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Original article

Fine mapping and interaction analysis of a linear rabies virus neutralizing epitope

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Abstract

A novel human antibody AR16, targeting the G5 linear epitope of rabies virus glycoprotein (RVG) was shown to have promising antivirus potency. Using AR16, the minimal binding region within G5 was identified as HDFR (residues 261–264), with key residues HDF (residues 261–263) identified by alanine replacement scanning. The key HDF was highly conserved within phylogroup I *Lyssaviruses* but not those in phylogroup II. Using computer-aided docking and interaction models, not only the key residues (Asp30, Asp31, Tyr32, Trp53, Asn54, Glu99, Ile101, and Trp166) of AR16 that participated in the interaction with G5 were identified, the van der Waals forces that mediated the epitope–antibody interaction were also revealed. Seven out of eight presumed key residues (Asp30, Asp31, Tyr32, Trp53, Asn54, Glu99, and Ile101) were located at the variable regions of AR16 heavy chains. A novel mAb cocktail containing AR16 and CR57, has the potential to recognize non-overlapping, non-competing epitopes, and neutralize a broad range of rabies virus.

Keywords: Rabies virus; G5-HDF; Fine mapping; Modeling; Interaction

1. Introduction

Rabies caused by the rabies virus (RV) affects the central nervous system, causing encephalopathy, and is considered to be virtually 100% fatal once symptoms are evident [1]. RV belongs to the family *Rhabdoviridae* and genus *Lyssavirus*. The viral genome is a non-segmented negative-strand RNA that produces five monocistronic mRNAs encoding the nucleoprotein, phosphoprotein, matrix protein, glycoprotein, and viral RNA-dependent RNA polymerase [2]. The glycoprotein is capable of inducing and binding virus-neutralizing antibodies, which confers immunity against a lethal infection challenge with the virus [3,4].

According to World Health Organization (WHO) guidelines, category 3 exposures to rabies, which are defined as either single or multiple transdermal bites or contamination of mucous membranes with saliva of a rabid animal, require rabies postexposure prophylaxis (PEP) [5]. Rabies PEP includes administration of both vaccine and anti-rabies immunoglobulin (RIG) [6]. Although human rabies Ig (HRIG) is exclusively used in developed countries, it has inherent potential health risks. HRIG can display batch-to-batch variation and may be of limited availability in cases of sudden mass exposures [7]. Equine anti-rabies immunoglobulin (ERIG) is produced in developing countries but its quality falls

Abbreviations: RV, rabies virus; RVG, rabies virus glycoprotein; BG, bacterial ghost; ELISA, enyzme-linked immunosorbent assay; PBS, phosphate-buffered saline; HRP, horseradish peroxidase; PBS, phosphate-buffered saline; mAb, monoclonal antibody; FAVN, fluorescent antibody virus-neutralizing test; Ig, immunoglobulin; DMEM, Dulbecco's modified Eagle medium; FBS, fetal bovine serum.

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far short of the requirement, and it can produce adverse effects such as anaphylactic shock [5]. The need to replace these hyperimmune serum preparations is widely recognized [8].

Human monoclonal antibodies (mAbs) have been shown to protect rodents from a lethal RV challenge [9–11], and the mAb that recognizes the G5 epitope represents one of strategies against RV [12]. G5 is a linear epitope within the glycoprotein of rabies virus [3]. The G5 epitope is a highly conserved region of RVG, indicating that synthetic peptides encompassing this region may induce production of a broadly reactive virus-neutralizing antibody in immunized animals and human beings [3].

In our previous study, a novel human single chain variable fragment (ScFv) of mAb (clone AR16) was obtained from a phage display library with a repertoire of approximately 10^8 specificities [13], and this antibody recognized the G5 epitope of RV specifically. To overcome the instability of ScFv, a novel isomer-3 domain disulfide-stabilized antibody fragment (3d-dsFv) which had a 6xHis tag was developed, and it possessed higher affinity and stability. The refolded 3d-dsFv was able to specifically neutralize the RV CVS-11 strain [14].

In the present study, we identified the minimal binding region of linear epitope G5, using the 3d-dsFv product of mAb clone AR16 (referred to as AR16 hereafter) and Pepscan technology. Subsequently, different residues were introduced into synthetic peptides that mimicked the epitope and these were tested for loss of mAb binding. The binding ability between AR16 and the mutant epitope was analyzed *in vitro*, and bioinformatic analysis was used to reveal the nature of the interaction between AR16 and the variants of the G5 epitope. Finally, we adopted a novel monoclonal antibody cocktail which included AR16 and the human mAb CR57 [9,15] to analyze its additive neutralizing effect against rabies *in vitro*.

2. Materials and methods

2.1. Cells, viruses and antibodies

BSR cells were provided by the State Key Laboratory of Pathogens and Biosecurity, Beijing, China. BSR cells were grown with 5% CO_2 at 37 °C in Dulbecco's modified Eagle medium (DMEM, Gibco) supplemented with 10% fetal bovine serum (FBS). The CVS-11 strain of RV was gifted by Xianzhu Xia from Changchun, China. A monolayer of BSR cells was infected with CVS-11 for 1 h with 5% CO_2 at 37 °C. Fresh DMEM supplemented with 2% FBS was added and cells were incubated for another 72 h. Culture supernatants were harvested and stored at −80 °C. Anti-RVG mAb CR57 was provided by North China Pharmaceutical Group Corporation [9,15,16]. The 3d-dsFv product of clone AR16 was named as AR16 in this article [14].

2.2. Preparation and detection of anti-RVG AR16 antibodies

The AR16 mAb was prepared and purified conveniently by its 6xHis tag on the C terminus as described previously [14]. Renaturation was performed *in vitro*, purified by nickel-nitrilotriacetic acid (Ni-NTA) column (HisTrap, Amersham), and stored at −80 °C. Subsequently, the neutralizing titers of AR16 were detected by the rapid fluorescent focus inhibition test (RFFIT) as described previously [14].

2.3. Analysis of key residues and amino acids within G5 linear epitope

Six 8-mer overlapping peptides were synthesized (DQLVNLHD, LVNLHDFR, NLHDFRSD, HDFRSDEI, FRSDEIEH, and SDEIEHLV), and solved in 0.01 mM phosphate-buffered saline (PBS, pH 7.0) and coated (1 mg/ml) in 96-well plate overnight. The plate was then blocked with 3% bovine serum albumin (BSA) for 1 h at 37 °C and washed with 0.1% PBS-Tween20 (PBS-T). AR16 protein was added at a concentration of 0.1 mg/ml into each well, and incubated overnight at 4 °C. After washing with PBS-T, HRP conjugated anti-His antibody (1:5000, Pierce) was added (secondary antibody) and incubated for 1 h at 4 °C. For color development, 3,3′,5,5′-tetramethyl benzidine dihydrochloride (TMB-2HCl, Sigma) was used after washing with PBS-T. The reaction was stopped with 2 M H_2SO_4 and read under 450 nM. The 18-mer peptide DQLVNLHDFRSDEIEHLV within RVG was used as positive control, and a non-specific 8-mer peptide was used as negative control (non-specific peptide for short in figure).

Alanine replacement scanning of the key peptide was performed by ELISA subsequently. Four 8-mer peptides (with alanine replacement in different sites of the key peptide) were synthesized and coated (1 mg/ml) overnight in a 96-well plate at 4 °C. AR16 was used as the primary antibody, and the HRP conjugated anti-His antibody (1:5000, Pierce) was used as the secondary antibody. The 8-mer peptide LVNLHDFR was used as positive control, and the non-specific 8-mer peptide was used as negative control (non-specific peptide for short in figure).

2.4. Key epitope alignment

The minimal binding region of the AR16-specific epitope on RVG was aligned using glycoprotein amino acid sequences of the 220 rabies virus isolates and 7 *Lyssavirus* genotypes (1–7) (data from Genbank).

2.5. Analysis of AR16 binding to variants of the G5 epitope of RVG

ELISA was used to determine binding of AR16 (containing a 6xHis tag) to two 8-mer synthesized mutant peptides, LVNLHDFH and LVNLHDFN. The peptides were coated in the wells of a 96-well plate. AR16 was used as the primary antibody, and HRP conjugated anti-His antibody (1:5000, Pierce) was used as the secondary antibody. The 8-mer peptide LVNLHDFR was used as a positive control, and a non-related 12-mer peptide was used as a negative control.