VIROLOGICA SINICA

Electronic Supplementary Material

A Highly Attenuated Mumps Virus Strain of Genotype F Generated by Passaging in Vero Cells

Yajing Zhang[1] • Lixia Xie[1] • Benjie Chai[2] • Juncheng Ruan[1] • Yulin Gu[1] • Biao Niu[1] • Yachun Zhang[1] • Zhenfang Fu[2]✉ • Qi An[1]✉ • Dayong Tian[1,2]✉

1. R&D Department, Shanghai King-cell biotechnology Co., Ltd., Shanghai 201506, China
2. State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, Wuhan 430070, China

Supporting information to DOI: 10.1007/s12250-020-00292-5

Table.S1 The source of all 29 MuV strains.

No.	Name	sample source	Sample type	genotype
MuV-1#	33MU01	ZHEJIANG	throat swab	F
MuV-2#	33MU02		throat swab	F
MuV-3#	32MU01B	JIANGSU	throat swab	F
MuV-4#	32MU02B		throat swab	F
MuV-5#	32MU03B		throat swab	F
MuV-6#	32MU04B		throat swab	F
MuV-7#	32MU05B		throat swab	F
MuV-8#	32MU06B		throat swab	F
MuV-9#	32MU07B		throat swab	F
MuV-10#	32MU08B		throat swab	F
MuV-11#	32MU09B		throat swab	F
MuV-12#	32MU10B		throat swab	F
MuV-13#	32MU11B		throat swab	F
MuV-14#	42MU03	HUBEI	throat swab	F
MuV-15#	11MU41	BEIJING	throat swab	F
MuV-16#	61MU01B	SHANXI	throat swab	F
MuV-17#	61MU05B		throat swab	F
MuV-18#	61MU08B		throat swab	F
MuV-19#	61MU22B		throat swab	F
MuV-20#	61MU23B		throat swab	F
MuV-21#	61MU25B		throat swab	F
MuV-22#	1006	GUANGDONG	throat swab	F
MuV-23#	1007		throat swab	F
MuV-24#	44MU17		throat swab	F
MuV-25#	44MU12		throat swab	F
MuV-26#	44MU05A		throat swab	F
MuV-27#	44MU08A		throat swab	F
MuV-28#	44MU11A		throat swab	F
MuV-29#	44MU18A		throat swab	F

Supplementary methods

Cells, virus, antibodies and animals

Vero cells, purchased from American type culture collection (ATCC), were maintained in Dulbecco's modified Eagle's medium (DMEM, Gibco) containing 10% FBS (Royacel). Six weeks old female BALB/c and One-day-old Wistar rats were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd.

Virus Titration

Virus titration was performed with the cytopathic effect. Vero cells in a 96-well plate were inoculated with serial 10-fold dilutions of virus and incubated at 34 °C for 168h. Antigen-positive foci were counted under a microscope

(OLYMPVS CKX53) and virus titer was calculated as cytopathic effect foci per milliliter ($CCID_{50}$/mL). All titrations were carried out in octuplicate.

Plaque Purification Technique

Vero cells in 6-well plates cultured at high confluence (> 90%), were incubated with a serial 10-fold dilutions of viruses at 34 ℃ for 1 h. The growth medium was changed to DMEM with 2% FBS, 1% P/S, and 1% low-melting-point agarose. After 5–7 days, DMEM with 2% FBS, 1% P/S, 0.0.02% Neutral red and 1% low-melting-point agarose were added on the 6-well plates again. One day later, Plaques were picked in the well with only one or two plaques.

Microneutralizing antibody test

Groups of 15 female Balb/c mice aged 6 weeks were immunized with a single dose of 4×10^5 $CCID_{50}$/Mouse of QS-F-P3, QS-F-P30 or mock immunized with the same volume of DMEM by i.m. route; The mice were immunized twice，after 21 days the mice were boost. And peripheral blood samples were collected after boost 14 days and sera were isolated for analysis. Neutralization titers were measured using the Micro neutralization test. Briefly, 50 μL of serial 2-fold dilutions of test serum was prepared on 96-well microplates in 50 μL volumes DMEM. Each sample was added to four adjacent wells. A 50 μL volume of QS-F P3 suspension, containing 25–100 $CCID_{50}$, was added to each well. Then the microplates were incubated at 37 ℃ for 1 h in an incubator with 5% CO_2. Following incubation, 100 μL of the cell suspension, containing 2×10^4 Vero cells, was added to each well, and the microplates were incubated at 34 ℃ in an incubator with 5% CO_2 for 168 h.

Rat Neurovirulence test

Wistar rats were inoculated intracerebrally injected (i.c.) with DMEM, QS-F-P3 or QS-F-P30, respectively, at a dose of 100 $CCID_{50}$ in a volume of 10 μL (n=10-16) within 24 hr of birth. The inoculation site was located in the left parietal area of the skull, (~2 mm left of midline and midway between the bregma and lambda). The rats were euthanized with chloral hydrate at 25 dpi. Brains were removed and fixed in 4% paraformaldehyde for 24 hours. Fixed brains were cut in half in the sagittal plane along the anatomical midline. A single 80-μm-thick section was obtained from each hemisphere at a depth of 0.5–1.0 mm from the surface by Vibratome (LEICA VT1200S, Germany). Then, the neurovirulence score (hydrocephalus severity) is expressed as a percentage, the quotient of the cross-sectional area of the entire brain (excluding the cerebellum) and the cross-sectional area of the lateral ventricle by using ImageJ assay.