Cemcv, P1FMDV, BHK, M, gFMDV, pEMCVΔ2A, pMC1 48hpt, BSR-T7, pMC1 27hpt. Bands are labeled P1-FMDV, VP0-FMDV, VP2-FMDV.]
IV. Production of EMCVΔ2A-P1FMDV
Conclusion

Virulence attenuation of EMCVΔ2A:

on BHK-21
on human primary astrocytes
(With first demonstration of human primary astrocytes sensitivity to EMCV.)
on mice
The EMCVΔ2A virus safe base for a recombinant.

Transfection of pMC1 (recombinant cDNA):
Cytopathic effects and expression of P1-FMDV
cellular particle production?
To be confirmed…
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Promoting Scientific Cooperation between China and Europe to Combat Epizootic Diseases