

Titration of Rabies Virus - CVS 11 Strain, Subsequent Replication in Speckled Mammalian Cell Lines

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ABSTRACT

Rabies virus (RV) and its related viruses are the leading cause of the fatal rabies disease in humans and animals, worldwide. Rabies is a disease that affects the central nervous system. Although rabies encephalitis remains untreatable, the disease is preventable by vaccination. Even though there are effective vaccines available, eradication of rabies has remained problematic due to the high cost of production and administration of the vaccines. The increasing global incidence of rabies in wildlife and the recent discovery of new genotypes of rabies-causing viruses have accelerated the need for the development of effective vaccines against the disease. The rabies virus can be cultivated in a wide variety of host cells. Cultivation of rabies virus is extremely important for producing large quantities of vaccine. The production of rabies vaccine in cell culture has resulted in highly purified vaccines with improved potency and safety. The present research paper discusses the data regarding the susceptibility of various continuous cell lines like DF-1, QT-35, Neuro2a, and BHK to rabies virus and compares the virus infectivity (in terms of titre) with MNA cell line, which is the most commonly used cell line for rabies vaccine production. Neuro-2a is also a mouse neuroblastoma cell line supposed to have similar sensitivity like MNA. DF-1 is an immortalized chicken embryonic fibroblast cell line and is also expected to have a susceptibility to rabies virus like primary avian embryo fibroblast. Studies were also made to check the susceptibility of QT – 35 which is a quail fibroblast cell line to rabies virus infection.

Key Words: Rabies Virus, Cell Lines, Titration, Vaccine

INTRODUCTION

Rabies is a fatal viral disease caused by the Rabies virus and affects all warm blooded vertebrates. It is a neglected zoonotic disease that is globally wide spread and represents a serious health problem in developing countries (Rupprecht *et al.*, 2002). Every year over 7 million people receive post exposure prophylaxis, and an estimated 55,000 people die from rabies. Ninety-nine percent of these deaths occur in developing countries due to rabid dog population (Cleaveland, 2002). Rabies virus belongs to the *Rhabdoviridae* family and is a non-segmented, single-stranded negative-sense RNA virus. The most common mode of rabies virus transmission is through the bites of an already infected animal. Although rabies encephalitis remains untreatable, the disease is preventable by vaccination. However the incidence of rabies in humans and animals remains relatively high especially, in poor areas of developing countries. Even though there are effective vaccines available, eradication of the rabies virus has remained problematic due to the high cost of production and administration of the vaccines. The increasing global incidence of rabies in wild life and the recent discovery of new rabies-causing viruses are accelerating the development of safer,

cheaper and effective vaccines against the disease (Nadin-Davis and Loza-Rubio, 2006). For Rabies virus assay, a number of plaque assays are used, among which the reliable and sensitive method is Fluorescent Focus assay (Smith, 1978). The FA technique employed for determination of the progress of infection in cell culture is one where in the fixed stained cells could be predominantly stained with nucleoprotein (Abelseth and Atanasiu, 1996). The introduction of monoclonal antibodies to the rabies field has provided specific probes for determination of antigenic variation among rabies and rabies related viruses. These antibodies are directed mainly against the N-Protein and are used on fixed infected cell cultures.

MATERIALS AND METHODS

Collection of Cell lines and their revival

Five cell lines were selected based on transfection rate (Ikeda *et al.*, 2002) and used for viral cultivation. The cell lines were BHK-Baby Hamster Kidney (MCB-I), QT-35-Quail cell line (MCB- I), DF-1- Immortalized Chicken Embryo Fibroblast Cell Line, MNA-Mouse Neuro Blastoma Cell Line, NEURO 2A-Neurobased Cell Line

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were obtained from ATCC and maintained at -196°C (LN2) in R&D Cell culture lab, Indian Immunological Ltd., Hyderabad. The cryo-preserved cell lines were revived (Morris, 1995) and then the sterility was checked by inoculating into SCDM (Soybean Casein Digest Medium) and FTM (Fluid Thioglycolate Medium). After sterility the cells were sub-cultured for propagation of the cell lines (Freshney, 1993).

Cell counting

Cell counting was done using the modified Rosenthal hemocytometer and number of cells calculated by using the following formula:

Cells/mL = No. of cells counted from five squares X correction factor (10^3) X dilution factor (10^2)

Virus strain used

CVS 11 Strain of rabies virus was used for titration study and it's GenBank Taxonomy No. is 11294. CVS stands for Challenge Virus Standard, and is the mouse-brain strain of fixed rabies virus (WHO, 1973). It was first isolated in 1882 from cow in France (Heaton et al., 1999) and is highly neurotropic in nature (Thoulouze et al., 1997).

Steps in rabies virus Titration

Cell suspensions were prepared with 4×10^{-5} (count per ml) for BHK and DF-1 cell lines, 6×10^{-5} (count per ml) for MNA and QT-35. Ten fold serial dilutions of CVS-11 (Rabies virus) were carried out (10^{-1} to 10^{-6}).

The prepared cell suspensions (100 µl) of BHK, MNA,

QT-35, DF-1, and NEURO-2A were added to the 96-well plate followed by 50 micro liter of diluted virus. Plate was incubated for 24 hours at 37 °C. After 24 h of incubation, the wells were fixed with 70 % acetone for 10 min at -20 °C. After fixing, residual acetone was flipped off and the plate was air dried for 15 min. and then 25 µl of antiRabies FITC conjugate (Chemi-Con-DFA reagent) was added to each well and incubated for 30 min at 37 °C in a humidified atmosphere. Finally the plates were washed twice with PBS-A (each wash for 10 min). A number of fluorescing fields were observed and 10 arbitrary fields were counted using fluorescent microscope and titres were calculated using Reed and Munch formula. Titers obtained for various cell lines were compared (Ravi et al., 2002).

Studied activity

Determined the susceptibility of various continuous cell lines such as DF-1, QT-35, Neuro2a and BHK to rabies virus infection and compared the virus infectivity in terms of titre with MNA cell line.

RESULTS AND DISCUSSION

All cell lines used in this study were confirmed to be free of microorganisms such as bacteria, fungi and yeast by inoculating into SCDM (Soybean Casein Digest medium) and FTM (Fluid Thioglycolate medium). CVS-11 strain of rabies virus used in this study showed titre value of 3.7 using MNA cell line.

Table-1. Titer values of rabies virus on different cell lines.

S. No.	No. of Trials	Types of Cell Lines				
		QT-35	BHK	MNA	DF-1	NEURO2A
1.	1	3.50	3.71	3.56	3.0	-
2.	2	3.40	3.28	3.38	-	-
3.	3	3.63	3.32	3.56	3.50	-
4.	4	3.56	3.78	3.63	3.63	-
5.	5	4.56	3.63	-	3.57	4.32
6.	6	3.73	3.37	-	3.72	3.76
7.	7	-	3.42	-	-	-
8.	8	-	3.57	-	3.75	-
9.	9	3.25	-	-	-	-
10.	10	3.56	3.58	-	-	-
11.	11	-	3.64	-	-	-
12.	12	-	3.84	-	-	-
Range of titre	--	3.25-4.56	3.28-3.84	3.38-3.63	3.0-3.75	3.76-4.32
Mean of titre	--	3.64	3.55	3.53	3.52	4.04

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The titre range of 3.25 to 4.56, 3.28 to 3.84, 3.38 to 3.63, 3 to 3.75 and 3.72 to 4.32 was obtained for QT-35, BHK, MNA, DF-1 and NUERO-2A cell line respectively. The mean titres of 3.64, 3.55, 3.53, 3.52 and 4.04 were obtained for QT-35, BHK, MNA, DF-1 and NUERO-2A cell line respectively and the results were mentioned in Table 1. The obtained results clearly states that the susceptibility of QT-35, BHK, DF-1 and Neuro-2A cell line is almost similar to MNA cell line. MNA (Mouse neuroblastoma) cell line is now widely used in rabies virus study and production of rabies vaccine. These MNA cells are especially used, since they share a number of characteristics with human neurons, including gross microscopic and fine structural neuron like morphology, and the presence of micro tubular proteins, neuro transmitter and electrically excitable cell membranes with Acetylcholine receptors (Ruddet, 1980). BHK cell line (Baby hamster kidney cell line) could be successfully used for both postmortem and anti mortem diagnosis and results could be obtained quickly than with the mouse inoculation test and the use of this cell line for routine rabies diagnosis is previously reported (McMorris et al, 1974). QT-35-Quail cell line (MCB- I), was established from methylcholanthrene-induced tumors in Japanese quail, and used for vaccine production (Kelly, 2010). Neuro-2a is also a mouse neuroblastoma cell line supposed to have similar sensitivity like MNA. DF-1 is an immortalised chicken embryonic fibroblast cell line and QT – 35 which is a quail fibroblast cell line were also showed susceptibility to rabies virus infection. Since all the cell lines showed equal susceptibility to rabies virus infection, all of them were suggested in accelerating the development of effective rabies vaccine.

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