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Optimal cell density and multiplicity of infection for the propagation of human rotavirus in monkey kidney cells

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Abstract

The human rotavirus titer was optimal at an infection cell density of about 4–8 $\times$ 10^4 cells cm^{-2} in monkey kidney cell cultures. The highest viral titers (3.8 $\times$ 10^7 $TCID_{50}$ ml^{-1} and 3.7 $\times$ 10^7 $TCID_{50}$ ml^{-1}) were obtained at an multiplicity of infection of 0.05 in well plate and T-flask, respectively.

Introduction

Rotaviruses of the family Reoviridae are now recognized as major pathogens causing acute diarrheal diseases in the young of numerous species, including humans (Wyatt *et al.* 1980). It is estimated that, in the U.S.A., over one million children under age five experience a severe rotavirus diarrheal episode, resulting in over 20,000 hospitalizations and 150 deaths annually (Hoshno & Kapikian 1994). Several strategies have been explored for developing a rotavirus vaccine, including using live attenuated rotavirus and human-bovine rotavirus reassortant (Clark *et al.* 1996).

The optimal propagation of human rotavirus is one of the key elements for cost-effective vaccine development. Several groups have reported that parameters such as cell type, temperature, protease, and trypsin concentration, and vessel type can affect rotavirus propagation in monkey kidney cell cultures (Birch *et al.* 1983, Ward *et al.* 1984, Konno *et al.* 1993). We recently reported that medium additives such as DEAE-dextran, DMSO, and cholesterol play an important role in human rotavirus propagation from rhesus monkey kidney cells (Park *et al.* 1998).

However, to the best of our knowledge, the effect of cell density at infection on rotavirus propagation has not been reported. In addition, the one report in the literature on the effect of multiplicity of infection (MOI) on human rotavirus propagation was insufficient to explain the effect clearly (Offit *et al.* 1983). In our study, we clarify the effects of cell density at infection and MOI on human rotavirus propagation in monkey kidney cell cultures.

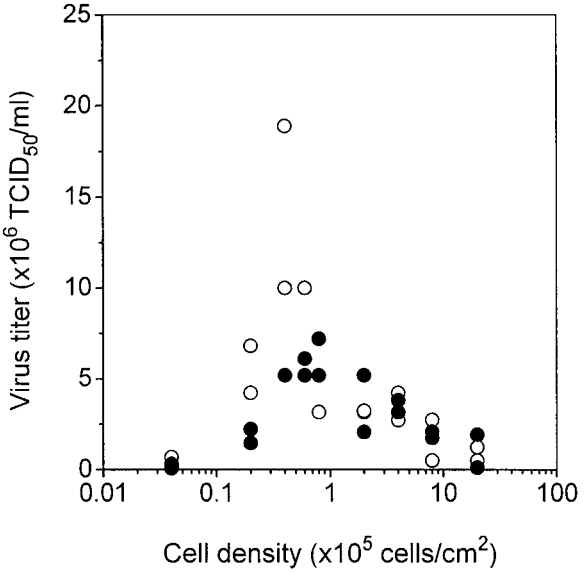

Fig. 1. Effect of cell density at infection on rotavirus titers in well-plates and T-flasks. The rotavirus titer per ml of medium 3 days post-infection was determined at a fixed MOI of 0.5. ○, well plate; ●, T-flask

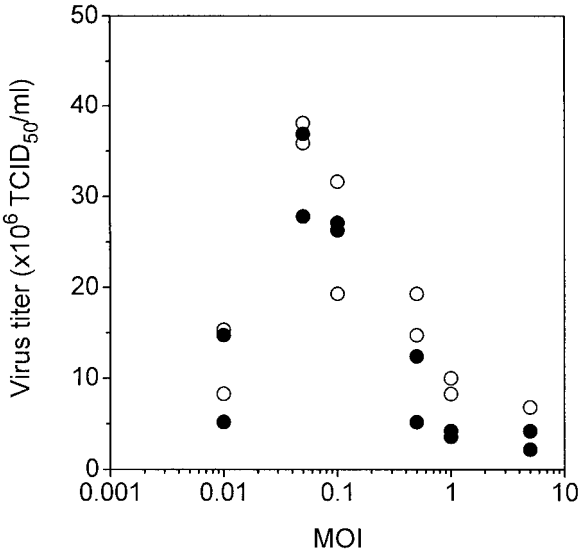

Fig. 2. Effect of multiplicity of infection (MOI) on rotavirus titers in well plates and T-flasks. The rotavirus titer per ml of medium 3 days post-infection was determined at an infection cell density of 6 $\times$ 10^4 cells cm^{-2}, respectively. ○, well plate; ●, T-flask

Materials and methods

Cell line and virus

Rhesus monkey kidney (MA-104) cells were maintained at 37 °C by weekly passage in Dulbecco's modified Eagle's medium (DMEM) containing 10% (v/v) fetal bovine serum (FBS, Gibco BRL), 3.5 g $NaHCO_3$ l^{-1} and 10 ml antibiotic-antimycotic (Gibco BRL) l^{-1}. The antibiotic-antimycotic consisted of 25 mg amphotericin B l^{-1}, 10 U penicillin l^{-1} and 10 g streptomycin sulfate l^{-1}. The human rotavirus Wa strain (ATCC VR2018) was propagated at 37 °C on a monolayer of MA-104 cells at a low multiplicity of infection (MOI) in serum-free medium containing trypsin. The viruses were stored at 4 °C in the form of culture supernatant.

Infection conditions for well plates and T-flasks

MA-104 cells were allowed to attach for 1 h in 6-well plates and T-flasks. Virus was added at MOIs ranging from 0.01 to 5. Then, in 6-well plate and T-flask runs, the medium containing virus was replaced with 1.5 ml and 5 ml, respectively, of medium identical to that described above, except that it contained 5 μg trypsin ml^{-1} and no FBS. The cells in the 6-well plates and T-flasks were incubated at 37 °C in a CO_2 (5%) incubator. Samples were taken 3 days post-infection to determine virus titers.

Analytical methods

The viable cell concentration was determined using a hemacytometer. Viral titers were measured by 50% tissue culture infectious dose ($TCID_{50}$) as described elsewhere (Reed & Muench 1938). Prior to viral titer determination, rotavirus was activated with trypsin (5 μg ml^{-1}) for 30 min before infection.

Results and discussion

Cell density at infection significantly influenced rotavirus propagation. The well-plates and T-flasks were seeded with MA-104 cells at concentrations ranging between 0.4 $\times$ 10^4 and 2 $\times$ 10^6 cells cm^{-2}. The rotavirus titer per ml of medium 3 days post-infection was determined at a fixed MOI of 0.5. At densities lower than 4 $\times$ 10^4 cells cm^{-2} or higher than 8 $\times$ 10^4 cells cm^{-2}, rotavirus propagation was hampered as shown in Figure 1. The optimal rotavirus titer per ml was obtained with cultures seeded at about 4–8 $\times$ 10^4 cells cm^{-2}. The cell density effect is thought to be caused by factors such as cell-to-cell contact inhibition, nutrient limitation, and the presence of metabolic wastes.

Comparing rotavirus titers after infection at MOIs of 0.01, 0.05, 0.1, 0.5, 1 and 5 with an infection cell density of 6 $\times$ 10^4 cells cm^{-2}, the highest rotavirus titers (3.8 $\times$ 10^7 $TCID_{50}$ ml^{-1} and 3.7 $\times$ 10^7 $TCID_{50}$ ml^{-1} in well plates and T-flasks, respectively) were obtained at an MOI of 0.05, three days after infection. Rotavirus titers were not improved at MOIs higher than 0.05. These high viral titers at an MOI of 0.05 seem to result from more active secondary infection. At an MOI of 0.01, secondary infection appears to be insufficient to bring the virus propagation to a comparable level. There is a report that the rotavirus titer at an MOI of 0.1 is better than that at an MOI of 0.01 (Offit *et al.* 1983). However, our results were 19% to 37% higher at an MOI of 0.05 (3.8 $\times$ 10^7 $TCID_{50}$ ml^{-1} and 3.7 $\times$ 10^7 $TCID_{50}$ ml^{-1}) than at an MOI of 0.1 (3.2 $\times$ 10^7 $TCID_{50}$ ml^{-1} and 2.7 $\times$ 10^7 $TCID_{50}$ ml^{-1}) in well plates and T-flasks, respectively. This shows that the above-mentioned literature report on the effect of MOI is incomplete, since it examined only two values of MOI (0.01 and 0.1).

Our findings suggest that rotavirus propagation is strongly dependent upon MOI and cell density at in-

fection. This information is expected to be useful to produce large quantities of rotavirus at a reduced cost for vaccine development.

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