Journal of General Virology

Identification of T-Cell Epitopes in African Swine Fever Virus CD2v and C-type Lectin Proteins

--Manuscript Draft--

Manuscript Number:	JGV-D-18-00393R2
Full Title:	Identification of T-Cell Epitopes in African Swine Fever Virus CD2v and C-type Lectin Proteins
Article Type:	Short Communication
Section/Category:	Animal - Large DNA Viruses
Keywords:	ASFV; cellular epitope; CD2v; C-type lectin
Corresponding Author:	Daniel L. Rock University of Illinois at Urbana Urbana, IL UNITED STATES
First Author:	Galina Burmakina
Order of Authors:	Galina Burmakina
	Alexander Malogolovkin
	Edan R Tulman
	Weidong Xu
	Gustavo Delhon
	Denis Kolbasov
	Daniel L. Rock
Manuscript Region of Origin:	UNITED STATES
Abstract:	African swine fever (ASF) is an emerging disease threat for the swine industry worldwide. No ASF vaccine is available and progress is hindered by lack of knowledge concerning the extent of ASF virus (ASFV) strain diversity and the viral antigens conferring type-specific protective immunity in pigs. Previously, we demonstrated that ASFV serotype specific proteins CD2v (EP402R) and/or C-type lectin (EP153R) are important for protection against homologous ASF infection. Here, we identified six discrete T-cell epitope regions present on CD2v and C-type lectin using IFN-γ ELISpot assay and PBMCs from ASF immune animals, indicating cellular reactivity to these proteins in the context of ASFV infection and protective immunity. Notably, three of the epitope regions map to previously described serotype-specific signature regions of these proteins. Improved understanding of ASFV protective antigens, relevant epitopes and their diversity in nature will facilitate ASFV subunit vaccine design and development.
Author Comments:	
Opposed Reviewers:	

Abstract

African swine fever (ASF) is an emerging disease threat for the swine industry worldwide. No ASF vaccine is available and progress is hindered by lack of knowledge concerning the extent of ASF virus (ASFV) strain diversity and the viral antigens conferring type-specific protective immunity in pigs. Previously, we demonstrated that ASFV serotype specific proteins CD2v (EP402R) and/or C-type lectin (EP153R) are important for protection against homologous ASF infection. Here, we identified six discrete T-cell epitope regions present on CD2v and C-type lectin using IFN-γ ELISpot assay and PBMCs from ASF immune animals, indicating cellular reactivity to these proteins in the context of ASFV infection and protective immunity. Notably, three of the epitope regions map to previously described serotype-specific signature regions of these proteins. Improved understanding of ASFV protective antigens, relevant epitopes and their diversity in nature will facilitate ASFV subunit vaccine design and development.

Keywords

C-type lectin, CD2v, T-cell epitopes, African swine fever virus, protective immunity

Abbreviations

ASF, African swine fever; ASFV, African swine fever virus; HAI, hemadsorption-inhibition; M-II, monocyte infection-inhibiting; HAU, hemadsorbing unit; DPC, days post challenge; IFN-γ, interferon gamma; PHA, phytohemagglutinin; AA, amino acids; SLA, swine leukocyte antigen

ASF is an acute viral hemorrhagic disease affecting domestic swine with mortality rates approaching 100% (1-3). Devastating ASF outbreaks and continuing epidemic in the Caucasus

47 region, the Russia Federation, the Baltic states, countries of Eastern Europe and now China
48 (2007 – to date) highlight the significance of this disease threat (4, 5). No ASF vaccine is
49 available, though protection against homologous virus challenge has been observed (6-12).
50 Vaccine development and disease control progress is hindered by lack of knowledge concerning
51 the ASF virus (ASFV) antigens responsible for inducing protective immunity and concerning the
52 diversity of these protective antigens in nature.

53 Protective immunity against ASFV remains poorly defined. As is the case with most viral
54 infections, both humoral and cellular immune responses appear to be important for protection.
55 While the passive transfer of anti-ASFV antibodies is protective, the effector mechanisms remain
56 undefined (13-15). ASFV neutralizing antibodies have been described (16-19), but their cross
57 neutralization *in vitro* does not correlate with ASFV cross protection in pigs (17, 20). ASF
58 protective immunity may be serotype-specific, as viruses within a hemadsorption-inhibition
59 (HAI) serogroup appear to cross protect against one another while viruses outside the serogroup
60 do not (21- 24; Malogolovkin et al. unpublished data). Interestingly, anti-ASFV "monocyte
61 infection-inhibiting (M-II) antibodies" inhibit ASFV replication in macrophage cell cultures (7)
62 but only against homologous ASFV strains and in a manner correlating with cross-protective
63 immunity in vivo (25, 26).

64 Multiple data support a role for cellular immune responses in ASFV protective immunity.
65 Lymphocyte depletion of pigs indicate that cytotoxic CD8+ lymphocytes are important for
66 ASFV clearance and protection (27), and that protective effects are correlated with ASFV strain-
67 specific CD8+ T-cell responses (28, 29). Additionally, lack of detectable anti-ASFV antibodies
68 at the time of challenge in DNA-vaccinated and partially protected animals has been interpreted
69 as support for the role of cellular immunity in protection (28, 29). Thus, no definitive correlates

of protection are established and no specific viral protein(s) has been shown sufficient for induction of robust protective immunity in pigs.

Recently, we have shown that two ASFV-encoded proteins, CD2v (EP402R) and/or C-type lectin (EP153R), are sufficient for mediating serologic specificity as determined by HAI (30). ASFV CD2v is the only known viral homolog of cellular CD2, a T-Cell protein involved in co-regulation of cell activation. CD2v is the ASFV hemagglutinin and has been implicated previously in protective immunity (31, 32, 33). Pigs immunized with CD2v developed HAI and M-II antibodies and were partially protected from challenge with the homologous virulent virus strain (34). CD2v expression was also required for partial protection conferred by subunit ASF vaccine constructs (28, 35). Additional support for a CD2v role in protective immunity comes from vaccine studies using ASFV chimeric viruses; homologous CD2v and/or the adjacent C-type lectin protein were necessary for protection against homologous ASFV infection (36). Thus, CD2v and C-type lectin proteins may represent significant protective antigens for ASFV that should be targeted for vaccine design and development; the viral protein domains and epitopes associated with protective host responses remain to be defined. Recent studies indicate that heterologous expression of CD2v and C-type lectin proteins in swine can induce specific T-cell responses (37, 38). Here, we have identified T-cell epitope regions from CD2v and C-type lectin proteins which induce T-cell responses in the context of ASF protective immunity.

To ensure that T-cell responses against CD2v and C-type lectin proteins were evaluated in the context of ASF protective immunity, ASFV immune animals were generated using a previously described vaccination protocol (36). The virulent ASFV isolate Congo K-49, an HAI serogroup 2 virus, and cell culture-passed, attenuated derivative Congo KK-262 virus, were used for the inoculation of animals in biosecure animal facilities (Federal Research Center of Virology

93 and Microbiology, Pokrov, Russia) in accordance with Russian legislation and under the
94 supervision of the Center's Research Ethics Committee. In three independent experiments,
95 Landrace and Large White pigs (30 to 35 kg) were mock vaccinated or vaccinated
96 intramuscularly with 10^6 hemadsorbing units (HAU) of attenuated Congo KK-262 and boosted
97 with the same dose at 21 days post-vaccination. Three weeks later, animals were challenged
98 intramuscularly with 10^3 HAU of virulent Congo K-49 and monitored for 30 days. Clinical signs,
99 survival rate and time-to-death were recorded as described previously (36). Blood samples were
100 collected at regular intervals post vaccination and for 30 days post challenge (DPC). Quantitative
101 PCR of ASFV in blood samples was performed as previously described (39). ASFV ELISA
102 assays were performed as recommended by the manufacturer (IDScreen® African Swine Fever
103 Indirect, France) using serum collected just prior to challenge infection.
104 Across three independent experiments, animals immunized with Congo-attenuated (KK-
105 262) virus demonstrated solid levels of protection when challenged with virulent Congo K-49;
106 immunized animals survived infection exhibiting only transient fever responses and reduced
107 viremia compared to control animals (Table 1). At the time of challenge, all KK-262 immunized
108 animals were serologically positive for ASFV-specific antibodies. From these ASFV Congo K-
109 49 immune animals, peripheral blood mononuclear cells (PBMC) were isolated seven to ten days
110 post-challenge by density gradient centrifugation using Histopaque 1077 (Sigma-Aldrich). Buffy
111 coats were collected, resuspended in RPMI-1640 with 10% fetal bovine serum and either used
112 immediately or stored frozen at −80°C until use in *in vitro* cellular assays.
113 To examine swine T-cell responses against discrete regions of the ASFV CD2v and C-type
114 lectin proteins, IFN-γ ELISpot assays were designed to assess responses of immune cells to short
115 peptides of each protein. To assess individual, potentially antigenic sequences of the CD2v and

C-type lectin proteins, a library of 132 overlapping (each by 11 amino acids) 15-mer peptides were designed based on a conceptual fusion of Congo K-49 C-type lectin and CD2v protein sequences (Supplemental Table 1). Peptides were synthesized by Genemed Synthesis, Inc (San Antonio, USA), resuspended in DMSO (100 mg/ml), aliquoted and stored at −80°C for later use as antigens in *in vitro* assays.

To assess reactivity of immune cells against K-49 CD2v and C-type lectin protein peptides, PBMC were assayed using IFN-γ ELISpot. ELISpot kit and protocols (BD Bioscience #551849) were used incorporating a mouse anti-Pig IFN-γ capture antibody (BD Bioscience #559961) and biotin-labeled mouse anti-pig IFN-γ primary antibody (BD Bioscience #559958). PBMC ($1.2x10^6$ cells/well) were cultured in duplicate with pooled or individual CD2v or C-type lectin peptides (2 to 5 μg/ml) for 18-20 h (37 C and 5% CO_2) in plates coated with capture antibody. Cells were removed, biotinylated detection antibody was added, and plates were developed with streptavidin–peroxidase and substrate using manufacturer's recommended protocols. Frequencies of IFN-γ- secreting cells recognizing individual CD2v or C-type lectin peptides were calculated by subtracting the number of spots in unstimulated wells from numbers in peptide-stimulated wells, and expressed as number of responding cells/10^6 PBMC. Controls included both unstimulated (negative control) and PHA-stimulated (positive control) immune cells, as well as peptide-stimulated cells from an ASFV naïve animal. Quantitative data were analyzed using the Two-way ANOVA test as implemented in GraphPad Prizm 7 (La Jolla, USA, graphpad.com).

ELISpot assays indicated reactivity against CD2v and C-type lectin protein peptides in ASFV-immune swine cells, but not in unstimulated cells or peptide-stimulated cells from naïve animals. Initial screenings of immune cells were conducted against pools of overlapping CD2v and C-type lectin peptides. Peptides from pools demonstrating initial reactivity were then used

individually to fine-map T-cell epitope regions demonstrating reactivity for each protein using PBMCs from multiple animals (Fig.1 and Table 2).

Six discrete T-cell epitope regions (Regions I-VI) were identified within C-type lectin and CD2v proteins, consisting of two to four overlapping peptide each (Table 2). Regions I and II were located in the carboxyl-terminal, extracellular domain of the C-type lectin protein (Fig. 1), a region previously identified as containing a serogroup-specific signature (30). In regions I and II, positive reactivity was observed for multiple overlapping peptides in approximately 70% of animals tested (Table 2). Four T-cell epitope regions (III-VI) were identified in CD2v, two located within the amino-terminal immunoglobulin-like domain and two within the proline-rich cytoplasmic domain of the protein (Fig. 1). Positive reactivity was observed for three to four overlapping peptides each within regions III-VI, and responses to individual peptides were detected in 40–100% of the animals tested (Table 2).

To examine relative potential for different epitope regions to contribute to serospecific cross-protective immunity, immunoreactive peptide sequences and consensus sequences of the six T-cell epitope regions identified for Congo K-49 (serogroup 2 virus) were compared with C-type lectin and CD2v sequences from ASFV identified as non-serogroup 2 based on HAI signature sequence comparison as previously described (30). Epitope conservation and cluster analysis were carried out with the Immune Epitopes database and analysis resources available on www.iedb.org.

Regions I and II, mapping to the carboxyl–terminal, extracellular domain of the C-type lectin protein, exhibited the greatest degree of variability with 27-80% amino acid (AA) identity between Congo K-49 peptides (or epitope region consensus) and sequences of non-serotype 2 viruses. Regions III and IV, mapping to the immunoglobulin domain of CD2v, were less variable

with a range of approximately 40-80% AA identity observed. Notably, Regions V and VI, mapping within the proline-rich cytoplasmic domain of CD2v were more conserved (79-100% AA identity) than regions I-IV between ASFV serotypes (Fig 1 and Table 2). A previously identified CD2v T-cell epitope for the ASFV isolate E75, a serogroup 4 virus, appears to partially overlap Region III (4 AA with 50% identity) as well (28); whether this represents a true Region III epitope or an additional adjacent epitope on the ASFV E75 CD2v protein remains to be determined. Nevertheless, this region of the protein appears to be a reactive T-cell epitope in two HAI serologically distinct viruses.

Without empirical data, identification of T-cell epitopes is limited to prediction based on computational models and algorithms. To compare performance of C-type lectin and CD2v epitope prediction against mapped reactive peptides, artificial neural network and support vector machine methods were used to predict potential T-cell epitopes using the CTLPred web server (40). Only two of the T-cell epitope regions identified experimentally in this study were predicted computationally as containing T-cell epitopes. One epitope in Region III and multiple overlapping epitopes (9 AA each) within Region III, were predicted as shown in (Table 2).

Overall, results described here have identified six novel T-cell epitope regions on ASFV serotype-specific proteins CD2v and C-type lectin with multiple overlapping peptides for each epitope region being recognized by approximately 50-100% of immune animals tested. Robust responses of T-cells to these epitopes, in immune animals seven to ten days post challenge, suggest their significance for the observed protective host response (Table1).

T-cell responses observed in these experiments potentially include both $CD8^+$ and $CD4^+$ responses. T-cell epitope regions identified ranged from 19 to 27 AA; sizes consistent with presentation via either SLA I or SLA II molecules where optimal peptide sizes range from 8-10

185 AA and 18-20 AA, respectively (41, 42). Conceivably, these regions could contain multiple
186 epitopes; for example, computer predictions identified four overlapping T-cell epitopes (of 9
187 AA) in Region III within the CD2v protein.

188 The modest degree of conservation observed for epitope regions I through IV with
189 homologous regions from other ASFV serogroups and unassigned viruses together with the fact
190 that Regions I, II and IV are located within previously identified serogroup-specific signature
191 regions of these proteins (30) suggest that T-cell epitopes also are specific for a given viral
192 serotype and that T-cell host responses may be associated with the serotype-specific protection
193 observed.

194 While CD2v has been implicated previously as a potential protective ASFV antigen (28,
195 34-36), only recently has the C-type lectin protein been considered a candidate (36). The robust
196 host responses to epitope Regions I and II located within the carboxyl-terminal regions of the C-
197 type lectin protein are notable for two reasons: the two epitope regions are significantly variable
198 between ASFV serogroups, and a high percentage of immune animals tested (54-76%)
199 responded to individual peptides contained within these regions. Given this result and prior
200 vaccine studies where homologous CD2v and/or C-type lectin protein were necessary for
201 protection against homologous ASFV infection (36), additional evaluation of this protein as a
202 protective antigen is warranted.

203 Surprisingly, strong T-cell responses were observed for epitope regions V and VI
204 contained within the proline–rich cytoplasmic domain of CD2v (Fig. 1, Table 2). Despite repeat
205 variation in the CD2v cytoplasmic domain, sequences representing the reactive peptides
206 identified here are in fact highly conserved among ASF viruses and across HAI serogroups. The

significance of this response for protective immunity or possible immunopathology in vaccinated but unprotected animals remains to be determined.

In summary, we have identified novel T-cell epitopes on ASFV serotype-specific proteins CD2v and C-type lectin. Improved understanding of ASFV protective antigens, relevant epitopes and their diversity in nature will facilitate ASFV subunit vaccine design and development.

Acknowledgements:

This project was supported by the Russian Science Foundation (grant 16-16-00090), the National Pork Board (grant 13-102) and by the USDA National Institute of Food and Agriculture (grant 2013-67015-21335).

Conflicts of interest:

The authors declare that there are no conflicts of interest.

Ethical statement:

All animal procedures were conducted in accordance with Russian legislation and under the supervision of the Research Ethics Committee of the Federal Research Center of Virology and Microbiology, Pokrov, Russia.

References

1. **Montgomery RE.** On a form of swine fever occuring in British East Africa (Kenya Colony). *J Comp Pathol Therapeutics* 1921; 34:159-191.

2. **Coggins L.** African swine fever virus. Pathogenesis. *Prog Med Virol* 1974; 18:48-63.

3. **Mebus CA.** African swine fever. *Adv Virus Res* 1988; 35:251-269.

4. **Gogin A, Gerasimov V, Malogolovkin A, Kolbasov D.** African swine fever in the North Caucasus region and the Russian Federation in years 2007-2012. *Virus Res* 2013; 173:198-203.

5. **Sanchez-Vizcaino JM, Mur L, Martinez-Lopez B.** African swine fever (ASF): five years around Europe. *Vet Microbiol* 2013; 165:45-50.

6. **Detray DE.** Persistence of viremia and immunity in African swine fever. *Am J Vet Res* 1957; 18:811-816.

7. **Malmquist WA.** Serologic and immunologic studies with African swine fever virus. *Am J Vet Res* 1963; 24:450-459.

8. **Ruiz GF, Carnero ME, Bruyel V.** Immunological responses of pigs to partially attenuated ASF and their resistance to virulent homologous and heterologous viruses. In *FAO/CEC Expert Consultation in ASF Research*, 1981; pp. 206-216. Edited by P. J. Wilkinson. Rome.

9. **Zsak L, Lu Z, Kutish GF, Neilan JG, Rock DL.** An African swine fever virus virulence-associated gene NL-S with similarity to the herpes simplex virus ICP34.5 gene. *J Virol* 1996; 70:8865-8871.

10. **Gomez-Puertas P, Rodriguez F, Oviedo JM, Brun A, Alonso C** *et al***.** 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* 1998; 243:461-471.

11. **Lewis T, Zsak L, Burrage TG, Lu Z, Kutish GF** *et al***.** An African swine fever virus *ERV1-ALR* homologue, *9GL*, affects virion maturation and viral growth in macrophages and viral virulence in swine. *J Virol* 2000; 74:1275-1285.

12. **Leitão A, Cartaxeiro C, Coelho R, Cruz B, Parkhouse RM** *et al*. The non-haemadsorbing African swine fever virus isolate ASFV/NH/P68 provides a model for defining the protective anti-virus immune response. *J Gen Virol* 2001; 82:513-523.

13. **Schlafer DH, Mebus CA, McVicar JW.** African swine fever convalescent sow: subsequent pregnancy and effect of colostral antibody on challenge inoculation of their pigs. *Am J Vet Res* 1984a; 45:361-1366.

14. **Schlafer DH, Mebus CA, McVicar JW.** African swine fever in neonatal pigs: passively acquired protection from colostrum or serum from recovered pigs. *Am J Vet Res*. 1984b; 45:1367-1372.

15. **Onisk DV, Borca MV, Kutish GF, Kramer E, Irusta P** *et al*. Passively transferred African swine fever virus antibodies protect swine against lethal infection. *Virology* 1994; 198:350-354.

16. **Ruiz-Gonzalvo F, Caballero C, Martinez J, Carnero ME.** Neutralization of African swine fever virus by sera from African swine fever-resistant pigs. *Am J Vet Res* 1986a; 47:1858-1862.

17. **Zsak L, Onisk DV, Afonso CL, Rock DL.** Virulent African swine fever virus isolates are neutralized by swine immune serum and by monoclonal antibodies recognizing a 72-kDa viral protein. *Virology* 1993;196:596–602.

18. **Borca MV, Irusta P, Carrillo C, Afonso CL, Burrage T** *et al*. African swine fever virus structural protein p72 contains a conformational neutralizing epitope. *Virology* 1994a; 201:413–418.

19. **Gómez-Puertas P, Rodríguez F, Oviedo JM, Ramiro-Ibáñez F, Ruiz-Gonzalvo F** *et al***.** Neutralizing antibodies to different proteins of African swine fever virus inhibit both virus attachment and internalization. *J Virol* 1996; 70:5689–5694.

20. **Neilan JG, Zsak L, Lu Z, Burrage TG, Kutish GF et al.** Neutralizing antibodies to African swine fever virus proteins p30, p54, and p72 are not sufficient for antibody-mediated protection. *Virology* 2004; 319:337–342.

21. **Vishnjakov I, Mitin N, Karpov G, Kurinnov V, Jashin A.** Differentiation African and classical swine fever viruses. *Veterinariya* 1991; 4:28-31.

22. **Sereda AD, Solovkin SL, Fugina LG, Makarov VV.** [Immune reactions to the African swine fever virus]. *Vopr Virusol* 1992; 37:168-170.

23. **Balyshev VM, Fedorishhev IV, Salina MV.** Study of serotype interactions of ASF virus strains both in vitro and in vivo. In *Virusniye bolezni zhivotnikh* 1995, p. 230. Vladimir.

24. **Sereda AD, Balyshev VM.** [Antigenic diversity of African swine fever viruses]. *Vopr Virusol* 2011; 56:38-42.

25. **Ruiz-Gonzalvo F, Carnero ME, Caballero C, Martinez J.** Inhibition of African swine fever infection in the presence of immune sera in vivo and in vitro. *Am J Vet Res* 1986b; 47:1249–1252.

26. **Knudsen RC, Genovesi EV, Whyard TC.** In vitro immune serum-mediated protection of pig monocytes against African swine fever virus. *Am J Vet Res* 1987; 48:1067-1071.

27. **Oura CA, Denyer MS, Takamatsu H, Parkhouse RM.** In vivo depletion of CD8+ T lymphocytes abrogates protective immunity to African swine fever virus. *J Gen Virol* 2005; 86:2445–2450.

28. **Argilaguet JM, Pérez-Martín E, Nofrarías M, Gallardo C, Accensi F** *et al*. DNA vaccination partially protects against African swine fever virus lethal challenge in the absence of antibodies. *PLoS One* 2012; 7:e40942.

29. **Lacasta A, Monteagudo PL, Jiménez-Marín Á, Accensi F, Ballester M** *et al*. Live attenuated African swine fever viruses as ideal tools to dissect the mechanisms involved in viral pathogenesis and immune protection. *Vet Res* 2015; 46:135. doi: 10.1186/s13567-015-0275-z.

30. **Malogolovkin A, Burmakina G, Tulman ER, Delhon G, Diel DG** *et al*. African swine fever virus CD2v and C-type lectin gene loci mediate serological specificity. *J Gen Virol* 2015; 96**:**866-873.

31. **Rodríguez JM, Yáñez RJ, Almazán F, Viñuela E, Rodriguez JF.** African swine fever virus encodes a CD2 homolog responsible for the adhesion of erythrocytes to infected cells. *J Virol* 1993; 67:5312-5320.

32. **Borca MV, Kutish GF, Afonso CL, Irusta P, Carrillo C** *et al*. An African swine fever virus gene with similarity to the T-lymphocyte surface antigen CD2 mediates hemadsorption. *Virology* 1994b; 199:463-468.

33. **Ruiz-Gonzalvo F, Coll JM.** Characterization of a soluble hemagglutinin induced in African swine fever virus-infected cells. Virology 1993; 196:769-777.

34. **Ruiz-Gonzalvo F, Rodrıguez F, Escribano JM.** Functional and immunological properties of the baculovirus expressed hemagglutinin of African swine fever virus. *Virology* 1996; 218:285–289.

35. **Argilaguet JM, Pérez-Martín E, López S, Goethe M, Escribano JM** *et al*. BacMam immunization partially protects pigs against sublethal challenge with African swine fever virus. *Antiviral Res* 2013; 98:61–65.

36. **Burmakina G, Malogolovkin A, Tulman ER, Zsak L, Delhon G** *et al*. African swine fever virus serotype-specific proteins are significant protective antigens for African swine fever. *J Gen Virol* 2016; 96:866-873.

37. **Lopera-Madrid J, Osorio JE, He Y, Xiang Z, Adams LG** *et al*. Safety and immunogenicity of mammalian cell derived and Modified Vaccinia Ankara vectored African swine fever subunit antigens in swine. *Veterinary Immunology and Immunopathology* 2017*;* 185:20–33.

38. **Jancovich JK, Chapman D, Hansen DT, Robida MD, Loskutov A *et al*.** Immunization of pigs by DNA prime and recombinant vaccinia virus boost to identify and rank African Swine Fever Virus immunogenic and protective proteins. *J Virol* 2018; 92(8). pii: e02219-17. doi: 10.1128/JVI.02219-17.

39. **King DP, Reid SM, Hutchings GH, Grierson SS, Wilkinson PJ** *et al*. Development of a TaqMan PCR assay with internal amplification control for the detection of African swine fever virus. *J Virol Methods* 2003; 107:53-61.

40. **Bhasin M, Raghava GPS.** Prediction of CTL epitopes using QM, SVM and ANN techniques. *Vaccine* 2004; 22:3195-3201.

41. **Trolle T**, **McMurtrey CP**, **Sidney J**, **Bardet W**, **Osborn SC** *et al*. The length distribution of class I-restricted T cell epitopes is determined by both peptide supply and MHC allele-specific binding preference. *J Immunol* 2016; 196:1480-1487.

42. **Kobayashi H**, **Celis E**. Peptide epitope identification for tumor-reactive CD4 T cells. *Curr Opin Immunol* 2008; 20:221–227.

Table 1. Vaccination with attenuated Congo KK-262 induces protection against virulent Congo K-49 challenge

	n	Mortality (%)	Mortality TTD[#]	TTF[##]	Pre-challenge serology, % positive[§]	Maximal viral load (genomes/ml)[§§]
Experiment 1						
KK-262/K-49[*]	7	0	-	4.4 (0.9)	100	5.4e+002 (0.3)
K-49[**]	3	100	4.3 (0.5)	3.3 (0.5)	0	5.9e+008 (0.1)
Experiment 2						
KK-262/K-49	3	0	-	3.3 (0.5)	100	4.6e+002 (0.4)
K-49	3	100	6.6 (0.6)	3.3 (0.5)	0	7.8e+008 (0.2)
Experiment 3						
KK-262/K-49	3	0	-	3.6 (0.5)	100	7.0e+002 (0.5)
K-49	3	100	6.3 (0.6)	3 (0.0)	0	9.1e+009 (0.2)

[#] TTD, time to death, in mean days post-challenge, with standard error (SE) in parenthesis.

[##] TTF, time to onset of fever, in mean days post-challenge, with SE in parenthesis.

[§] As determined by ELISA (IDScreen® African Swine Fever Indirect, France); percent (%) of animals with positive result.

[§§] Mean maximal viral load in $\log_{10}$ viral genomic copies (ml blood^{-1}), with SE in parenthesis.

[*] Animals were vaccinated with Congo-attenuated virus (KK-262) followed by challenge with Congo-virulent virus (K-49).

[**] Animals were mock vaccinated followed by challenge with Congo-virulent virus (K-49).

390

391

392 **Table 2. T-cell epitopes in ASFV Congo K-49 CD2v and C-type lectin proteins.**

Epitope Region[1]	Peptide No[2]	Peptide sequence[3]	No. animals positive/tested (%)[4]	Min-Max aa Id, other serogroups[5]	Predicted/ Previously mapped [6]
	29	SFLNLTKLYHHHSHY	10/13 (76%)	47%-60%	
I	30	LTKLYHHHSHYWVNY	9/13 (69%)	47%-73%	
(Lec)	31	YHHHSHYWVNYSLNN	9/13 (69%)	40%-80%	
	32	SHYWVNYSLNNNYSV	7/13 (54%)	33%-80%	
	Cons	SFLNLTKLYHHHSHYWVNYSLNNNYSV		42%-74%	
II	37	KYNLNRKKSHYTDLL	6/8 (75%)	27%-33%	
(Lec)	38	NRKKSHYTDLLFICS	6/8 (75%)	27%-40%	
	Cons	KYNLNRKKSHYTDLLFICS		27%-55%	
	51	INSETEGIFWNFYNN	2/4 (50%)	33%-67%	
III	52	TEGIFWNFYNNTFNT	2/4 (50%)	40%-70%	FYNNTFNTI
(CD2v)	54	YNNTFNTIATCGKKN	2/5 (40%)	33%-80%	
	Cons	INSETEGIFWNFYNNTFNTIATCGKKN		26%-67%	
	66	TYQLVYSRNRINYTI	3/5 (60%)	47%-73%	VYSRNRINY
IV	68	NRINYTINLLLPVTS	2/4 (50%)	53%-87%	SRNRINYTI
(CD2v)	69	YTINLLLPVTSPIIT	2/4 (50%)	53%-93%	NRINYTINL
	Cons	TYQLVYSRNRINYTINLLLPVTSPIIT		48%-81%	RINYTINLL
					F3:SVDSPTITY
	117	PLNPSPPPKPCPPPK	3/7 (43%)	73%-100%	
V	118	SPPPKPCPPPKPCPP	5/7 (71%)	93%-100%	
(CD2v)	119	KPCPPPKPCPPPKPC	5/7 (71%)	93%-100%	
	120	PPKPCPPPKPCPPPK	3/7 (43%)	93%-100%	
	Cons	PLNPSPPPKPCPPPKPCPPPKPCPPPK		74%-100%	
	127	YSPPKPLPSIPLLPN	2/4 (50%)	53%-100%	
VI	128	KPLPSIPLLPNIPPL	4/4 (100%)	73%-100%	
(CD2v)	129	SIPLLPNIPPLSTQN	2/4 (50%)	87%-100%	
	130	LPNIPPLSTQNISLI	2/4 (50%)	87%-100%	
	Cons	YSPPKPLPSIPLLPNIPPLSTQNISLI		74%-100%	

393 [1] Epitope Region, T-cell epitope regions identified by ELISPOT reactivity to multiple,
394 overlapping peptides. Regions I-II represent sequences in C-type lectin-like (Lec) protein,
395 Regions III-VI represent sequences in CD2v (CD2v).
396 [2] Peptide no., (see Supplemental Table 1).
397 [3] Peptide sequence, amino acid sequence of reactive peptide. Cons, consensus of all peptide
398 sequences in the region.
399 [4] No. animals positive/tested (%), total number of swine testing positive versus the total number
400 of swine tested over three independent experiments, with the percent positive indicated in
401 parentheses.
402 [5] Min-Max aa Id, other serogroups. Lower and upper range of amino acid identity (aa Id)
403 between peptide and consensus sequences and sequences in ASFV which phylogenetically fall
404 outside of the lectin/CD2v serogroup 2 cluster (30).
405 [6] Predicted/Previously mapped. *In silico*-predicted epitopes matching within empirically
406 identified epitope regions I-VI. Also listed is the F3 epitope previously identified in ASFV strain
407 E-75 CD2v (28), overlapping here in epitope region II and with conserved amino acids indicated
408 by underlining.
409
410
411

Fig. 1. Identification of T-cell epitopes in ASFV serotype-specific proteins.

Pigs were immunized with attenuated Congo KK-262 and subsequently challenged with virulent Congo K-49. PBMCs were isolated 7 to 10 days post-challenge, incubated with CD2v and C-type lectin overlapping 15-mer peptides, and assayed by IFN-γ-ELISpot as described in the text. Peptide numbers are indicated in the x-axis and polypeptide regions to which the peptides map are shown schematically below. Black and grey bars represent number of tested pigs and ELISpot-reactive pigs, respectively. Roman numbers (I-VI) above bar clusters indicate the six T-cell epitope regions identified. Small circles above the bars represent average numbers of spot forming units (SFU) from 1.2e+006 PBMCs. The dotted line represents the mean SFU in control (non-stimulated) PBMCs plus two SE.

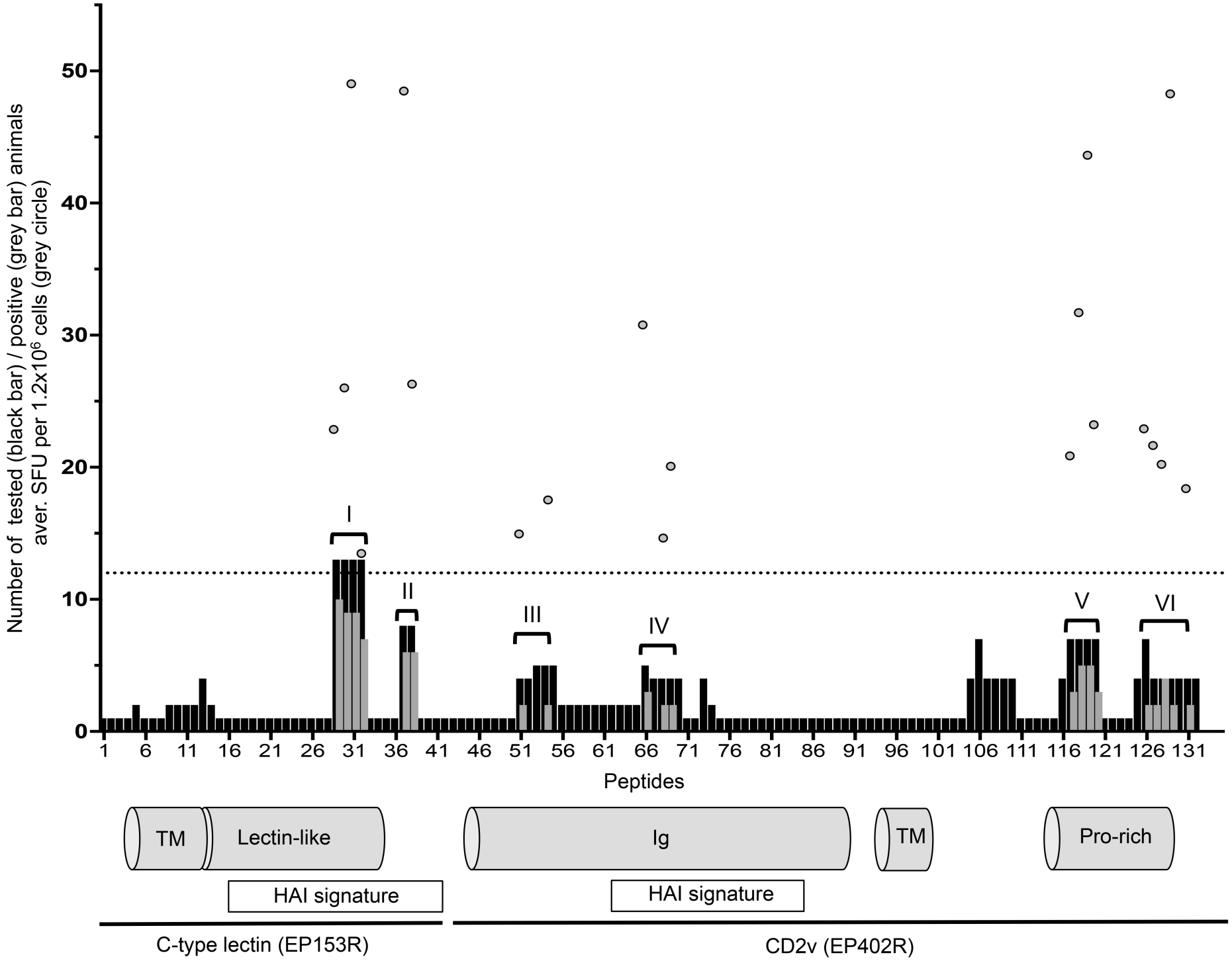

Figure 1

Supplemental Table 1. ASFV strain K-49 C-type lectin and CD2v fusion protein peptides used for T-cell epitope mapping (Burmakina et al., 2018)

Peptide number	Peptide sequence	Peptide number	Peptide sequence
1	MAFLNKKYIGLINKK	67	VYSRNRINYTINLLL
2	NKKYIGLINKKEGLK	68	NRINYTINLLLPVTS
3	IGLINKKEGLKKKID	69	YTINLLLPVTSPIIT
4	NKKEGLKKKIDDYSI	70	LLLPVTSPIITYNCT
5	GLKKKIDDYSILIIG	71	VTSPIITYNCTQSLI
6	KIDDYSILIIGILIG	72	IITYNCTQSLITCEK
7	YSILIIGILIGTNIL	73	NCTQSLITCEKTNGT
8	IIGILIGTNILSLII	74	SLITCEKTNGTNIRL
9	LIGTNILSLIINIIG	75	CEKTNGTNIRLFLNL
10	NILSLIINIIGEINK	76	NGTNIRLFLNLNDTI
11	LIINIIGEINKPICY	77	IRLFLNLNDTINEYT
12	IIGEINKPICYQNDD	78	LNLNDTINEYTNKSF
13	INKPICYQNDDKIFY	79	DTINEYTNKSFLNYY
14	ICYQNDDKIFYCPKD	80	EYTNKSFLNYYWNSS
15	NDDKIFYCPKDWVGY	81	KSFLNYYWNSSELNN
16	IFYCPKDWVGYNNVC	82	NYYWNSSELNNIFLA
17	PKDWVGYNNVCYYFS	83	NSSELNNIFLATCII
18	VGYNNVCYYFSNDNG	84	LNNIFLATCIINNTL
19	NVCYYFSNDNGNNYT	85	FLATCIINNTLNSAN
20	YFSNDNGNNYTTADN	86	CIINNTLNSANTTKV
21	DNGNNYTTADNKCKQ	87	NTLNSANTTKVINCT
22	NYTTADNKCKQLNNS	88	SANTTKVINCTNPLL
23	ADNKCKQLNNSTLAN	89	TKVINCTNPLLKSYQ
24	CKQLNNSTLANNLTD	90	NCTNPLLKSYQNYFL
25	NNSTLANNLTDLLNL	91	PLLKSYQNYFLENIH
26	LANNLTDLLNLTSFL	92	SYQNYFLENIHTLFY
27	LTDLLNLTSFLNLTK	93	YFLENIHTLFYMIIF
28	LNLTSFLNLTKLYHH	94	NIHTLFYMIIFIVSG
29	SFLNLTKLYHHHSHY	95	LFYMIIFIVSGITIS
30	LTKLYHHHSHYWVNY	96	IIFIVSGITISIFIS
31	YHHHSHYWVNYSLNN	97	VSGITISIFISIITF
32	SHYWVNYSLNNNYSV	98	TISIFISIITFLSLR
33	VNYSLNNNYSVPLID	99	FISIITFLSLRKRKK
34	LNNNYSVPLIDSKYN	100	ITFLSLRKRKKHVEE
35	YSVPLIDSKYNLNRK	101	SLRKRKKHVEEIESP
36	LIDSKYNLNRKKSHY	102	RKKHVEEIESPPPSE
37	KYNLNRKKSHYTDLL	103	VEEIESPPPSESNEE
38	NRKKSHYTDLLFICS	104	ESPPPSESNEEDISH
39	SHYTDLLFICSKGGG	105	PSESNEEDISHDDTT
40	DLLFICSKGGGGSII	106	NEEDISHDDTTSIHE
41	ICSKGGGGSIIKLIF	107	ISHDDTTSIHEPSPR
42	GGGGSIIKLIFLICF	108	DTTSIHEPSPREPLL
43	SIIKLIFLICFKIVL	109	IHEPSPREPLLPKPY
44	LIFLICFKIVLSINY	110	SPREPLLPKPYSRYQ

45	ICFKIVLSINYWVRY
46	IVLSINYWVRYNDTV
47	INYWVRYNDTVTLNS
48	VRYNDTVTLNSNINS
49	DTVTLNSNINSETEG
50	LNSNINSETEGIFWN
51	INSETEGIFWNFYNN
52	TEGIFWNFYNNTFNT
53	FWNFYNNTFNTIATC
54	YNNTFNTIATCGKKN
55	FNTIATCGKKNNVCE
56	ATCGKKNNVCECSNY
57	KKNNVCECSNYDNSL
58	VCECSNYDNSLYNIT
59	SNYDNSLYNITNNCS
60	NSLYNITNNCSLTIF
61	NITNNCSLTIFPNNT
62	NCSLTIFPNNTKIFN
63	TIFPNNTKIFNTTYQ
64	NNTKIFNTTYQLVYS
65	IFNTTYQLVYSRNRI
66	TYQLVYSRNRINYTI
111	PLLPKPYSRYQYNTP
112	KPYSRYQYNTPIYYM
113	RYQYNTPIYYMRPST
114	NTPIYYMRPSTQPLN
115	YYMRPSTQPLNPSPP
116	PSTQPLNPSPPPKPC
117	PLNPSPPPKPCPPPK
118	SPPPKPCPPPKPCPP
119	KPCPPPKPCPPPKPC
120	PPKPCPPPKPCPPPK
121	CPPPKPCPPPKPCPP
122	KPCPPPKPCPPPKPC
123	PPKPCPPPKPCPSPE
124	CPPPKPCPSPESYSP
125	KPCPSPESYSPPKPL
126	SPESYSPPKPLPSIP
127	YSPPKPLPSIPLLPN
128	KPLPSIPLLPNIPPL
129	SIPLLPNIPPLSTQN
130	LPNIPPLSTQNISLI
131	PPLSTQNISLIHVDR
132	TQNISLIHVDRII