Preparation and preliminary application of a monoclonal antibody against the African swine fever virus D205R protein

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Abstract

Background

African swine fever (ASF) is a lethal infectious disease that causes significant economic losses to the pig industry worldwide. However, few vaccines or drugs were available to prevent or control ASF to date. The D205R protein (pD205R) is a non-structural protein of the African swine fever virus (ASFV) that is abundantly expressed in virus-infected macrophages. However, the antigenic epitopes of pD205R remain unclear.

Results

The main aim of this study was to investigate the new antigenic epitope of pD205R, providing a new tool for the prevention and diagnosis of ASF. Recombinant pD205R was firstly expressed with prokaryotic system and purified with Ni affinity chromatography. Hybridoma cell fusion, clone purification, and immunological assays were then used to develop a monoclonal antibody (mAb) against pD205R. Alanine scanning indicated that S167, W173, L174, G175, P178, and D180 were important for binding to mAb 19A5, and homologous and structural analysis revealed that these residues were highly conserved across all assessed ASFV strains and located on the protein surface.

Conclusion

The results of this study are expected to provide new insights into the development of vaccines and the establishment of serological diagnostic methods for ASF.

Background

African swine fever virus (ASFV) is a highly infectious and devastating swine pathogen that causes clinical symptoms such as fever, respiratory distress, skin cyanosis, and marked bleeding of the lymph nodes, kidneys, and gastrointestinal mucosa [1]. African swine fever (ASF), which is caused by ASFV infection, was first reported in Kenya in 1921 and introduced to China in 2018 [2], and it has since spread to multiple countries, causing huge economic losses to the global pork industry [3]. The World Organization for Animal Health (WOAH) classifies ASF as a legally reported animal disease [4], and the morbidity and mortality rates of ASF are nearly 100% in domestic pigs [5].

ASFV is a large, double-stranded DNA virus and is the only member of the *Asfarviridae* family and the only DNA arbovirus [6]. The virus is 175 ~ 215 nm in diameter, exhibiting 20-hedral symmetry with a capsid membrane [7]. The genomic size is approximately 170 ~ 190 kb, encoding more than 160 proteins [8]. Owing to the large size of viral genome, high complexity of the encoded proteins, and poor understanding of immune escape mechanism, there is few commercial vaccine or effective drugs to

control ASFV transmission [9]. Therefore, prevention of ASF mainly rely on early, rapid, and accurate diagnosis, coupled with animal slaughter and strict sanitation strategies. Considering no vaccination procedure is available practically, serological testing is indeed an efficient way to estimate ASFV infection.

WOAH considers enzyme-linked immunosorbent assay (ELISA) to be an important serological testing for the diagnosis of ASF [10]. Several commercial ELISA kits are currently available for the detection of ASF antibodies, including antibodies against the capsid protein p72 [11], polyprotein pp62 [12], and transmembrane protein p54 [13] among others. These structural proteins are common markers for serological testing of ASF.

However, it is noteworthy that inactivated vaccines have been proved noneffective, even with state-of-the-art adjuvants. Although ASFV-specific antibodies against p72 and p30 were detectable in vaccinated pigs prior to challenge, no protective effect of immunization was observed [14]. Thus, serological testing against any single viral protein may be not accurate enough. Efforts to discover novel markers for serological testing are still of great importance for ASF prevention.

Few studies have focused on the development of serological assays for nonstructural proteins. ASFV encodes more than one hundred nonstructural proteins that are involved in viral replication, host cell metabolic regulation, and immune escape [15]. Knockdown of the nonstructural proteins DP148R, DP71L, and DP96R would render ASFV less virulent and are potential live attenuated vaccine candidates [16]. Thus, discovering nonstructural proteins as markers for serological testing may also meet the demand for differentiation of pigs infected by wild-type virus from ones vaccinated with gene-deletion vaccines.

Non-structural protein pD205R of ASFV is encoded by the D205R gene. pD205R shows sequence similarity with eukaryotic RNA polymerase II subunit 5 (RPB5) and the characteristic structural domain of this protein family. RPB5 interacts with transcription initiation factor IIB (TFIIB) and is a potential target for transcriptional activator interactions [17]. ASFV also encodes proteins analogous to the TFIIB factors; therefore, it has been hypothesized that pD205R is involved in ASFV transcription-related processes [18]. pD205R is abundantly expressed in ASFV-sensing macrophages and may play an important role in viral gene transcription and mRNA processing [19]. Currently, most studies on pD205R use polyclonal antibody, and no monoclonal antibody (mAb) has been prepared. pD205R mAb is helpful to study the structure, function, and molecular mechanism of D205R. In addition, D205R mAb may also be useful for the diagnosis of ASF.

To investigate the antigenic epitope of pD205R to provide a new tool for the prevention and diagnosis of ASF, prokaryotic recombinant pD205R was expressed with *E.coli* system and purified with Ni affinity chromatography. mAb against pD205R was also prepared and the linear B-cell epitope was characterized. These results are expected to be useful for studying the function of pD205R and for developing new diagnostic methods for ASF infection.

Results

Prokaryotic expression and purification of recombinant His-tagged ASFV pD205R

The recombinant protein His-ASFV-pD205R was successfully expressed in DE3 competent *E.coli*, (Fig. 1A, lane 2). Recombinant pD205R had a molecular weight of 44 kDa, which was consistent with expected size. The His-ASFV-pD205R protein was then purified using the Ni-Agarose His Purification Kit, and the purified protein showed a single band and had a final concentration of 0.5 mg/mL (Fig. 1A, lane 3). Western blotting showed that the recombinant protein was specifically recognized by anti-His antibodies (Fig. 1B) and ASFV-positive serum (Fig. 1C). The ability of recombinant pD205R to react with ASFV-positive serum indicates that the expressed protein has a structural similarity to the natural virus and also has the potential to be developed as a serological diagnostic target.

Determination of serum titer of immunized mice

Serum titers of immunized mice were measured using indirect ELISA. The titers of two immunized mice were higher than those of non-immunized mice, and mouse #1 had the highest serum titer (Fig. 2A). Next, pCMV-ASFV-pD205R was transfected into HEK293T cells, and recombinant Flag-ASFV-pD205R was successfully expressed (Fig. 2B). The eukaryotic recombinant protein was also recognized by the antiserum of immunized mouse #1 (Fig. 2C), indicating that the recombinant pD205R protein effectively induced an immune response and that immunized mouse #1 could be used for subsequent mAb preparation.

Identification of ASFV pD205R mAbs

Indirect ELISA showed that mAb 19A5 has a high affinity to recombinant His-ASFV-pD205R (Fig. 3A). To further confirm the immunogenicity of mAb 19A5, we performed indirect immunofluorescent assay (IFA) and western blotting on HEK293T cells transfected with pCMV-ASFV-pD205R using mAb 19A5 as the primary antibody. Results showed mAb 19A5 reacted with pCMV-ASFV-pD205R (Fig. 3B & C). Similarly, proteins expressed in PAM cells under viral infection were recognized by mAb 19A5 (Fig. 3D). Western blotting results showed that mAb 19A5 can specifically reacted with viral protein pD205R. The results showed that mAb 19A5 has good specificity and anti-ASFV pD205R property, and has good reactivity.

Identification of ASFV pD205R antigenic epitopes

To investigate the epitopes targeted by mAb 19A5, we performed alanine scanning to identify key amino acids (Fig. 4A). Western blotting revealed that all 41 five amino acid mutants were successfully expressed. In addition, residues 167 ~ 181 comprised the main epitope region can be recognized by mAb 19A5 (Fig. 4B). We further constructed single amino acid mutants. The results showed that S167, W173, L174, G175, P178, and D180 were the key epitope amino acids recognized by mAb 19A5 (Fig. 4C).

Conservation analysis and spatial distribution of the epitopes of ASFV pD205R

The sequences of pD205R in ASFV strains in domestic and international from GenBank (Table 1) were compared to analyze the residues recognized by mAb 19A5. Sequence analysis revealed that the residues were highly conserved across the different strains (Fig. 5A).

Table 1
ASFV strains collected from NCBI and used toalign the sequences of the identified epitopes

Gene accession	Title	Country	Year	Length(AA)
MK128995.1	China/2018/AnhuiXCGQ	China	2018	206
OP605386.1	20355/RM/2022_Italy	Italy	2022	206
OM481276.1	ABTCVSCK_ASF007	India	2022	206
LR812933.1	Arm/07/CBM/c2	Spain	2020	206
OP467597.1	ASF-MNG19	USA	2022	206
LR722600.1	ASFV CzechRepublic 2017/1	Germany	2017	206
MW049116.1	ASFV Korea/pig/Yeoncheon1/2019	South Korea	2019	206
LR722599.1	ASFV Moldova 2017/1	Germany	2017	206
MZ202520.1	ASFV strain K49	Russia	2021	206
MN393477.1	ASFV Wuhan 2019-2	China	2019	206
MT459800.1	ASFV/Kabardino-Balkaria 19/WB-964	Russia	2019	206
MN172368.1	ASFV/pig/China/CAS19-01/2019	China	2019	206
MW396979.1	ASFV/Timor-Leste/2019/1	Australia	2019	206
MN715134.1	ASFV_HU_2018	Hungary	2018	206
MT180393.1	ASFV_NgheAn_2019	Vietnam	2019	206
MZ614662.1	CADC_HN09	China	2021	206
ON380540.1	HB31A	China	2022	206
OL692744.1	IND/AR/SD-61/2020	India	2020	206
MW856068.1	MAL/19/Karonga	Tanzania	2021	206
ON409983.1	TAN/20/Morogoro	Tanzania	2022	206

The three-dimensional (3D) structure of the ASFV pD205R sequence was predicted using I-TASSER software and visualized in PyMol to verify the epitopes recognized by mAb 19A5. Structural visualization showed that mAb 19A5 recognized a linear epitope located on the α-helix on the surface of pD205R (Fig. 5B). I-TASSER predicted E87, N119, Y121, V172, G175, G176, and R177 as the possible ligand-binding sites for pD205R (Fig. 5C).

Discussion

ASF has had a huge impact on the global economy since its outbreak. However, few effective vaccines or drugs to prevent or control ASF have been developed. Many proteins have been developed as serological diagnostic targets, including P72, P54, and CD2v [20–22]. However, due to the large genome of ASFV and complexity of the encoded proteins, more diagnostic targets need to be explored.

In this study, the His-tagged pD205R protein was expressed in *E. coli* and the recombinant protein reacted strongly with anti-ASFV antibody serum, indicating that the His-tagged pD205R protein has a structure similar to the natural virus and also has the potential to be developed as a novel target for serological diagnostics.

The mAb 19A5 was successfully identified by the hybridoma technique. ELISA, western blotting and IFA results showed mAb 19A5 specifically recognized the pD205R and reacted with the wild-type ASFV pD205R. Western blotting results showed that mAb 19A5 can specifically reacted with viral protein pD205R. The results indicated that mAb 19A5 has good reactivity. Although western blotting results suggest that mAb 19A5 can recognize naturally infected viruses, we need to collaborate with qualified organizations to further investigate the reactivity of 19A5 with natural viruses by methods such as IFA in future studies.

Currently, there are few reports on the epitope of ASFV pD205R, therefore, it is crucial to characterize the epitope of ASFV pD205R. By alanine scanning technique, we found that residues 167 ~ 181 comprised the main epitope region recognized by mAb 19A5. S167, W173, L174, G175, P178, and D180 were the key epitopes recognized by mAb 19A5.

To further investigate the B cell epitope, we compared the ASFV pD205R strains in China and international and showed that the epitope identified by mAb 19A5 was all highly conserved. Epitope conservation may be a good target for ASFV serologic testing. Using a 3D model, we found that mAb 19A5 recognizes a linear epitope located in an α-helix on the surface of pD205R. This feature makes it easier to recognize viral antigens. pD205R ligand-binding sites and regions of enzymes or receptors were predicted, including E87, N119, Y121, V172, G175, G176, and R177. Among these, V172, G175, G176, and R177 are located in the antigenic epitope of mAb 19A5. These results suggest that the conserved epitopes of mAb 19A5 are good targets for the serological detection of ASFV.

In summary, this study generated mAb 19A5 against ASFV pD205R and identified a novel epitope that is highly conserved among different ASFV strains. Future work will focus on the establishment of serological diagnostic methods and the evaluation of the neutralizing and blocking abilities of mAb 19A5. The results of this study lay the foundation for the development of serological diagnosis and vaccine, but also provide mAb tool for further investigation of the function of pD205R.

Conclusions

In this study, we successfully generated immunogenic ASFV pD205R using a prokaryotic expression system. The novel B cell epitope of mAb 19A5 was identified and found to be highly conserved among different ASFV strains. S167, W173, L174, G175, P178, and D180 were the key epitopes recognized by mAb 19A5. This study provides a strong basis for studying the structure and function of pD205R and an effective detection method for the prevention and control of ASF.

Methods

Cells and reagents

HEK293T cells were provided by Wuhan Institute of Virology. Porcine kidney-15 (PK-15) cells were maintained in International Joint Research Center of National Animal Immunology. HEK293T cells and PK-15 cells were cultured in Dulbecco's modified Eagle's medium (Solarbio, China) supplemented with 10% fetal bovine serum (Gibco, USA). Mouse myeloma cells (SP2/0) were obtained from the American Type Culture Collection. SP2/0 cells were cultured in hybridoma cell serum-free medium (Basal Media, China). DH5α and BL21 competent *Escherichia coli* were purchased from Tsingke (China). ASFV-positive serum was purchased from the China Veterinary Drug Inspection Institute. ASFV against p72 was provided by the Key Laboratory of Animal Immunology, Henan Provincial Academy of Agricultural Sciences. Proteins from ASFV infected PAM cells were tested by the Harbin Veterinary Institute.

The pCMV-ASFV-pD205R plasmid was cloned in our laboratory. A Ni-Agarose His Purification Kit was purchased from CWBIO (China). The mouse monoclonal antibody isotyping ELISA kit was purchased from Proteintech (China). Tetramethylbenzidine (TMB) was purchased from Solarbio (China). Antibodies against Flag (66008-4-Ig) and His (66005-1-Ig) and horseradish peroxidase (HRP)-conjugated goat anti-mouse secondary antibody (SA00001-1) were purchased from Proteintech (China). DyLight 488 Goat Anti-Mouse IgG (A23210) was purchased from Abbkine (China). 2-(4-Amidinophenyl)-6-indolecarbamidine dihydrochloride (DAPI, C0065) was purchased from Solarbio (China).

Construction of the pD205R prokaryotic expression vector

ASFV *D205R* (GenBank entry MK795937) was amplified using primers with EcoRI and XhoI restriction sites (Table 2). After amplification and purification, the target gene was inserted into the pET-32a vector, the ligation product was transformed into competent *E.coli* DH5α, and plasmid-containing cells were selected using ampicillin. The recombinant plasmids were extracted and validated using restriction endonuclease digestion, and positive samples were further confirmed by DNA sequencing (Sunya, Shanghai, China). The confirmed plasmid was named pET-32a-ASFV-pD205R and stored at -20°C.

Table 2
Sequences of primers used in pD205R prokaryotic expression plasmid.

Plasmids	Sequences (5'–3')
pET32a-ASFV-D205R	F: TCGGATCCGAATTCATGGCCATGCAAAGTTA
	R: TGGTGGTGCTCGAGAATTTTGGACTTGGTGAT

Preparation of recombinant ASFV-pD205R protein

The recombinant plasmid pET-32a-ASFV-pD205R was transformed into competent *E.coli* BL21, and logarithmic phase cells were induced with IPTG (0.8 mmol/L) and then incubated at 37°C for 6 h. Enriched bacterial cultures were collected and lysed using an autoclave homogenizer. Supernatants and precipitates were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and analyzed by western blotting with anti-His antibodies or positive serum samples. Finally, the recombinant protein pD205R was purified using the Ni-agarose His Purification Kit.

Generation of mAb against ASFV pD205R and isotype determination

BALB/c female mice at 6 ~ 8 weeks of age purchased from Henan Provincial Laboratory Animal Center were subcutaneously injected with 10 μg of purified recombinant ASFV pD205R emulsified with Freund's complete adjuvant. Immunization was then reinforced with incomplete Freund's adjuvant emulsified antigen, injected every two weeks for a total of two immunizations. After the last immunization, mice serum titers were measured by ELISA, the mouse with the highest serum titer was hyperimmunized with 30 μg of pD205R. Three days later, the mouse was executed and splenocytes were isolated and fused with SP2/0 cells using 50% (w/v) PEG1420 to obtain hybridoma cells. The fused cells were cultured in 96-well plates with HAT selection medium for 7 days, and hybridoma cell supernatants were analyzed by indirect ELISA using recombinant ASFV pD205R as the screening antigen. Hybridoma clones secreting anti-pD205R antibodies were subcloned into single-cell clones using three rounds of restriction dilution. Finally, antibody isotypes were identified using a mouse mAb isotype ELISA kit according to the manufacturer's instructions. All the mice studies were performed under ABSL2 facility. At the end of the experiment, all the mice were first anesthetized with isofluorane and then euthanized by cervical dislocation.

Indirect ELISA

Purified pD205R (200 ng/well) was plated in 96-well ELISA plates and incubated at 37°C for 3 h. Wells were washed twice with PBS containing 0.05% Tween-20 (PBST), 1% bovine serum albumin (BSA) was placed in each well, and the plates were incubated at 37°C for 2 h. Positive serum from pD205R immunized mouse and negative serum from non-immunized mouse were used as controls. The supernatants of hybrid tumor cells were added to the plates, which were incubated at 37°C for 1 h and washed four times with PBST. HRP-conjugated goat anti-mouse IgG was added. The plates were

incubated at 37°C for 1 h and washed four times with PBST. H_2SO_4 (2 mol/L) was used to terminate the reaction and TMB chromogenic substrate solution was used for color development. The absorbance of each well was measured at 450 nm using a microplate reader (Gallop, China).

Western blotting

To verify the specifical interaction between mAb and pD205R, pCMV-ASFV-pD205R with a Flag label (Flag-ASFV-pD205R) was transfected into HEK-293T cells. Proteins from ASFV infected PAM cells were tested by the Harbin Veterinary Institute. After 24 h of transfection, the liquid in the well plate was aspirated, lysate was added and the cells were stirred, then the cells were transferred to EP tubes and placed in a metal bath at 95°C for 30 min before being analyzed by western blotting. The proteins were separated by 12.5% SDS-PAGE and transferred to polyvinylidene difluoride membranes. Membranes were blocked with 5% skimmed milk for 1 h at room temperature and incubated with mouse anti-Flag antibody, ASFV-positive serum, or hybrid cell supernatant as primary antibodies for 1 h at room temperature. After four washes with PBST, the membranes were incubated with HRP-conjugated goat anti-mouse IgG for 1 h at room temperature. After the final wash step, the membranes were imaged using a digital imaging system.

IFA

The pCMV-ASFV-pD205R plasmid was transfected into PK-15 cells. After 20 h, the cells were fixed with 4% paraformaldehyde for 20 min, permeabilized with 0.1% Triton X-100 for 10 min, and blocked with 5% BSA for 1 h. Transfected cells were incubated with mouse anti-Flag antibody and hybrid cell supernatants as primary antibodies for 1 h. After three times washing with PBST, the cells were incubated with fluorescein DyLight 488 goat anti-mouse IgG for 1 h and washed 3 times with PBST. After DAPI staining, cells were imaged using a fluorescence microscope (Olympus, Tokyo, Japan).

Identification of the antigenic epitopes

Flag-tagged pD205R mutants were constructed using standard molecular biology techniques. Alanine scanning was performed to identify key amino acids. Western blotting of the mAb 19A5 epitope region was followed by the construction of single amino acid mutants to identify the key amino acids bound to mAb 19A5. The primers used are listed in Table 3.

Table 3
Sequences of primers used to construct mutants

Plasmids	Sequences (5'–3')
pD205R-2-6AA	F: GGCGCGGCAGCGGCATTTACGTATATTTACGAGTTTATTGAAT
	R: TGCCGCTGCCGCGCCCATGAATTCAATCGATAGAACCGAGGTG
pD205R-7-11AA	F: GCTGCGGCTGCTGCCGAGTTTATTGAATATCGTAAGATGGTGC
	R: GGCAGCAGCCGCAGCTAACTTTTGCATGGCCATGAATTCAATC
pD205R-12-16AA	F: GCGGCTGCTGCAGCTCGTAAGATGGTGCTGTTGGAAGAAAAGG
	R: AGCTGCAGCAGCCGCGTAAATATACGTAAATAACTTTTGCATG
pD205R-17-21AA	F: GCTGCGGCGGCGGCGTTGGAAGAAAAGGTACCATATGATAAGT
	R: CGCCGCCGCCGCAGCATATTCAATAAACTCGTAAATATACGT
pD205R-22-26AA	F: GCGGCAGCAGCGGCACCATATGATAAGTTTGTTCAAATGGTAC
	R: TGCCGCTGCTGCCGCCAGCACCATCTTACGATATTCAATAAAC
pD205R-27-31AA	F: GCAGCTGCTGCGGCTGTTCAAATGGTACTTAATACAGGATTTT
	R: AGCCGCAGCAGCTGCTACCTTTTCTTCCAACAGCACCATCTT
pD205R-32-36AA	F: GCTGCAGCGGCAGCTAATACAGGATTTTTTCGTATTAACGCGG
	R: AGCTGCCGCTGCAGCAAACTTATCATATGGTACCTTTTCTTCC
pD205R-37-41AA	F: GCTGCAGCAGCTGCTCGTATTAACGCGGAGACGCTGAATCACG
	R: AGCAGCTGCTGCAGCAAGTACCATTTGAACAAACTTATCATAT
pD205R-42-46AA	F: GCTGCTGCCGCGGCGACGCTGAATCACGGAATCGTATCCGTGT
	R: CGCCGCGGCAGCAGCAAAAAATCCTGTATTAAGTACCATTTG
pD205R-47-51AA	F: GCGGCGGCTGCCGCAATCGTATCCGTGTTTATCTTTGGAGC
	R: TGCGGCAGCCGCCGCCTCCGCGTTAATACGAAAAAATCCTGT
pD205R-52-56AA	F: GCCGCAGCCGCGGCTATCTTTGGAGCAAATGGCAAGTACGTTC
	R: AGCCGCGGCTGCGGCTCCGTGATTCAGCGTCTCCGCGTTAAT
pD205R-57-61AA	F: GCCGCTGCAGCAGCTGGCAAGTACGTTCACCACGGAGGCGAC
	R: AGCTGCTGCAGCGGCAAACACGGATACGATTCCGTGATTCAGC
pD205R-62-66AA	F: GCCGCGGCCGCTGCCCACGGAGGCGACATGAGAACGCTTTT
	R: GGCAGCGGCCGCGGCATTTGCTCCAAAGATAAACACGGATACG
pD205R-67-71AA	F: GCCGCAGCCGCCGCGAGAACGCTTTTAACGAATACGCTTAATG

Plasmids	Sequences (5'–3')
	R: CGCGGCGGCTGCGGCGTGAACGTACTTGCCATTTGCTCCAAAG
pD205R-72-76AA	F: GCAGCGGCTGCAGCGAATACGCTTAATGAAAAAAAACATTATG
	R: CGCTGCAGCCGCTGCCATGTCGCCTCCGTGGTGAACGTACTTG
pD205R-77-81AA	F: GCTGCGGCTGCTGCAAAAAAACATTATGAAGAATTAATTTT
	R: TGCAGCAGCCGCAGCCGTTAAAAGCGTTCTCATGTCGCCTCCG
pD205R-82-86AA	F: GCAGCAGCTGCTGCAGAATTAATTTTAATCGTTGATAAGCCCG
	R: TGCAGCAGCTGCTGCTTCATTAAGCGTATTCGTTAAAAGCGTT
pD205R-87-91AA	F: GCAGCAGCTGCAGCCGTTGATAAGCCCGTTTTAAGCAAAAAAAAT
	R: GGCTGCAGCTGCTGCTTCATAATGTTTTTTTTCATTAAGCGT
pD205R-92-96AA	F: GCTGCTGCGGCCGCTTTAAGCAAAAAAAATATTTTAGATAT
	R: AGCGGCCGCAGCAGCGATTAAAATTAATTCTTCATAATGTTTT
pD205R-97-101AA	F: GCAGCCGCAGCAGCTATTTTAGATATAATCGTCGAGCAGCGCG
	R: AGCTGCTGCGGCTGCAACGGGCTTATCAACGATTAAAATTAAT
pD205R-102-106AA	F: GCTGCAGCTGCAGCCGTCGAGCAGCGCGCTGCAAATCCCACG
	R: GGCTGCAGCTGCAGCATTTTTTTTGCTTAAAACGGGCTTATC
pD205R-107-111AA	F: GCCGCGGCGGCCGCTGCAAATCCCACGATTGTAATAAACATAT
	R: AGCGGCCGCCGCGGCGATTATATCTAAAATATTTTTTTTGCTT
pD205R-112-116AA	F: GCAGCTGCCGCGGCTGTAATAAACATATATCCCTACCACCTGT
	R: AGCCGCGGCAGCTGCAGCGCGCTGCTCGACGATTATATCT
pD205R-117-121AA	F: GCAGCAGCCGCAGCTCCCTACCACCTGTTCTGCATTAACATTC
	R: AGCTGCGGCTGCTGCAATCGTGGGATTTGCAGCGCGCTGCTCG
pD205R-122-126AA	F: GCCGCCGCCGCGGCCTGCATTAACATTCCCAAGGTGAGTGCC
	R: GGCCGCGGCGGCGGCATATATGTTTATTACAATCGTGGGATTT
pD205R-127-131AA	F: GCCGCTGCCGCTGCCAAGGTGAGTGCCATTCCTAAACATAAAC
	R: GGCAGCGGCAGCGGCGAACAGGTGGTAGGGATATATGTTTATT
pD205R-132-136AA	F: GCGGCGGCTGCCGCTCCTAAACATAAACTAATTACTCAGGAGG
	R: AGCGGCAGCCGCCGCGGGAATGTTAATGCAGAACAGGTGGTAG
pD205R-137-141AA	F: GCTGCAGCTGCAGCAATTACTCAGGAGGAGGCGCAGGAGTTTT

Plasmids	Sequences (5'–3')
	R: TGCTGCAGCTGCAGCAATGGCACTCACCTTGGGAATGTTAATG
pD205R-142-146AA	F: GCTGCTGCGGCGGCGGCGCAGGAGTTTTTAGGTCGCGAATAT
	R: CGCCGCCGCAGCAGCTAGTTTATGTTTAGGAATGGCACTCACC
pD205R-147-151AA	F: GCGGCGGCGGCTGCAGGTCGCGAATATCTGCAACCGCAGGACC
	R: TGCAGCCGCCGCCGCCTCCTCCTGAGTAATTAGTTTATGTTT
pD205R-152-156AA	F: GCTGCCGCAGCTGCGCAACCGCAGGACCTCATGCAAATTAGCG
	R: CGCAGCTGCGGCAGCTAAAAACTCCTGCGCCTCCTCCTGAGT
pD205R-157-161AA	F: GCAGCGGCGGCCGCCATGCAAATTAGCGCGTCAGACCCCCCGG
	R: GGCGGCCGCCGCTGCCAGATATTCGCGACCTAAAAACTCCTGC
pD205R-162-166AA	F: GCGGCAGCTGCCGCGTCAGACCCCCCGGTGGTCTGGCTGGGAG
	R: CGCGGCAGCTGCCGCGAGGTCCTGCGGTTGCAGATATTCGCG
pD205R-167-171AA	F: GCAGCCGCCGCGGCGGTCTGGCTGGGAGGAAGACCGGGAGACT
	R: CGCCGCGGCGGCTGCCGCGCTAATTTGCATGAGGTCCTGCGGT
pD205R-172-176AA	F: GCCGCGGCGGCAGCAAGACCGGGAGACTTTGTGCAAATTGAGC
	R: TGCTGCCGCCGCGGCCACCGGGGGGTCTGACGCGCTAATTTGC
pD205R-177-181AA	F: GCAGCGGCAGCCGCTGTGCAAATTGAGCGGCCCTCAGAGACAG
	R: AGCGGCTGCCGCTGCTCCTCCCAGCCAGACCACCGGGGGGTCT
pD205R-182-186AA	F: GCGGCAGCTGCGGCGCCCTCAGAGACAGCTATGCACGCTGTTG
	R: CGCCGCAGCTGCCGCAAAGTCTCCCGGTCTTCCTCCCAGCCAG
pD205R-187-191AA	F: GCCGCAGCGGCAGCTATGCACGCTGTTGTTATCCGCTTTATC
	R: AGCTGCCGCTGCGGCCCGCTCAATTTGCACAAAGTCTCCCGGT
pD205R-192-196AA	F: GCGGCCGCTGCTGCTATCCGCTTTATCACCAAGTCCAAAATTT
	R: AGCAGCAGCGGCCGCAGCTGTCTCTGAGGGCCGCTCAATTTGC
pD205R-197-201AA	F: GCCGCCGCTGCCGCCAAGTCCAAAATTTCCTAGAACTAGTGACT
	R: GGCGGCAGCGGCGGCAACAACAGCGTGCATAGCTGTCTCTGAG
pD205R-202-205AA	F: GCGGCCGCAGCTTCTAGAACTAGTGACTACAAGGACGACGATG
	R: AGCTGCGGCCGCGGTGATAAAGCGGATAACAACAGCGTGCAT
pD205R-167AA	F: ATGCAAATTAGCGCGGCAGACCCCCCGGTGGTCTGGCTGGGAG

Plasmids	Sequences (5'–3')
	R: GACCACCGGGGGGTCTGCCGCGCTAATTTGCATGAGGTCCTGC
pD205R-168AA	F: CAAATTAGCGCGTCAGCCCCCCCGGTGGTCTGGCTGGGAGGAAG
	R: CCAGACCACCGGGGGGGCTGACGCGCTAATTTGCATGAGGTCC
pD205R-169AA	F: ATTAGCGCGTCAGACGCCCCGGTGGTCTGGCTGGGAGGAAGAC
	R: CAGCCAGACCACCGGGGCGTCTGACGCGCTAATTTGCATGAGG
pD205R-170AA	F: AGCGCGTCAGACCCCGCGGTGGTCTGGCTGGGAGGAAGACCGG
	R: TCCCAGCCAGACCACCGCGGGGTCTGACGCGCTAATTTGCATG
pD205R-171AA	F: GCGTCAGACCCCCCGGCGGTCTGGCTGGGAGGAAGACCGGGAG
	R: TCCTCCCAGCCAGACCGCCGGGGGGTCTGACGCGCTAATTTGC
pD205R-172AA	F: TCAGACCCCCCGGTGGCCTGGCTGGGAGGAAGACCGGGAGACT
	R: TCTTCCTCCCAGCCAGGCCACCGGGGGGTCTGACGCGCTAATT
pD205R-173AA	F: GACCCCCCGGTGGTCGCGCTGGGAGGAAGACCGGGAGACTTTG
	R: CGGTCTTCCTCCCAGCGCGACCACCGGGGGGTCTGACGCGCTA
pD205R-174AA	F: CCCCCGGTGGTCTGGGCGGGAGGAAGACCGGGAGACTTTGTGC
	R: TCCCGGTCTTCCTCCCGCCCAGACCACCGGGGGGTCTGACGCG
pD205R-175AA	F: CCGGTGGTCTGGCTGGCAGGAAGACCGGGAGACTTTGTGCAAAT
	R: GTCTCCCGGTCTTCCTGCCAGCCAGACCACCGGGGGGTCTGAC
pD205R-176AA	F: GTGGTCTGGCTGGGAGCAAGACCGGGAGACTTTGTGCAAATTG
	R: AAAGTCTCCCGGTCTTGCTCCCAGCCAGACCACCGGGGGGTCT
pD205R-177AA	F: GTCTGGCTGGGAGGAGCACCGGGAGACTTTGTGCAAATTGAGC
	R: CACAAAGTCTCCCGGTGCTCCTCCCAGCCAGACCACCGGGGGG
pD205R-178AA	F: TGGCTGGGAGGAAGAGCGGGAGACTTTGTGCAAATTGAGCGGC
	R: TTGCACAAAGTCTCCCGCTCTTCCTCCCAGCCAGACCACCGGG
pD205R-179AA	F: CTGGGAGGAAGACCGGCAGACTTTGTGCAAATTGAGCGGCCCT
	R: AATTTGCACAAAGTCTGCCGGTCTTCCTCCCAGCCAGACCACC
pD205R-180AA	F: GGAGGAAGACCGGGAGCCTTTGTGCAAATTGAGCGGCCCTCAG
	R: CTCAATTTGCACAAAGGCTCCCGGTCTTCCTCCCAGCCAGACC
pD205R-181AA	F: GGAAGACCGGGAGACGCTGTGCAAATTGAGCGGCCCTCAGAGAC

Plasmids	Sequences (5'–3')
	R: CCGCTCAATTTGCACAGCGTCTCCCGGTCTTCCTCCCAGCCAG

Bioinformatic analysis of the pD205R protein

To assess the conservation of the identified ASFV pD205R epitopes in different ASFV strains, we compared the pD205R sequences of different ASFV strains using MEGA7. The reference sequences of the different ASFV strains are listed in Table 1. The spatial location of the ASFV pD205R epitope was predicted and clarified using I-TASSER (https://seq2fun.dcmb.med.umich.edu//I-TASSER/) and visualized using PyMol.

Abbreviations

ASF: African swine fever

pD205R: The D205R protein

ASFV: African swine fever virus

WOAH: The World Organization for Animal Health

ELISA: Enzyme-linked immunosorbent assay

RPB5: RNA polymerase II subunit 5

TFIIB: Transcription initiation factor IIB

IFA: Indirect immunofluorescent assay

mAb: Monoclonal antibody

PK-15: Porcine kidney-15 cells

Declarations

Additional Files Legends

Supplementary files are the original images of Fig. 1, Fig. 2, Fig. 3 and Fig. 4.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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Authors' contributions

ZL and HR: Data collation and writing-original manuscript. YZ: Expression and purification of prokaryotic proteins. BZ: Screening of monoclonal antibodies. SS: Screening of antigenic epitopes. SH: Data collation. WH: Data analysis. BW: Conceptualization of the article. YZ and GZ: project management, writing-reviewing and editing.

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Ethics Declarations

Ethics approval and consent to participate

All animal experiment protocols conformed to the rules of the animal ethics procedures and guidelines of the Institutional Animal Care and Use Committee (IACUC). All methods were carried out in accordance with relevant guidelines and regulations. The studies were reported in accordance with ARRIVE guidelines.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing financial interests.

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Figures

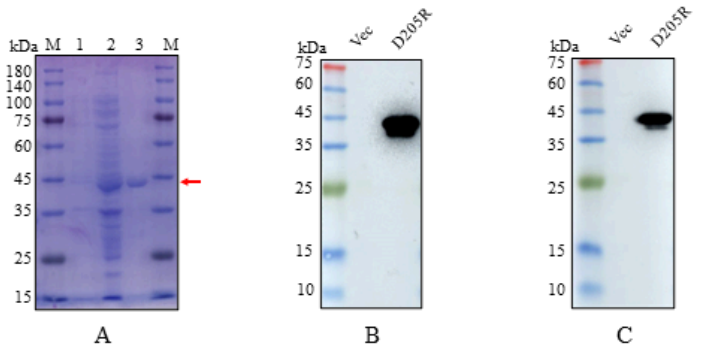

Figure 1

Expression and purification of recombinant His-tagged pD205R. (A) SDS-PAGE analysis of His-tagged ASFV pD205R expression, followed by Coomassie brilliant blue stain. M is protein ladder; lane 1, Negative control; lane 2, whole bacterial cell lysates after induction with IPTG; lane 3, the purified ASFV pD205R. (B) Western blotting of the recombinant pD205R with anti-His mAb. (C) Western blotting of the recombinant pD205R with ASFV positive serum.

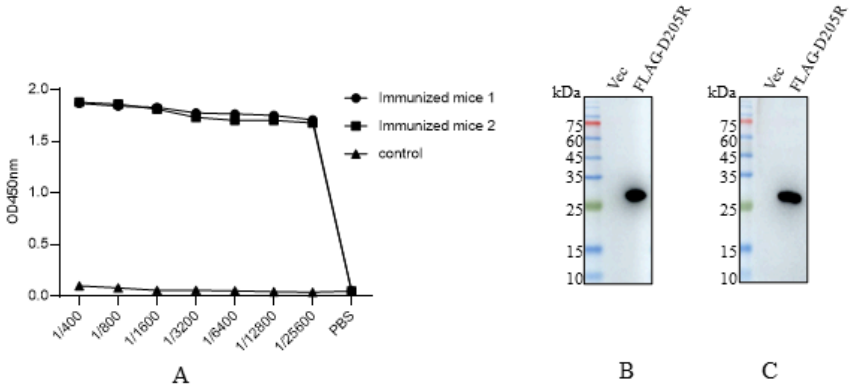

Figure 2

Identification of the immunogenicity of the pD205R. (A) The serum titers of the mice were determined by indirect ELISA. (B) Western blotting detect eukaryotic expression of the recombinant pD205R with anti-Flag mAb. (C) Western blotting detect eukaryotic expression of the recombinant pD205R with mouse # 1 serum.

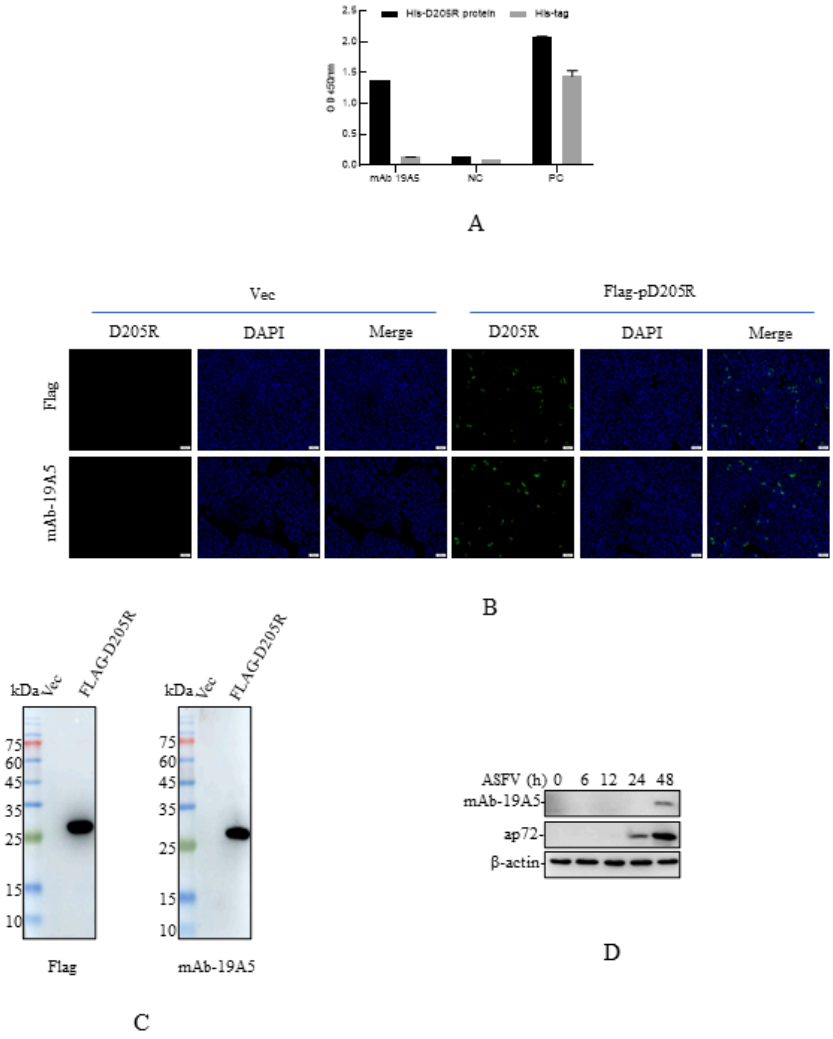

Figure 3

Identification of pD205R mAb. (A) Determination of mAb titer by indirect ELISA. (B) Reactivity of mAb was analyzed by IFA. (C) Reactivity of mAb was analyzed by western blotting. (D) Reactivity of mAb to ASFV by western blotting.

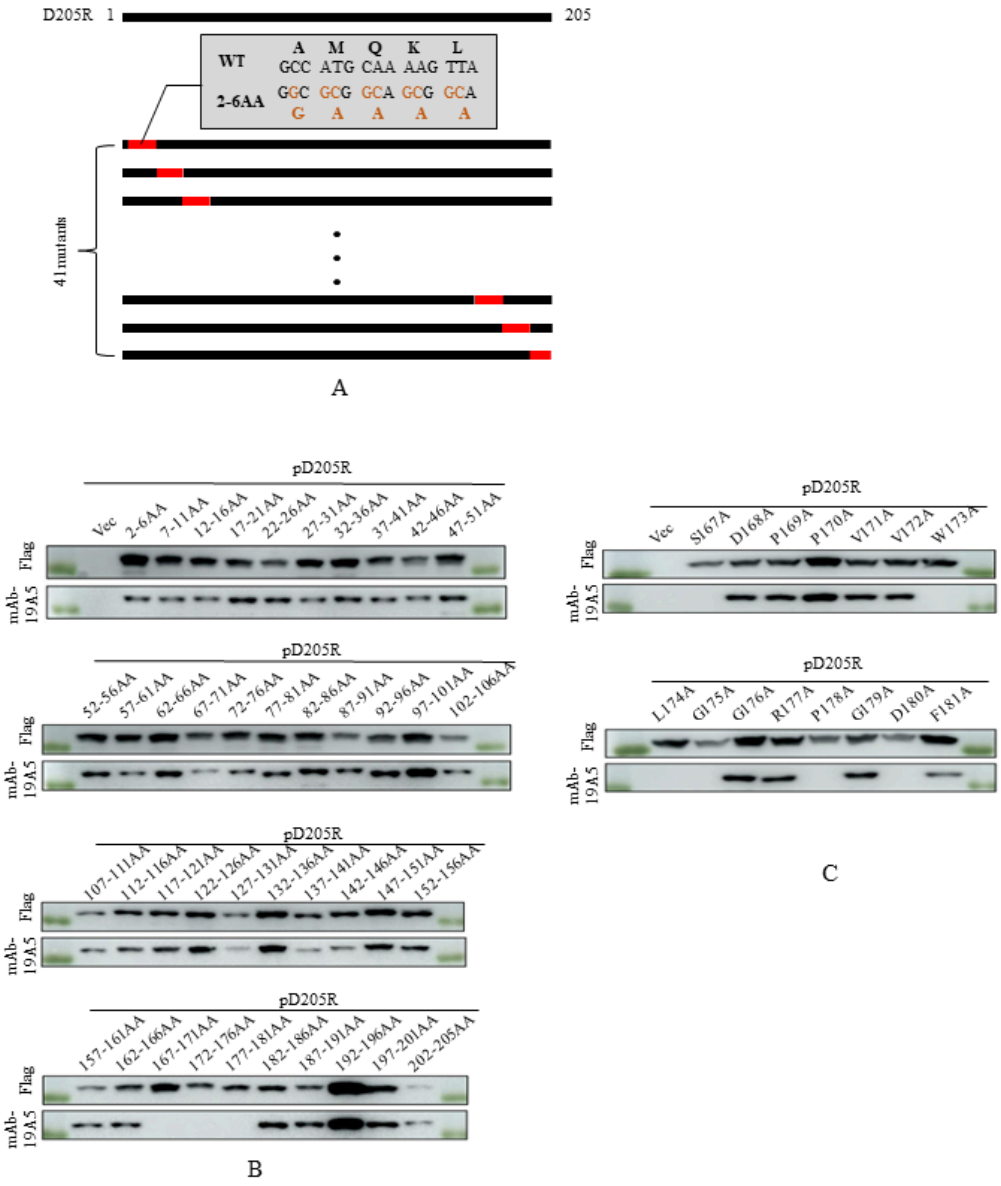

Figure 4

B-cell epitopes analysis of pD205R. (A) Schematic diagram of the pD205R mutants. (B) Preliminary mapping of B-cell epitopes. (C) Key epitope amino acids for mAb by western blotting.

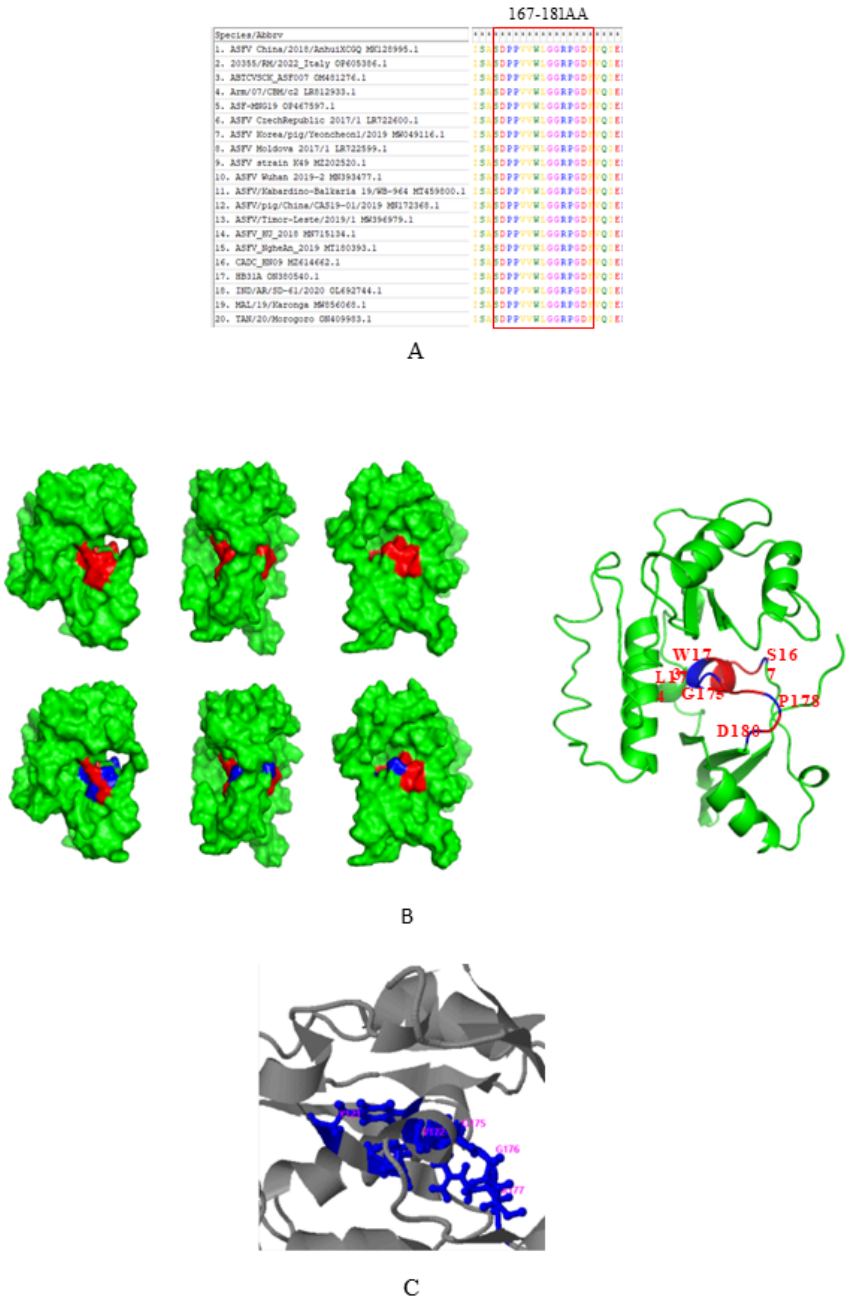

Figure 5

Conservation analysis and spatial distribution of pD205R B-cell epitopes. (A) Conservation analysis of pD205R B-cell epitopes. The ASFV China/2018/AnhuiXCGQ strain and some other ASFV strains collected from GenBank. Only residues of the B-cell epitopes region are shown. The red box indicates the identified epitopes. (B) Spatial distribution of epitopes recognized by mAb 19G5 on pD205R. mAb 19G5-recognized

essential amino acids are blue, non-essential amino acids are red. (C) Prediction of the ligand binding sites of pD205R.

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