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Technical note

In vitro neutralization of equid herpesvirus 1 mediated by recombinant antibodies

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Abstract

A phage antibody display library of single chain fragment variables (scFv) was applied to develop anti-equid herpesvirus-1 (EHV-1) glycoprotein D (gD) neutralizing antibodies. To enrich for specific scFvs, the phage antibody library was panned against epitope derived from the N-terminal part of EHV-1 gD. Unique clones were differentiated by BstNI fingerprinting and further characterized by sequencing and immunoreactivity. The neutralizing effect of each clone was assessed by plaque reduction assay. Three clones with neutralizing effect were isolated.
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1. Introduction

Equid herpesvirus-1 (EHV-1), a member of the alphaherpesvirus subfamily, is the major causative agent of acute respiratory disease, abortions and neurological disease in horses. Cell-associated viremia during an acute infection has been shown to be a prerequisite for abortion and paresis caused by replication of EHV-1 in endothelial cells in the pregnant uterus and central nervous system (CNS). Following viremia, EHV-1 can be isolated from peripheral blood mononuclear cells, primarily T lymphocytes and, to a lesser extent, monocytes for up to 3 weeks post-infection (Bumgardner et al., 1982).

The role of virus neutralizing (VN) antibodies in EHV-1 infection is still controversial. In some experiments, intranasal EHV-1 challenge led to viremia and virus shedding in nasal mucus despite circulating VN antibodies (Burrows et al., 1984), whereas in other studies (Dolby et al., 1995) viremia was not recorded. Virus neutralizing antibodies are long lived, enduring for up to 1 year after infection, largely type specific and directed primarily against viral glycoproteins.

Glycoprotein D (gD) is one of the most important glycoproteins associated with EHV-1 and is considered to be the major target molecule of the host's immune system. Antibodies against this glycoprotein appear soon after infection and exhibit neutralizing properties. One of the epitopes inducing production of neutralizing

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antibodies (Flowers and O'Callaghan, 1992) seems to be a promising target for antiviral therapy and a specific target for detection of EHV-1 infections.

In this report, we describe the use of a synthetic phagemid library (Griffiths et al., 1994) for the generation of scFv antibodies with neutralizing properties. Since our previous effort (Molinkova and Celer, 2006) to generate neutralizing scFv antibodies against gD of EHV-1 was unsuccessful, we adopted a novel strategy consisting of scFv selection against a synthetic peptide representing the neutralizing epitope of EHV-1 gD. Neutralizing scFv clones were characterized by sequencing, measurement of the affinity constant and reactivity, using ELISA and immunofluorescence tests.

2. Materials and methods

2.1. Synthetic peptide

N-biotinylated peptide (biotin-CEKAKRAVRGRQ DRPKEF P) corresponding to the neutralization epitope of glycoprotein D (Flowers and O'Callaghan, 1992) was synthesized (Clonestar, Bristol, UK), dissolved in 8% (1 mg/ml) dimethyl sulfoxide and stored at −80 °C.

2.2. Selection of the phage display library

We used the human synthetic scFv Griffin.1 library (MRC Laboratories, Cambridge, UK) in phagemid format (Griffiths et al., 1994). The panning procedure has been described previously (Blazek et al., 2004). Briefly, 100 nM of biotinylated peptide was added to 1 ml of phage library (10^{13} pfu/ml) and mixed on a rotator for 30 min. The phage–peptide mix was then added to equilibrated Dynabeads M280 (Dynal, Oslo, Norway). After washing in PBS+2% Tween-20+2% milk, phages were eluted with 1 ml of 100 mM triethylamine, neutralized with 0.5 ml of 1 M Tris (pH 7.4) and used to infect *E. coli* TG1cells. Three rounds of selection were performed.

Individual colonies, producing recombinant phages from different rounds of selection, were grown in a microtiter plate, infected with helper phages and tested for gD specific phage production. The binding activity of individual clones of recombinant phages was checked using an ELISA with recombinant gD (100 μg/ml) as antigen.

2.3. Production and purification of soluble antibody fragments

HB2151 non-suppressor *E. coli* cells (which allow scFv soluble expression) were infected with the previously selected gD specific phages and induced for soluble scFv expression by isopropyl β-D-thiogalactopyranoside. ScFv purification was performed by immobilized metal affinity chromatography using Ni–NTA agarose (Qiagen, Hilden, Germany). The selected scFv clones were analyzed by sequencing. Alignment of nucleotide sequences, of rearranged V genes with their closest germline V, D and J segments, was performed using a V base DNA plot (http://vbase.mrc-cpe.cam.ac.uk).

2.4. Kinetic characterization of scFv antibodies

The Spreeta sensor (Texas Instruments, Dallas, TX) was modified with a self-assembled monolayer by incubation with cystamine (20 mg/ml) for 2 h. After activation using glutaraldehyde (3% in phosphate buffer, pH 7.0, 1 h), streptavidin (250 μg/ml in phosphate buffer, 12 h) was covalently immobilized, and the Schiff bonds were stabilized by sodium borohydride (20 mg/ml, 30 min). Finally, the biotinylated peptide antigen was attached to the sensing surface. Measurements with the Spreeta sensor were realized in flow-through mode using an MP3 peristaltic pump (Gilson, Villeurbanne, France) and a flow rate of 35 μl/min. An individual measurement consisted of a 5 min flow of buffer, 10 min sample and 10 min buffer zones. Regeneration of the surface was carried out using glycine-HCl (50 mmol/l, pH 2.0) for 3 min. The relative change of the refractive index Δn_{rel} was collected using the Spreeta software program (ver. 5.31). The kinetic rate constants k_a (association) and k_d (dissociation) were obtained from the Δn_{rel} vs. time (t) curve fitted to the equation:

$$\begin{aligned} \Delta n_{\mathrm{rel}} &= n_0 + \frac{k_{\mathrm{a}} c n_{\max}}{k_{\mathrm{a}} + k_{\mathrm{d}}}\{1 - \exp[-(k_{\mathrm{a}} c + k_{\mathrm{d}})t]\} \\ &= n_0 + n_{\mathrm{eq}}[1 - \exp(-k_{\mathrm{obs}} t)] \end{aligned} \quad (1)$$

using Origin (Microcal, Northampton, MA). The symbol c is the molar concentration of scFv (M_{r}=30 kDa), n_0 is the initial signal and $n_{\max}$ is the binding capacity of the sensing surface. The values of the rate constants were obtained by linear regression from the dependency of k_{obs} on c:

$$k_{\mathrm{obs}} = k_{\mathrm{a}} c + k_{\mathrm{d}} \quad (2)$$

K_{A} represents the ratio k_a/k_d.

2.5. Cell line and virus strain

The Madin–Darby bovine kidney (MDBK) cell line was maintained in Dulbecco's modified Eagle's medium (DMEM) (Sigma, St. Louis, MO) containing 4.5 mg/ml

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