Epitope mapping of African swine fever virus (ASFV) structural protein p30

yao zhang[1], Aiping Wang[1], Jingming Zhou[1], Hongliang Liu[1], Yumei Chen[1], Peiyang Ding[1], Xifang Zhu[1], Chao Liang[1], Yanhua Qi[1], and Gaiping Zhang[1]

[1]Zhengzhou University

February 22, 2024

Abstract

Since African swine fever (ASF) was first reported in 1921, it has brought huge economic losses to the world pig industry. No vaccine or therapy is available. Rapid and effective diagnostics are key steps in managing ASF. We generated three monoclonal antibodies (mAbs) against the African swine fever virus (ASFV) phosphoprotein p30 and designated these as 7D2, 8C8 and 2F6. Epitope mapping revealed that mAb 7D2 recognized ^{26}VFHAG SLYNW ^{35} of p30, and mAb 8C8 and 2F6 recognized ^{1}MDFIL NISMK MEVIF KTDLR ^{20} of p30. Furthermore, epitope ^{1}MDFIL NISMK MEVIF KTDLR ^{20} and ^{26}VFHAG SLYNW ^{35} could be well recognized by ASFV-positive sera from natural infected pigs, suggesting that they were natural linear B-cell epitope. Conservation analysis indicated that epitope ^{1}MDFIL NISMK MEVIF KTDLR ^{20} and ^{26}VFHAG SLYNW ^{35} were highly conserved among the different strains of ASFV. This is the first research to characterize specific mAbs against p30 protein. These findings may facilitate further understanding the function of p30 protein and development of diagnostic tools.

Epitope mapping of African swine fever virus (ASFV) structural protein p30

Yao Zhang [1] , Aiping Wang [12] , Jingming Zhou [1 2] , Hongliang Liu [1 2] , Yumei Chen [1 2] , Peiyang Ding [1] , Xifang Zhu [1] , Chao Liang[1] , Yanhua Qi [1] , Gaiping Zhang[1*]

1 School of Life Sciences, Zhengzhou University, Zhengzhou, Henan, China.

2 Henan Zhongze Biological Engineering co LTD, Zhengzhou, Henan, China.

Summary

Since African swine fever (ASF) was first reported in 1921, it has brought huge economic losses to the world pig industry. No vaccine or therapy is available. Rapid and effective diagnostics are key steps in managing ASF. We generated three monoclonal antibodies (mAbs) against the African swine fever virus (ASFV) phosphoprotein p30 and designated these as 7D2, 8C8 and 2F6. Epitope mapping revealed that mAb 7D2 recognized ^{26}VFHAG SLYNW^{35} of p30, and mAb 8C8 and 2F6 recognized^{1}MDFIL NISMK MEVIF KTDLR^{20} of p30. Furthermore, epitope ^{1}MDFIL NISMK MEVIF KTDLR^{20} and ^{26}VFHAG SLYNW^{35} could be well recognized by ASFV-positive sera from natural infected pigs, suggesting that they were natural linear B-cell epitope. Conservation analysis indicated that epitope^{1}MDFIL NISMK MEVIF KTDLR^{20} and^{26}VFHAG SLYNW^{35} were highly conserved among the different strains of ASFV. This is the first research to characterize specific mAbs against p30 protein. These findings may facilitate further understanding the function of p30 protein and development of diagnostic tools.

Key words: African swine fever virus; p30 protein; linear B cell epitope; monoclonal antibody

Introduction

African swine fever virus is one of the most threatening diseases for swine industry of the world. World Organization for Animal Health (OIE) list ASFV as a notifiable disease(Sanchez-Vizcaino, Mur, Gomez-Villamandos, & Carrasco, 2015). Since ASFV was first identified in Kenya in 1921, it has spread to most sub-Saharan countries, Europe, the Caribbean, South America and China(Muñoz-Moreno, Galindo, Cuesta-Geijo, Barrado-Gil, & Alonso, 2015).. African swine fever is a highly contagious and lethal viral disease of swine has had a significant socioeconomic impact in the developed and developing word, making it a global animal health priority(Agüero et al., 2003). African swine fever caused by African swine fever virus has caused huge economic losses to the world pig industry. Since it was first found in Portugal, up to now, there have been three transcontinental spreading. On the third transcontinental spread occurred into China in 2018, from 2018 to 2019, more than 169,000 pigs were slaughtered(Zhou et al., 2018).

African swine fever virus (ASFV) is the only one of the*Asfarviridae* family, it belongs to a 170-194 kb double-stranded DNA virus(Chapman et al., 2011). The genome encodes for more than 150 polypeptides, at least 50 of which result in production of the structural proteins that comprise the different domains of the viral particles(Heimerman et al., 2018). ASFV has more than 100 unique proteins, such as p30, p54, p72, pp220, p17, CD2V, p10, p12(Jia, Ou, Pejsak, Zhang, & Zhang, 2017). ASFV contains at least 68 different structural proteins and 21 cellular proteins(Alí, Tania, Milagros, & Germán, 2018). Among the structural proteins, p30 also named p32 which appears early during ASFV infection(Alonso et al., 2001; Barderas et al., 2001). P30 protein was determined to be a highly immunogenic protein and stimulates the highest level of antibody response during ASFV post infection in pig (Giménez-Lirola et al., 2016).

ASFV p30 protein is an early phosphorylated protein encoded by*CP204L* gene which is one of the most immunogenic proteins(Afonso et al., 1992; Prados, Viñuela, & Alcamí, 1993). P30 protein located in the inner membrane of the viral envelope(Kolontsov, Ustin, Shubina, Piria, & Makarov, 1992). ASFV p30 protein could induce neutralizing antibodies in immunized pigs, and p30 was shown to be involved in various steps of virus attachment and internalization(Giménez-Lirola et al., 2016). P30 protein is highly immunogenic, which can be expressed at 2 to 4 hours after infection, and then continue the whole infection cycle(Gómez-Puertas et al., 1998). Therefore, the expression of ASFV p30 indicates that the virus has entered but not coated, and the virus gene has been expressed. Related studies have shown that p30 protein is the most diagnostic antigen. So, the diagnosis based on monoclonal antibody of p30 is of great significance for early prevention and control of ASF(Petrovan et al., 2019).

In our study, we expressed and purified p30 protein, then produced three mAbs against p30 protein. Two novel linear p30 B cell epitopes,^{1}MDFILNISMKMEVIFKTDLR^{20} and^{26}VFHAGSLYNW^{35} were subsequently identified using these three mAbs. All of them could be recognized by anti-ASFV positive sera. Moreover,^{1}MDFILNISMKMEVIFKTDLR^{20} and^{26}VFHAGSLYNW^{35} are highly conserved among 19 genotypes. These findings provide valuable information for virus diagnosis.

2. Materials and methods

2.1 Strains, reagents, cell lines

The SP2/0 myeloma cells were kept by the Henan Provincial Key Laboratory of

Animal Immunology (Zhengzhou, China), and SP2/0 cells were cultured in Roswell Park Memorial Institute 1640 (RPMI 1640, Gibco) medium supplemented with 10% fetal bovine serum (FBS, Gibco, USA). ASFV-positive sera (Convalescent sera from surviving pigs naturally infected with ASFV) were a kind gift from Professor Hu of Institute of Military Veterinary Medicine, Academy of Military Medical Sciences, Changchun, China. Human embryonic kidney 293T (HEK293T) cells were obtained from ATCC (Manassas, VA, USA) and stored in DMEM medium (Solarbio, Beijing, China) supplemented with 10% (v/v)fetal bovine serum

(FBS, Gibco, USA).*Escherichia coil* BL21 (DE3) competent cells were purchased from Takara Biomedical Technology (Beijing China). Animals care and study procedure followed the guideline of the Animal Research Ethics Board of Zhengzhou University.

2.2. Expression and purification of the ASFV p30 recombinant protein

In response to efficient expression, we constructed three co-expression system with chaperone plasmids. The chaperone plasmids include pKJE7, pGro7 and pTf16. And then chosen the best one. Eventually we found that 0.1 mmol/L of IPTG, 12 h of expression time, and 18 of the temperature, 0.4 mg/mL of L-arabinose are the best condition to obtain the highest yield of soluble p30 protein. The soluble recombinant protein was purified by His Trap FF and His Trap Q HP affinity column.

The purified p30 protein was mixed with 5 x loading buffer, the mixture was boiled for 10 min. Following separation by sodium dodecyl sulfate-poly-acrylamide gel electrophoresis (SDS-PAGE), proteins were transferred onto a polyvinylidene fluoride membrane. The membrane was blocked overnight with 5% skim milk at 4 and incubated with anti-His tag mAb (Solarbio, Beijing, China) 1:2000 for 1 h. After washing three times with PBST, and then probed with a 1:2000 HRP-conjugated goat anti-mouse IgG antibody at 37 for 1 h. The reactivity was visualized using AEC reagents.

2.3. Construction of pcDNA3.1-p30

According to the genome information of strain BA71V (GenBankYP_009704045) Georgia 2007/1, the full-length ASFV p30 protein gene with *Bam* HI and *Xho* I restriction sites was synthesized and subcloned into the pcDNA3.1 vector (Invitrogen, Shanghai, China). The recombinant construct was named pcDNA3.1-p30 and confirmed by PCR and sequencing.

2.4. Monoclonal antibody generation and characterization

Monoclonal antibodies against p30 were produced as described below. Briefly, female, 6 weeks old, BALB/c mice were immunized subcutaneously on week 0 with 50 μg purified p30 mixed with the same amount of complete Freund' adjuvant. Two booster immunizations with p30 emulsified in incomplete Freund' adjuvant were administrated on weeks 2 and 4. The mice were administered 100 μg of p30 protein without adjuvant intraperitoneally. Mice were euthanized 3 days later after the last administration, and harvested spleen cells were fused with SP2/0 cells using polyethylene glycol (PEG 1500). After HAT/HT medium selection, positive hybridomas cells were screened by ELISA. Then, the supernatant was collected to identify p30-specific antibodies with ELISA assay. Positive clones were sub-cultured three times. The selected clones were cultured in the peritoneal cavities of BALB/c mice primed with paraffin to obtain ascites fluid.

2.5. Indirect enzyme-linked immunosorbent assay

Ninety-six-well plates were coated overnight at 4 with the purified p30 protein (4 μg/mL, 100 μL/well) in 0.05 M carbonate-bicarbonate buffer (CBS, pH 9.6). After washing with PBST (1×PBS with 0.05% Tween 20, pH 7.4), the plates were incubated with 5% skim milk at 37 for 2 h. Then, the first antibodies diluted with PBST were incubated at 37 for 30 min. After the incubation, 100 μL/well of HRP-conjugated goat anti-mouse IgG antibody was added at a dilution of 1:5,000, and the sample was incubated at 37 for 30 min, followed by washing three times. Tetramethylbenzidine (TMB) was added and the signal was collected at 450 nm.

2.6. Immunofluorescence assay (IFA)

HEK293T cells were seeded into 96-well cell culture plates at a density of 4×10^4 cells/well and incubated overnight at 37 with 5% CO_2. When the cells were 70-80% confluent, the recombinant plasmid pcDNA3.1-

p30 was transfected into the cells using Lipofectamine(r) 2000 and cultured at 37 for 48 h. After cultured, the plates were fixed with methanol containing 3% paraformaldehyde and permeabilized with 0.3% Triton X-100 for 15 min at room temperature (RT). Next, the plates were blocking with 5% skim milk at 37 for 2 h. After three times washes, anti-p30 mAbs were incubated at 37 for 30 min, the plates were washed three times with PBS. Subsequently, the plates incubated with FITC labeled goat anti-mouse IgG (1:100 in PBS) for 30 min at 37 . Cells were simultaneously stained by 4',6-diamidino-2-phenylindole (DAPI, Solarbio, Beijing, China). After the final wash, the fluorescence signals were visualized by fluorescence microscopy. Mice immune and pre-immune sera were used as positive and negative controls.

2.7. Peptide design and synthesis

Bioinformatics tools were applied to analyze the entire sequence of p30 protein (201 aa). The secondary structure prediction was performed on Ramachandran server

(*http://www.ebi.ac.uk/thornton-srv/databases/pdbsum/Generate.html*). The B cell epitope regions were predicted using the online tool BepiPred (*BepiPred-2.0 (dtu.dk)*, and the epitope threshold was set up at 0.5 as previously reported(Petrovan et al., 2019). The cleavage sites were selected in the rigid regions to conduct overlapping peptides of p30 protein without destroying the protein flexible structure. Based on a comprehensive consideration of bioinformatics prediction, 13 overlapping peptides (with an offset of 5 amino acids) covering the entire p30 protein and the further truncated peptides were synthesized by GL Biochem (Shanghai, China).

2.8. Dot-blot assay and peptide ELISA

Dot-blot hybridization for identification of linear epitopes was based on the method described by previously described method(Chen, Wu, Huang, Cheng, & Chang, 2015). Dot immunoblotting assay was employed to determine the epitopes recognized by mAbs. Polypeptide were prepared dissolving 1mg of solid in 100 μL of DMF (HPLC grade), 2.5 mg of bovine serum albumin (BSA) was mixed with 1.5mg of EDC in 1.25 mL of PBS. And then, the above solution was mixed slowly and after 10 min, 0.5mg of EDC was added, and stired at 4 for 18 h. The reacted mixture was dialyzed for 3 days. Centrifuged at 3500 r/min for 10 min, the supernatant was kept at -20 . ASFV p30 protein peptides were spotted onto nitrocellulose membranes (Pierce, USA), and blocked with 5% skim milk in PBST at 4 overnight. After washing 5 times with PBST, the membranes were incubated with anti-p30 antibody at 37 for 1 h. HRP-conjugated goat anti-mice IgG antibody was used as the secondary antibody, and the results were observed by incubating with AEC reagent.

In peptide ELISA, 96-well plates were coated with 6 μg/mL peptide-BSA conjugates (100 μL/well) in 0.05 M CBS buffer (pH 9.6) at 37 2 h. Then, the plates were blocked with 5% skim milk in PBST. Next, mAbs anti-p30 were used as the primary antibodies. BSA was used as negative control. HRP-conjugated goat anti-mouse IgG was used as the secondary antibody. The reactions were developed using TMB. The OD values of each well were measured at 450 nm using an ELISA microplate reader.

2.9. Reactivity of the linear B cell epitopes with ASFV-positive serum

To assess whether the linear B cell epitopes could be recognized by ASFV-positive serum, the BSA and peptides were then conjugated using the EDC reaction. The reactivity of the synthesized peptides with ASFV-positive swine serum was determined by the above-mentioned dot-blot.

3. Results

3.1. Expression and purification of the recombinant p30 protein

Target proteins were purified from the supernatants of bacterial lysis product and analyzed by means of SDS-PAGE and Western blot assay. As shown in Fig. 1a and Fig. 1b, p30 protein was successfully expressed, with molecular weights of 34 kDa. The Western blot (Fig. 1b), demonstrates that the purified p30 protein could be recognized by anti-His tag mAb.

3.2. Screening and features of mAbs against p30 protein

To generate anti-p30 mAbs, two female BALB/c mice were separately immunized with purified p30 protein at two-week interval. The two mice with the higher antibody titers were used to generate mAbs by the hybridoma technique. ELISA and western blotting results showed that all the mAbs specifically recognized p30 protein (Fig. 2a - b). Supernatants from the resulting hybridoma cells were screening yielded eleven primary hybridoma clones, which were subcloned to obtain 3 monoclonal cell lines, and named as 7D2, 2F6, and 8C8. Results from IFA further confirmed the reactivity binding of these mAbs to p30 protein (Fig. 3).

3.3. Mapping of the linear B-cell epitopes of p30 protein using mAbs

To identify specific B-cell epitope, 13 overlapping polypeptides from ASFV p30 protein were designed with an offset of 5 amino acids (Fig. 4a). We designed p30 fragments from the CP204L based on in silico predicted B-cell linear epitopes obtained using BepiPred. Results from dot-blot and peptide ELISA indicated that 2F6 and 8C8 reacted only with the L1 (1-20 aa), and 7E2 reacted only with L2 (16-35 aa). And then 3 overlapping polypeptides from L2 were designed with an offset of 5 amino acids (Fig. 4b). Three overlapping polypeptides from L1 were designed with an offset of 5 amino acids (Fig. 4c). Results from dot-blot and peptide ELISA indicated that residues 26-35 aa (L2-3) (Fig. 4c).

The identified epitope regions were further truncated as shown in the Fig. 4b. The details of the truncated peptides are listed in S1. Dot-blot and peptide ELISA results suggested that the linear B cell epitopes on ASFV p30 protein were located at residues^{1}MDFILNISMKMEVIFKTDLR^{20}and ^{26} VFHAGSLYNW^{35}.

3.4. Biological information analysis of the identified epitopes

To deeply analyze the conservation of the identified epitopes among different strains, we analyzed p30 sequences from 19 genotypes(Petrovan et al., 2019) (Table S2). In the epitope ^{1}MDFILNISMKMEVIFKTDLR^{20}, an amino acid substitution (M-V) at site 9 was observed only in one (GenBank accession ACJ61516.2) of 19 sequences. In the epitope^{26}VFHAGSLYNW^{35}, the amino acid alignment identified a total of 5 aa differences in the mAb 7D2 epitope region. One single aa difference, His_{28} to Asn_{28} between Georgia/07 and Genotype XIII, an amino acid substitution (N-T) at site 34 was observed in four genotype (GenBank accession ACJ61560.1, ADQ44025.1, ACJ61548.1, ACJ61546.1). The result implied that the^{26}VFHAGSLYNW^{35} might be less conserved than the epitope^{1}MDFILNISMKMEVIFKTDLR^{20}. Furthermore, p30 proteins of 19 representative p30 strains (Table S2). These results further confirmed that the two linear B cell epitopes identified in our work were highly conserved in diverse p30 strains (Fig. 5a-b).

To understand the structural mechanism of the epitopes identified by these three mAbs, the 3D structure was predicted using an online computer software program Phyre2 (*http://www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi?id=index*)(Kelley, Mezulis, Yates, Wass, & Sternberg, 2015). The result is shown in the Fig. 5c. Furthermore, the 3D model of p30 was obtained after molecular dynamics simulation and energy minimization. The visualization software PyMol was used to view the position of predicted linear epitopes and digestion-resistant peptides of p30 in the tertiary structure. To understand the spatial distribution of the identified epitopes, the 3D models of p30 protein

used for further analysis. As shown in Fig. 6c, all the identified linear epitope^{26}VFHAGSLYNW^{35}and ^{1}MDFILNISMKMEVIFKTDLR^{20} were exposed on the surface of the p30 protein (Fig. 5d).

3.5. Reactivity of the linear B cell epitopes with ASFV positive serum

Recognition of the epitope peptides by ASFV-positive serum were tested by dot-blot and ELISA. Results showed that the epitope^{26}VFHAGSLYNW^{35}and "^{1}MDFIL NISMK MEVIF KTDLR^{20}" could be recognized by ASFV-positive serum. These results suggested that epitope^{26}VFHAGSLYNW^{35} and^{1}MDFIL NISMK MEVIF KTDLR^{20} may be immunodominant epitopes (Fig. 6a-b).

4. Discussion

Since ASF was first reported in Kenya in 1921, it caused high mortality in domestic pigs(Davies et al., 2017). African swine fever (ASF) is the most serious epidemic disease in the pig industry, which is caused by the African swine fever virus (ASFV). Monoclonal antibodies (mAb) are key reagents for diagnostic detection of viral infection(Neilan et al., 2004). ASFV p30 protein is an early phosphorylated protein encoded by *CP204L* gene which is one of the most immunogenic proteins(Agüero et al., 2003; Zhou et al., 2018).

Monoclonal antibodies against ASFV p30 protein are powerful tools for mapping ASFV p30 protein epitopes and investigating antigenic(Petrovan et al., 2019). Undoubtedly, the gold standard for epitope definition is to determine the 3D structure of the antigen-antibody complex by X-ray or cryo-EM, but it is not readily applicable to many antigens and antibodies, for its laborious efforts with a low success rate(Gershoni, Roitburd-Berman, Siman-Tov, Tarnovitski Freund, & Weiss, 2007). A second approach to epitope mapping uses nuclear magnetic resonance (NMR) which gives a picture of the antigen-antibody complex in solution. Other means of epitope mapping include computational docking, binding analyses, alanine scanning mutagenesis (ASM), and saturating mutagenesis(Huynen, Filée, Matagne, Galleni, & Dumoulin, 2013). B-cell epitopes are classified as linear or conformational, though it has been reported that 90% of B cell-recognizing epitopes are conformational epitopes, the antigen internalizing process and antigen recognizing ability(Barlow, Edwards, & Thornton, 1986; Chang et al., 2019; Van Regenmortel MHV, 1996). Different methods have been used for the identify-cation of epitopes.

Escherichia coli (E. coli) is a remarkable host due to its rapid growth rate, requirement for inexpensive carbon sources, low cost and well-characterized genetic structure(Malakar & Venkatesh, 2012; Overton, 2014; Scaglia, Cassani, Pilu, & Adani, 2014). However, E. coli has some defects such as inability for posttranslational modifications, ineffective cleavage of the amino terminal methionine, inability to produce proteins containing complex disulfide bonds, and expression of proteins as insoluble inclusion bodies (IBs) (IBs). Thus, in our study , we choose three molecular chaperone to improve the the soluble expression of the target protein. A fundamental function of molecular chaperones is to inhibit unproductive protein interactions by recognizing and protecting hydrophobic surfaces that are exposed during folding or following proteotoxic stress(Balchin, Hayer-Hartl, & Hartl, 2020; Mamipour, Yousefi, & Hasanzadeh, 2017). we constructed three co-expression system with chaperone plasmids. After purified by His Trap FF and His Trap Q HP affinity column, obtain the pure protein. Monoclonal antibodies were produced against purified p30 protein and their epitopes investigated. The three antibodies we tested, all recognized the N-terminal amino acids of p30 protein, indicating that this section contains the main antigenic epitope of p30 protein.

In the present study, Vlad Petrovan, Fangfeng Yuan used the prokaryotically expressed p30 protein as immunogen to get three mAbs, and two of the mAbs recognize the C-terminal region of the protein, the other one could recognized a linear B cell epitope 61-93 amino acid(Petrovan et al., 2019). Wu, Lowe,

Rodriguez et al developed a panel of 21 mAbs against ASFV p30. With 14 out of the 21 mAbs, they defined 4 antigenic regions that contain at least 4 linear epitopes. Four linear epitopes contain 61-90aa, 96-115aa, 111-130aa, 143-160aa(Wu et al., 2020). Zhang, Liu, Wu, et al generated two mAbs against the ASFV p30 and found two novel linear B cell epitopes, 144-154 aa and 12-18 aa(Zhang et al., 2021).

5 Conclusion

In summary, three hybridoma cell lines producing mAbs against ASFV p30 protein were obtained, and all of them we identified for the first time two novel epitopes (^{1}MDFILNISMKMEVIFKTDLR^{20},^{26}VFHAGSLYNW^{35}) on p30 protein. Epitope mapping is an important technique used to determine regions within a protein that can be recognized by the immune system. Therefore, identifying the exact epitopes will create the opportunity to design epitope-based vaccines and genotype specific diagnostic tests.

Acknowledgments

The rearch was supported by National Key Research and Development Program od China (2019YFC1604501).

Conflict of Interest

The authors declare that they have no conflict of interests.

References

Afonso, C., Alcaraz, C., Brun, A., Sussman, M., Onisk, D., Escribano, J., & Rock, D. (1992). Characterization of p30, a highly antigenic membrane and secreted protein of African swine fever virus. *Virology, 189* (1), 368-373.*doi:10.1016/0042-6822(92)90718-5*

Aguero, M., Fernandez, J., Romero, L., Sanchez Mascaraque, C., Arias, M., & Sanchez-Vizcaino, J. (2003). Highly sensitive PCR assay for routine diagnosis of African swine fever virus in clinical samples.*Journal of clinical microbiology, 41* (9), 4431-4434.*doi:10.1128/jcm.41.9.4431-4434.2003*

Ali, A., Tania, M., Milagros, G., & German, A. (2018). A Proteomic Atlas of the African Swine Fever Virus Particle. *Journal of Virology, 92* (23). doi:*10.1128/JVI.01293-18*

Alonso, C., Miskin, J., Hernaez, B., Fernandez-Zapatero, P., Soto, L., Canto, C., . . . Escribano, J. M. (2001). African swine fever virus protein p54 interacts with the microtubular motor complex through direct binding to light-chain dynein. *J Virol, 75* (20), 9819-9827.*doi:10.1128/JVI.75.20.9819-9827.2001*

Balchin, D., Hayer-Hartl, M., & Hartl, F. U. (2020). Recent advances in understanding catalysis of protein folding by molecular chaperones.*FEBS Lett, 594* (17), 2770-2781.*doi:10.1002/1873-3468.13844*

Barderas, M., Rodriguez, F., Gomez-Puertas, P., Aviles, M., Beitia, F., Alonso, C., & Escribano, J. (2001). Antigenic and immunogenic properties of a chimera of two immunodominant African swine fever virus proteins. *Archives of Virology, 146* (9), 1681-1691.*doi:10.1007/s007050170056*

Barlow, D. J., Edwards, M. S., & Thornton, J. M. (1986). Continuous and discontinuous protein antigenic determinants. *Nature, 322* (6081), 747-748.*doi:10.1038/322747a0*

Chang, C., Cheng, I., Chang, Y., Tsai, P., Lai, S., Huang, Y., Chang, H. (2019). Identification of Neutralizing Monoclonal Antibodies Targeting Novel Conformational Epitopes of the Porcine Epidemic Diarrhoea Virus Spike Protein. *Scientific reports, 9* (1), 2529.*doi:10.1038/s41598-019-39844-5*

Chapman, D., Darby, A., Da Silva, M., Upton, C., Radford, A., & Dixon, L. (2011). Genomic analysis of highly virulent Georgia 2007/1 isolate of African swine fever virus. *Emerging infectious diseases, 17* (4), 599-605.*doi:10.3201/eid1704.101283*

Chen, C., Wu, M., Huang, Y., Cheng, C., & Chang, C. (2015). Recognition of Linear B-Cell Epitope of Betanodavirus Coat Protein by RG-M18 Neutralizing mAB Inhibits Giant Grouper Nervous Necrosis Virus (GGNNV) Infection. *PLoS One, 10* (5), e0126121.*doi:10.1371/journal.pone.0126121*

Davies, K., Goatley, L., Guinat, C., Netherton, C., Gubbins, S., Dixon, L., & Reis, A. (2017). Survival of African Swine Fever Virus in Excretions from Pigs Experimentally Infected with the Georgia 2007/1 Isolate. *Transboundary and Emerging Diseases, 64* (2), 425-431.*doi:10.1111/tbed.12381*

Gershoni, J., Roitburd-Berman, A., Siman-Tov, D., Tarnovitski Freund, N., & Weiss, Y. (2007). Epitope mapping: the first step in developing epitope-based vaccines. *BioDrugs : clinical immunotherapeutics, biopharmaceuticals and gene therapy, 21* (3), 145-156.*doi:10.2165/00063030-200721030-00002*

Gimenez-Lirola, L. G., Mur, L., Rivera, B., Mogler, M., Sun, Y., Lizano, S., Zimmerman, J. (2016). Detection of African Swine Fever Virus Antibodies in Serum and Oral Fluid Specimens Using a Recombinant Protein 30 (p30) Dual Matrix Indirect ELISA. *PLoS One, 11* (9).*doi: 10.1007/s00705-020-04915-w*

Gomez-Puertas, P., Rodriguez, F., Oviedo, J. M., Brun, A., Alonso, C., & Escribano, J. M. (1998). The African Swine Fever Virus Proteins p54 and p30 Are Involved in Two Distinct Steps of Virus Attachment and Both Contribute to the Antibody-Mediated Protective Immune Response. *Virology, 243* (2).*doi: 10.1006/viro.1998.9068*

Heimerman, M. E., Murgia, M. V., Wu, P., Lowe, A. D., Jia, W., & Rowland, R. R. (2018). Linear epitopes in African swine fever virus p72 recognized by monoclonal antibodies prepared against baculovirus-expressed antigen. *J Vet Diagn Invest, 30* (3), 406-412.*doi:10.1177/1040638717753966*

Huynen, C., Filee, P., Matagne, A., Galleni, M., & Dumoulin, M. (2013). Class A β -Lactamases as Versatile Scaffolds to Create Hybrid Enzymes: Applications from Basic Research to Medicine. *BioMed Research International, 2013* , 1-16.*doi: 10.1155/2013/827621*

Jia, N., Ou, Y., Pejsak, Z., Zhang, Y., & Zhang, J. (2017). Roles of African Swine Fever Virus Structural Proteins in Viral Infection.*J Vet Res, 61* (2), 135-143.*doi:10.1515/jvetres-2017-0017*

Kelley, L., Mezulis, S., Yates, C., Wass, M., & Sternberg, M. (2015). The Phyre2 web portal for protein modeling, prediction and analysis.*Nature protocols, 10* (6), 845-858.*doi:10.1038/nprot.2015.053*

Kolontsov, A., Ustin, A., Shubina, N., Piria, A., & Makarov, V. (1992). [Polypeptides p14 and p31 of the African swine fever virus–early proteins located on the membrane of the infected cell]. *Voprosy virusologii, 37* (3), 165-168.

Malakar, P., & Venkatesh, K. (2012). Effect of substrate and IPTG concentrations on the burden to growth of Escherichia coli on glycerol due to the expression of Lac proteins. *Applied microbiology and biotechnology, 93* (6), 2543-2549.*doi:10.1007/s00253-011-3642-3*

Mamipour, M., Yousefi, M., & Hasanzadeh, M. (2017). An overview on molecular chaperones enhancing solubility of expressed recombinant proteins with correct folding. *Int J Biol Macromol, 102* , 367-375.*doi:10.1016/j.ijbiomac.2017.04.025*

Muñoz-Moreno, R., Galindo, I., Cuesta-Geijo, M., Barrado-Gil, L., & Alonso, C. (2015). Host cell targets for African swine fever virus. *Virus Research, 209* , 118-127.*doi:10.1016/j.virusres.2015.05.026*

Neilan, J. G., Zsak, L., Lu, Z., Burrage, T. G., Kutish, G. F., & Rock, D. L. (2004). Neutralizing antibodies to African swine fever virus proteins p30, p54, and p72 are not sufficient for antibody-mediated protection. *Virology, 319* (2), 337-342.*doi:10.1016/j.virol.2003.11.011*

Overton, T. (2014). Recombinant protein production in bacterial hosts.*Drug discovery today, 19* (5), 590-601.*doi:10.1016/j.drudis.2013.11.008*

Petrovan, V., Yuan, F., Li, Y., Shang, P., Murgia, M. V., Misra, S., . . . Fang, Y. (2019). Development and characterization of monoclonal antibodies against p30 protein of African swine fever virus. *Virus Research, 269 .doi:10.1016/j.virusres.2019.05.010*

Prados, F., Viñuela, E., & Alcamí, A. (1993). Sequence and characterization of the major early phosphoprotein p32 of African swine fever virus. *Journal of Virology, 67* (5), 2475-2485.*doi:10.1128/jvi.67.5.2475-2485.1993*

Sanchez-Vizcaino, J. M., Mur, L., Gomez-Villamandos, J. C., & Carrasco, L. (2015). An update on the epidemiology and pathology of African swine fever. *J Comp Pathol, 152* (1), 9-21.*doi:10.1016/j.jcpa.2014.09.003*

Scaglia, B., Cassani, E., Pilu, R., & Adani, F. (2014). Expression ofArabidopsis thalianaS-ACP-DES3 inEscherichia colifor high-performance biodiesel production. *RSC Adv., 4* (108), 63387-63392.*doi:10.1039/c4ra13001d*

Van Regenmortel MHV. (1996). Mapping Epitope Structure and Activity: From One-Dimensional Prediction to Four-Dimensional Description of Antigenic Specificity. *Methods (San Diego, Calif.), 9* (3), 465-472.*doi:10.1006/meth.1996.0054*

Wu, P., Lowe, A., Rodríguez, Y., Murgia, M., Dodd, K., Rowland, R., & Jia, W. (2020). Antigenic regions of African swine fever virus phosphoprotein P30. *Transboundary and Emerging Diseases .doi:10.1111/tbed.13533*

Zhang, X., Liu, X., Wu, X., Ren, W., Zou, Y., Xia, X., & Sun, H. (2021). A colloidal gold test strip assay for the detection of African swine fever virus based on two monoclonal antibodies against P30.*Archives of Virology, 166* (3), 871-879.*doi:10.1007/s00705-020-04915-w*

Zhou, X., Li, N., Luo, Y., Liu, Y., Miao, F., Chen, T., .Hu, R. (2018). Emergence of African Swine Fever in China, 2018. *Transboundary and Emerging Diseases, 65* (6), 1482-1484.*doi:10.1111/tbed.12989*

Hosted file

`Figures.docx` available at https://authorea.com/users/440879/articles/541510-epitope-mapping-of-african-swine-fever-virus-asfv-structural-protein-p30

Hosted file

`Tables.docx` available at https://authorea.com/users/440879/articles/541510-epitope-mapping-of-african-swine-fever-virus-asfv-structural-protein-p30