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Identification of two conserved B-cell epitopes in the gp90 of reticuloendothelial virus using peptide microarray

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ABSTRACT

Since the gp90 protein of Reticuloendotheliosis virus (REV) plays vital roles in virus neutralization, so detailed analysis of REV-gp90 epitopes is important for the development of epitope based marker vaccines and diagnostic tools for REV infections. In this study, two monoclonal antibodies (mAbs) namely 6C12 and 09980 were used to map the epitopes in REVgp90 using peptide microarray and ELISA. Peptide microarray revealed that mAbs 6C12 and 09980 recognized ^{216}YHPLA^{220} and ^{230}DPQTSDILEA^{239} motifs, respectively. This result was confirmed by ELISA using synthetic peptides. Moreover, homology analysis indicated that mAbs defined epitopes are highly conserved among REV strains used in this study. The mAbs and their epitopes identified in this study may have potential applications in development of diagnostic techniques and epitope-based marker vaccines for control of REV infections.

1. Introduction

Reticuloendotheliosis virus (REV) is classified as a member of the genus Gammaretrovirus in the family *Retroviridae* and causes an immunosuppressive, oncogenic and runting-stunting syndrome in multiple avian hosts (Wang et al., 2012). REVs comprise a variety of strains, including nondefective REV-A, defective REV-T, spleen necrosis virus (SNV), chick syncytial virus (CSV), and duck infectious anaemia virus (DIAV) (Jiang et al., 2013; Miao et al., 2015).

REV genome consists of three structural genes (*gag*, *pol* and *env*) flanked by long-terminal repeats (LTRs) (Barbosa et al., 2007). The major mature *env* gene products of REVs are the surface glycoprotein (gp90) and the transmembrane protein (gp20) (El-Sebelgy et al., 2014). The gp90 protein of REV is associated with virus neutralization, which is known to be the major candidate antigen for vaccines and serological diagnosis (Xue et al., 2012). The different REV strains show only minor variations in the genetic sequences (Bohls et al., 2006), and they are antigenically similar, which suggested that a subunit vaccine expressing the gp90 gene of a single REV isolate may provide protective immunity against numerous REV-associated diseases (Li et al., 2012).

Epitope mapping is usually done with monoclonal antibodies (mAbs), though it can be done with polyclonal antisera in a rather less specific way. Only B-cell epitopes are relevant to the biochemical understanding of antibody-antigen interactions (Morris, 2007). Detailed analysis of epitopes is important for the understanding of immunological events, and the development of epitope based marker vaccines and diagnostic tools for various diseases (Khairy et al., 2017; Lin et al., 2010; Peng et al., 2008)

The gp90 protein containing both continuous and discontinuous epitopes functions as the immunodominant protein (Xue et al., 2012) and is responsible for eliciting REV antibodies. In this study, peptide microarray was performed to identify epitopes recognized by two mAbs (6C12 and 09980), which were previously developed against REV-gp90 in our lab. The information provided in this study may facilitate the development of specific serological diagnosis of REV infection, and will contribute to the rational design of vaccines by further understanding of the antigenic structure of gp90.

2. Materials and methods

2.1. Virus, cell and antibody

REV-HA1101 strain of REV was isolated from commercial layer chickens in Jiangsu, China (Miao et al., 2015). DF-1 cells were cultured in Dulbecco's modified Eagle medium (DMEM; GIBCO, USA) supplemented with 5% fetal bovine serum (FBS), 100 U/ml penicillin and

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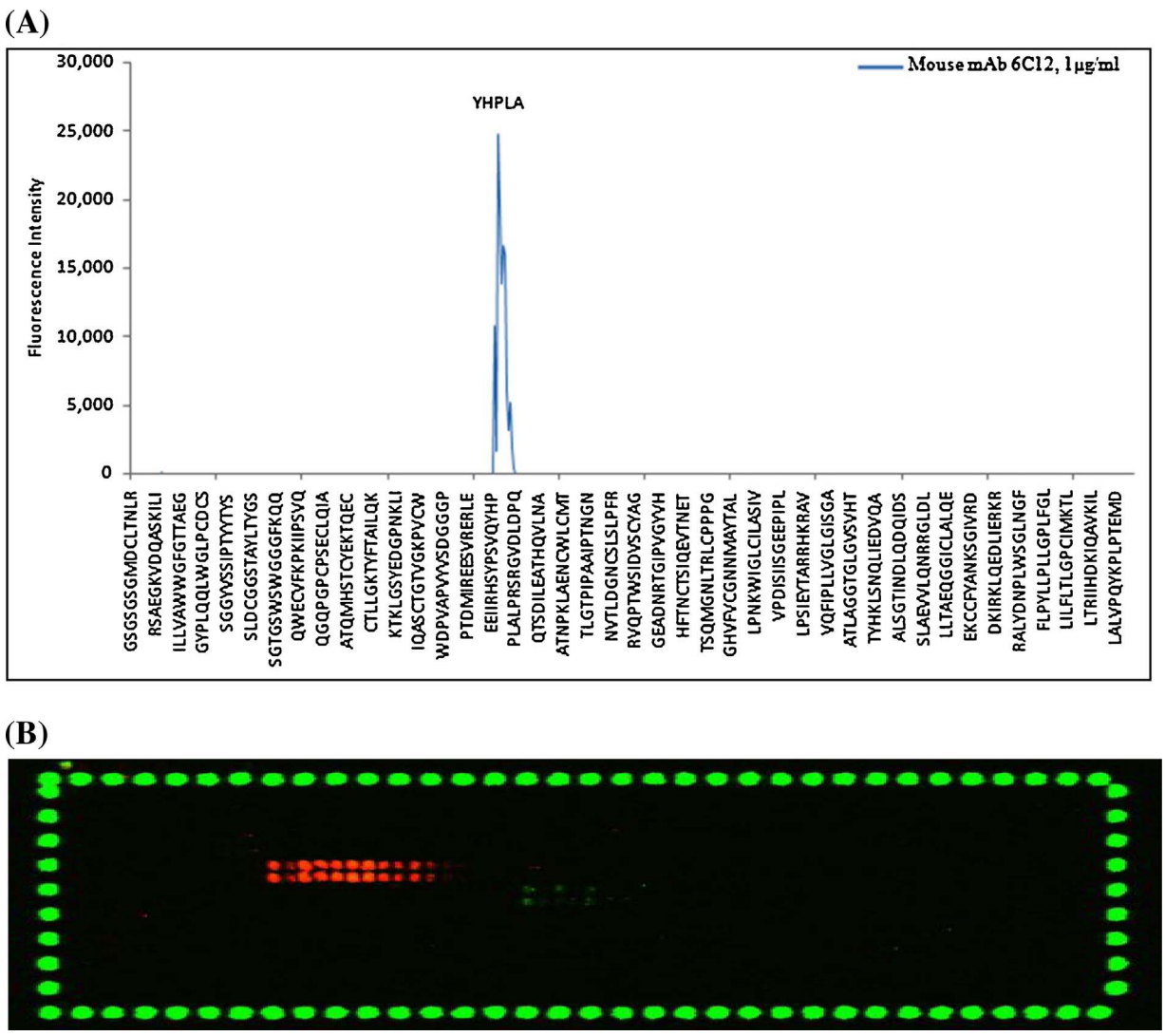

Fig. 1. Mapping epitopes for 6C12 mAb using peptide microarray. Result of the fluorescence signal intensity and its representation of a deduced protein sequence region (A). Well-defined frame of HA control peptides and weak cross reaction of control antibody (B).

100 µg/ml streptomycin at 37 °C and 5% CO2 atmosphere. REV-gp90-specific mAbs 6C12 and 09980 were prepared in our laboratory by immunizing BALB/c mice with the recombinant His-tagged gp90 protein according to normal procedure (Qin et al., 2001). The mAbs were characterized as IgG1 isotype and they reacted with prokaryotic expressing recombinant protein and tissue cultured REV-HA1101 and SNV in western blot and IFA, respectively.

2.2. Peptides

The C-and N-terminus of REV-gp90 were elongated with neutral GSGSGSG linkers to avoid truncated peptides. Then the protein sequence was translated into 15 amino acid peptides with a peptide–-peptide overlap of 14 amino acids. The resulting gp90 peptide microarrays contained 586 different peptides printed in duplicate (1172 peptide spots) to improve precision and to determine intra-assay variation. Each array was framed by additional HA (YPYDVPDYAG, 86 spots) control peptides. The chips were prepared by PepperPRINT (Heidelberg, Germany) and stored at 4 °C until use.

2.3. Immunoassay

The chips were rinsed with washing Buffer (PBS, pH 7.4 with 0.05% Tween 20) 3 × 1 min and nonspecific binding sites blocked with Rockland blocking buffer MB-070 for 30 min at RT.

Pre-staining of a gp90 peptide microarray copy was done with the secondary goat anti-mouse IgG (H + L) DyLight680 antibody (Thermo, Rockford, USA) diluted 1:5000 in incubation buffer (washing buffer with 10% blocking buffer) to investigate background interactions with the antigen-derived peptides that could interfere with the main assays. Subsequent incubation of other gp90 peptide microarray copies with mouse mAb 6C12 and 09980 at a concentration of 1 µg/ml in incubation buffer for 16 h at 4 °C with shaking at 140 rpm was followed by washing and staining with the secondary goat anti-mouse IgG (H + L) DyLight680 antibody (Thermo, Rockford, USA) diluted 1:5000 in incubation buffer for 45 min at RT. The additional HA control peptides framing the peptide microarrays were subsequently stained with mouse monoclonal anti-HA (12CA5) DyLight800 (1:2000) in incubation buffer for 45 min at RT and used as internal quality control to confirm the assay quality and the peptide microarray integrity. Then microarray copies were read-out at a scanning intensity of 5 (red) using LI-COR Odyssey Imaging System (PEPperPRINT, Heidelberg, Germany).

2.4. Data analysis

Quantification of spot intensities and peptide annotation were done