Influenza Virus-cell Fusion Inhibition Assay

Mayo Yasugi[1] and Kazuyoshi Ikuta[2*]

[1]Graduate School of Life and Environmental Sciences, Osaka Prefecture University, Osaka, Japan; [2]Department of Virology, Research Institute for Microbial Diseases, Osaka University, Osaka, Japan

*For correspondence: ikuta@biken.osaka-u.ac.jp

[Abstract] During viral infection to host cells, several viruses undergo the process of endocytosis and pH-dependent fusion. By fusion of viral membrane with host cellular membrane, the viral core invades to host cytoplasm. A part of monoclonal antibodies against viral membrane protein have potential to inhibit the viral fusion step. Here we describe *in vitro* influenza virus-cell fusion inhibition assay. The infected cells expressing viral membrane protein, such as hemagglutinin (HA), on cellular surface are incubated with monoclonal antibodies targeting viral membrane protein. Then the cells are incubated under low pH condition. If the antibody does not inhibit the fusion step, we can see multinucleated giant cells.

Materials and Reagents

1. Monkey kidney cell line CV-1 cells
2. MDCK-propagated Influenza B viruses (B/Florida/4/2006 and B/Malaysia/2506/2004) provided by the National Institute of Infectious Diseases, Japan
3. Minimal Essential Medium (MEM) (Sigma-Aldrich, catalog number: M4655)
4. Fetal Bovine Serum (FBS) (MP Biomedicals, catalog number: 2917054)
5. Phosphate buffered saline without Ca^{2+} and Mg^{2+} (PBS)
6. Dulbecco's Modified Eagle's Medium F12 (DMEMF12) (Life Technologies, catalog number: 11320-033)
7. Acetylated trypsin (Sigma-Aldrich, catalog number: T6763)
8. Giemsa stain solution (Wako Pure Chemical Industries, catalog number: 079-04391)
9. Methanol (Nacalai tesque, catalog number: 21915-93)
10. Bovine serum albumin (BSA) (Sigma-Aldrich, catalog number: A9576)
11. Specific pathogen
12. Monoclonal antibody
13. MEM powder (Nisshin EM, catalog number: 05901)
14. HEPES (Sigma-Aldrich, catalog number: H3375)
15. MES (Wako Pure Chemical Industries, catalog number: 344-08351)

16. Fusion medium (see Recipes)

Equipment

1. 96-well cell culture plate (Corning, catalog number: 3596)
2. CO_2 incubator (37 °C, 5% CO_2)
3. Light microscope

Procedure

1. (Day 1) CV-1 cells in 100 µl MEM including 10% FBS are passaged to 96-well plate at 2.5 x 10^5/ml and incubated in the CO_2 incubator for 24 h.
2. (Day 2) The cells are rinsed with 100 µl PBS once, adsorbed with 50 µl viruses in MEM at an MOI of 0.3 for 1 h in the CO_2 incubator.
3. The cells are rinsed with 100 µl PBS once, added 100 µl DMEMF12 including 0.2% BSA and incubated for 24 h in the CO_2 incubator.
4. (Day 3) The cells are rinsed with 100 µl MEM twice.
5. The cells in 100 µl MEM including 2.5 µg/ml acetyrated trypsin are incubated in the CO_2 incubator for 15 min.
6. They are rinsed with 100 µl MEM twice.
7. 50 µl MEM containing different concentration of monoclonal antibody (concentration is 100, 25, 6.25, 1.5, 0.4 and 0.1 µg/ml) is incubated with the cells in different wells for 30 min in the CO_2 incubator.
8. After aspiration of medium, 100 µl fusion medium with each of pH is added and then incubated for 2 min in the CO_2 incubator.
9. The cells are rinsed with 100 µl MEM twice.
10. The cells are suspended with 100 µl DMEMF12 including 0.2% BSA and incubated in the CO_2 incubator for 3 h.
11. After aspiration of medium, the cells are fixed using 100 µl absolute methanol for 30 sec.
12. After aspiration of methanol, the cells are stained with 100 µl diluted Giemsa staining solution (1 drop per ml dH_2O) for 30 min at room temperature.
13. After rinsing the cells on tap water, the cells are observed by microscope (Figure 1).

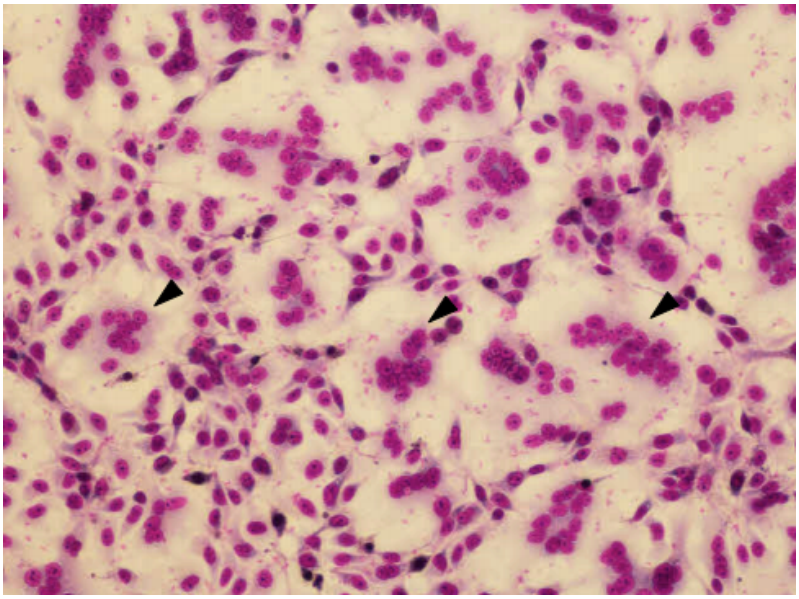

Figure 1. Giemsa staining of CV-1 cells in fusion inhibition assay. Arrow heads show multinuclear giant cells.

Recipes

1. Fusion medium
   Mix 2x MEM with 10 mM MES and 10 mM HEPES
   Adjust to pH 5.0, 5.5, 6.0, 6.5 and 7.0
   Mess up to 1x MEM
   Autoclaved

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