Inhibition of Newcastle Disease Virus Propagation In Vero Cells By a Recombinant Bacteriophage And A Synthetic Peptide

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Abstract
A recombinant filamentous phage bearing the peptide sequence CTLTTKL YC has been shown to interact tightly with Newcastle disease virus (NDV). The peptide, either alone or in the phage associated form, inhibited the hemolytic activity of the virus and also its propagation in embryonated chicken eggs. Here, we demonstrate that phage bearing the peptide sequence CTLTTKL YC and a synthetic peptide derived from the sequence inhibit the infection of Vero cells by NDV.

Keywords: Filamentous phage, peptide, inhibitory activities, cell culture.

Introduction
Newcastle disease causes a major loss to the poultry industry in many parts of the world. The causative agent of the disease is known as Newcastle disease virus (NDV) which is classified in the family of Paramyxoviridae [1] of the genus Avulavirus [2]. NDV has pleomorphic structures [3] and it is surrounded by a lipid bilayer envelope derived from the host cell membrane [4]. It contains a non-segmented negative stranded RNA genome of about 15 kb which encodes six major structural proteins: nucleocapsid (NP), phospho-(P), matrix (M), fusion (F), haemagglutinin-neuraminidase (HN) and large (L) proteins [5]. The lipid bilayer envelope contains the HN and F proteins [6]. The HN protein mediates the binding of virus to cell surface receptors through its haemagglutinating activity and it also displays neuraminidase activity [7]. The F protein is responsible for the fusion between the viral envelope and the target membrane. NDV can also be grown in cell culture which causes cell fusion, resulting from the co-expression of the F and HN proteins. The resulting syncytial formation is characteristic of its cytopathic effect [8]. A peptide with the sequence CTLTTKL YC that interacts tightly with NDV was previously identified by affinity selection from a random phage display peptide library [9]. The interaction between the phage carrying the peptide with NDV shows two different dissociation constants (Kd) in pico- and nanomolar range. Synthetic peptides containing the same sequence either in linear or constrained form inhibited NDV propagation in embryonated chicken eggs. In addition, this peptide inhibited the hemolytic activity of the virus. The present study was carried out in a Vero cell line to determine the inhibitory rate of NDV propagation by phage bearing the peptide CTLTTKL YC.

Materials and Methods
Propagations and Purification of NDV
NDV strain AF2240 was obtained from the Veterinary Research Laboratory (VRI), Ipoh, Malaysia. The propagation and purification of the virus were adapted from Yusoff et al. [10].

Preparation of Bacteriophage
Phage bearing the peptide CTLTTKL YC was obtained from a previous study [9]. The large scale preparation and purification of CTLTTKL YC-carrying phage were carried out according to the method described by Smith and Scott [11]. The phage titration method was adapted from Sambrook et al. [12].

Cells
The Vero cells (obtained from American Type Culture Collection) were maintained in RPMI 1640 medium supplemented with 5% (v/v) FBS (Fetal Bovine Serum), in 5% CO2 at 37°C for 72 h until the monolayer was confluent. Cells were trypsinized [0.25% (w/v) Trypsin], and resuspended with RPMI 1640 medium containing 5% serum. Cell suspension (1 ml) was then added to each well in a 6-well plate and incubated in 5% CO2, at 37°C, until the cells covered about 80% of the wells.

Infectivity Assay
The haemagglutination units (HAU) of NDV were determined as previously described [13]. A series of different HAU, ranges from 0 to 1000, was prepared and added with 2X antibiotic-antimycotic solution (Gibco BRL, USA; 20 µl), and then topped up to a final volume of 1 ml with PBSA buffer (0.4 g/l KH2PO4, 0.4 g/l KCl, 16 g/l NaCl, 4.32 g/l Na2HPO4 7H2O, pH 7.4). Confluent

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monolayers of Vero cells were grown in a 6-well tissue culture plate 2-3 days after passage 2, and used for the infectivity assay. Monolayers were washed with PBSA buffer and inoculated with virus suspension (1 ml) and incubated in an incubator at 37°C containing 5% CO₂. Cells were examined daily for 3 days and the percentage of cell detachment was determined by mixing the cells (100 µl) with equal volume of trypan blue (Sigma, USA). The cells were counted using a haemocytometer under a light microscope to determine the median tissue culture infectious dose (TCID₅₀).

Results and Discussion

Infectivity Assay

The infectivity assay was carried out to determine the median tissue culture infectious dose (TCID₅₀). Figure 1 shows the infectivity of the Vero cells by NDV strain AF2240 observed after 48 h post-infection. The cells remained as confluent monolayer at 0 HAU. Detachment of cells in culture infected by 100 HAU of NDV was about 10% after 24 h of incubation, and increased to about 50% after 48 h incubation. At 1000 HAU, 100% of the cells were detached due to viral infection. The TCID₅₀ value obtained was about 100 HAU based on the 48 h observation. No significant difference was observed in the number of detached cells between the 48 and 72 h incubation. Figure 2 shows the morphology of Vero cells after being infected by NDV at different HAU for 48 h. The uninfected cells have fibroblast-like morphology (Figure 2a) whereas the infected cells formed syncytia (Figures 2b and c). At the late stage, the infected cells died and detached from the wells.

Inhibitory Assay

Figure 3(a) shows the inhibition of cell detachment by phage after 24 h post-infection. The cells remained confluent at 0 HAU, detachment increased slowly to 5% at 40 HAU, and remained constant from 40 HAU to 100 HAU.
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HAU by using the CTLTTKLYC peptide-associated phage as an inhibitor. As for the negative control using M13 KE (phage M13 that does not carry any insert), the cellular detachment increased to about 10% at 80 HAU and 100 HAU. In the absence of phage, detachment increased up to 20% at 100 HAU. After 48 h and 72 h post-infection (Figures 3b and c), the inhibitory activity of the phage bearing the peptide reduced significantly compared to that of 24 h post-infection. This is probably due to the degradation of the peptide inhibitor at longer incubation period in the medium.

Figure 3: Inhibition of NDV propagation in Vero cell line using phage bearing the peptide with the sequence CTLTTKLYC at a series of different HAU (0, 20, 40, 60, 80 and 100 HAU). Results were observed after 24 h post-infection (a), 48 h post-infection (b), and 72 h post-infection (c). Experiments were performed in triplicate and the error bars indicate the standard deviation of the mean. (△) inhibition using phage bearing peptide CTLTTKLYC, (■) inhibition using M13 KE as a negative control, (○) NDV infected cell line.

Figure 4: Inhibition of cell detachment by a synthetic peptide. A cyclic peptide with the sequence CTLTTKLYC (1 mM) was incubated with NDV (40 HAU) and the mixture was used to infect Vero cells. Uninfected cells serve as a negative control.

Figure 4 shows the inhibition assay using only a synthetic peptide with the sequence CTLTTKLYC (1 mM). The result shows that cell detachment in the cell line infected by NDV (40 HAU) approached about 50% after 48 h post-infection. The percentage of cell detachment in the cell line inhibited with peptide CTLTTKLYC was about 28%. The uninfected cells showed the lowest detachment at about 7%. This suggests that the CTLTTKLY peptide alone in the absence of the phage was able to inhibit the propagation of NDV in Vero cells.

Previously, we have shown that embryonated chicken eggs can be used to study the inhibitory activity of peptides that inhibit the propagation of NDV [9]. To the best of our knowledge, there is currently no information available on the use of Vero cell line to study the inhibitory activity of fusion phages or peptides on NDV propagation. In this study, we demonstrate that the Vero cell line can also be used as an alternative system to study the inhibitory activity of free peptides as well as peptide-associated phages.

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References


