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Short communication

Identification of potent epitopes on hexon capsid protein and their evaluation as vaccine candidates against infections caused by members of *Adenoviridae* family

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ABSTRACT

Adenoviruses cause economically important diseases in vertebrates. Effective vaccines against adenoviral diseases are currently lacking. Here, we report a highly conserved epitopic region on hexon proteins of adenoviruses that generate a strong immune response when used as a virus-like-particle (VLP) vaccine, produced by inserting the epitopic region into the core protein of hepatitis B virus. For evaluation of its protective efficacy, the epitopic region from a representative adenovirus, fowl adenovirus serotype 4 (FAdV-4), was tested as a VLP vaccine which conferred 90% protection against challenge with a virulent FAdV-4 isolate in chickens. Importantly, such a high level of protection is not achieved when the epitopic region is employed as a part of a subunit vaccine. As the sequence and the structure of the epitopic region are highly conserved in hexon proteins of adenoviruses, the epitopic region could be employed as a promising VLP vaccine candidate against adenoviral diseases, in general.

1. Introduction

Adenoviridae is a family of viruses (adenoviruses) which consists of five genera, members of which cause infections in vertebrates. Adenoviruses are also known to cause economically important diseases such as egg-drop syndrome, inclusion body hepatitis, and hydropericardium syndrome (HPS) in chickens, inflicting significant damages to the poultry industry worldwide and necessitating the development of effective vaccines against adenoviral diseases [1].

Hexon is a major capsid protein of adenoviruses which is highly immunogenic in nature [2]. The hexon protein in adenoviruses comprises of pedestal regions (P1 and P2) which are mostly conserved among adenoviruses and seven hypervariable regions (HVR1-7) which differ among adenoviruses and are contained in three loops (L1, L2, and L4) [3,4]. Owing to its immunogenic nature, the hexon protein of adenoviruses has been used as an antigen for the development of vaccines against adenoviral infections. However, effective vaccines which can be readily produced and use hexon as an antigen for providing complete protection against adenoviral infections are currently lacking [5–7]. Recently, virus-like particles (VLPs) which are based on the core protein of hepatitis B virus (HBc) have gained considerable attention as vaccine carriers since HBc can be readily produced as VLPs in various expression

Abbreviations: ELISA, Enzyme-linked immunosorbent assay; FAdV-4, Fowl adenovirus of serotype 4; GndCl, Guanidinium chloride; HBc, Core protein of hepatitis B virus; HPS, Hydropericardium syndrome; MHC, Major histocompatibility complex; MIR, Major immunodominant region; PBS, Phosphate buffer saline; VLP, Virus-like particle.

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systems, can accommodate large exogenous antigens at its major immunodominant region (MIR), and can generate a humoral response in the properly folded (particulate) form [8]. To the best of our knowledge, VLPs which consist of HBc-fused hexon have not been evaluated as vaccine candidates against adenoviral infections yet. In this study, we have identified a highly conserved epitopic region located on hexon proteins of adenoviruses which generates a strong immune response when employed as a VLP vaccine. For evaluation of its protective efficacy, the epitopic region on the hexon protein of a representative adenovirus, fowl adenovirus of serotype 4 (FAdV-4), was tested as a VLP vaccine. FAdV-4 causes highly devastating HPS (up to 100% mortality) in chickens [9]. The data presented here indicate that the conserved epitopic region of FAdV-4 hexon in fusion with HBc provides up to 90% protection against HPS. As the sequence and the structure of the epitopic region are highly conserved in hexon proteins of adenoviruses, the vaccine strategy presented here could be generalized for providing protection against infections caused by other adenoviruses.

2. Materials and methods

2.1. Sequence and structure-based epitope prediction

In order to identify epitopic regions on hexon proteins of adenoviruses, we selected the hexon protein of FAdV-4 as a representative capsid protein of the *Adenoviridae* family. For prediction of epitopic regions, a protocol previously reported by our research group was followed [10] (see supplementary materials for details). The predicted epitopes were analyzed for sequence conservation in hexon proteins of adenoviruses by multiple sequence alignment. Highly conserved epitopic regions were structurally analyzed and used for immunization studies.

2.2. Purification and refolding of HBc-fused epitopic regions

Expression constructs encoding HBc-fused epitopic regions of FAdV-4 hexon (Ser19-Pro82, Asp348-Phe369, and Gly932-Phe956, respectively) or HBc (containing no FAdV-4 hexon epitope) with a polyhistidine (His_6) tag at the C-terminus were generated as described previously [11] (see supplementary materials for details). The proteins were expressed using *Escherichia coli* BL21 (DE3) cells, and purified through affinity chromatography using nickel-nitrilotriacetic acid columns (see supplementary materials for details). For on-column refolding of the target proteins, the column was washed with refolding buffers such that the concentration of guanidinium chloride (GndCl) was gradually decreased (from 6 M to 0 M) in five steps (Table S1). The refolded protein was eluted and dialyzed overnight at 4 °C to remove excess salts and imidazole. The integrity of purified proteins was assessed through western blotting (see supplementary materials for details).

2.3. Immunization studies in chickens

One-day-old 60 broiler chickens were purchased from a local commercial hatchery (Anchor Poultry Breeders, Faisalabad). The experiments were conducted under the regulations of the Institutional Animal Care and Use Committee of Animal Sciences Division, Nuclear Institute for Agriculture and Biology, Faisalabad, Pakistan. Chickens were randomly divided into six groups (A, B, C, D, E, and F) of 10 birds each. At 7 days of age, chickens from four (A, B, C, and D) groups were immunized subcutaneously at the neck. Chickens from groups A, B, and C were immunized with 100 μg of recombinant HBc-fused epitopes (HBc-hexon (Ser19-Pro82), HBc-hexon (Asp348-Phe369), and HBc-hexon (Gly932-Phe956), respectively) adjuvanted with Montanide™ ISA71 VG (1:1 (w/v) adjuvant: protein), as described previously [5]. Chickens from group D were immunized with 300 μL of a formalin-inactivated commercial vaccine (Angara NIAB) [12] as described previously [10]. At the same day, chickens from groups E and F were injected with 500 μL of phosphate buffer saline (PBS).

At the age of 21 days, chickens from groups A, B, C, and D were immunized with a booster dose of the same composition as used at the age of 7 days. At the 28th day, chickens, except from group F, were challenged with 500 μL of a pathogenic FAdV-4 isolate, having a biological titre of $10^{5.5}$ units of lethal dose 50 (LD50) per mL, as described previously [10]. For determination of antibody titres, blood samples were collected from the wing vein of chickens when the birds were 7, 14, 21, and 28 days old. Mortality and morbidity of all challenged chickens were recorded for 7 days post-infection. The dead birds were subjected to post-mortem examinations for observing typical signs and symptoms of HPS. After 7 days post-challenge, all the remaining alive birds were sacrificed and post-mortem examinations were carried out.

2.4. Histopathology of liver cells

Liver samples were collected from all experimental and control groups during post-mortem examinations. The samples were preserved in neutral buffered formalin, and the tissue samples were processed for histopathological examination through paraffin embedding techniques. The 3–5 μm thick tissues were cut by microtome and stained with the hematoxylin and eosin (H & E) staining technique [13].

2.5. Enzyme-linked immunosorbent assay (ELISA)

An indirect ELISA protocol was optimized by using purified HBc-fused epitopes as antigens, as described previously [10] (see supplementary materials for details). After optimization of ELISA using recombinant proteins, antibody titres of chickens immunized with selected HBc-fused epitopic regions (HBc-hexon (Ser19-Pro82), HBc-hexon (Asp348-Phe369), and HBc-hexon (Gly932-Phe956)) were determined using suitable dilutions of the respective protein and the corresponding serum sample (HBc-hexon (Ser19-Pro82), HBc-hexon (Asp348-Phe369), and HBc-hexon (Gly932-Phe956), respectively). The data were expressed as the antibody titre representing the highest dilution yielding three times the optical density of the negative control serum. ELISA assays were performed using purified FAdV-4 or HBc (with no FAdV-4 hexon epitope) as a coating antigen, as described for HBc-fused hexon epitopes.

3. Results

3.1. Selection of highly immunogenic regions of FAdV-4 hexon

For identification of potent epitopic regions on hexon proteins of adenoviruses, we selected FAdV-4 hexon as a representative capsid protein of the *Adenoviridae* family. Epitopic regions of the protein were first predicted using bioinformatics tools, as described previously by our research group [10]. A total of 24 linear epitopes (LE1 to LE24), 6 different 9-mer peptides as potential major histocompatibility complex (MHC) class-I binders, and 12 (15-mer) MHC class-II binders were predicted (Tables S2-S4). The analysis of the MHC class-I and MHC class-II binders for antigenicity using the VaxiJen v2.0 server predicted 15 peptides as probable antigens [14] (Tables S3, S4). The availability of a three-dimensional model of the FAdV-4 hexon protein allowed us to predict 7 discontinuous epitopes using the ElliPro program [15]

(Table S5). The model was structurally analyzed and three regions (Ser19-Pro82, Asp348-Phe369, and Gly932-Phe956) of the protein were finally selected for immunization studies due to their surface-exposed nature (Fig. 1A, B). Multiple sequence alignment of hexon proteins of adenoviruses reveals that Asp348-Phe369 is the most conserved epitopic region out of the three selected regions. Moreover, regions corresponding to Asp348-Phe369 share a similar fold (conformation) in hexon proteins of adenoviruses (Fig. S1).

3.2. Expression and purification of HBc-fused epitopic regions

The HBc-fused proteins were predominantly expressed as inclusion bodies in the *E. coli* system (Fig. S2). Therefore, the proteins were purified in the folded form through the on-column refolding procedure. The eluted proteins were analyzed through sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE) and western blotting, revealing protein bands on the gel at positions corresponding to the molecular weight of the proteins (Fig. S2). The total yield of the HBc-fused proteins ranged from 8 to 14 mg per litre of the culture after refolding and purification, resulting in the availability of sufficient quantities of the proteins for performing immunization studies in chickens. Furthermore, it was observed that the addition of 0.8% sarkosyl and 10% glycerol stabilized the refolded proteins for storage at 4 °C (for ~ 3 to 5 months) or at −20 °C for longer times.

3.3. Immunization studies

Upon injection in chickens, the purified HBc-fused proteins (Ser19-Pro82, Asp348-Phe369, and Gly932-Phe956) adjuvanted with Montanide™ ISA71 VG displayed protection against the pathogenic FAdV-4 challenge to different levels. The highest protection (90% protection) was offered by HBc-hexon (Asp348-Phe369) followed by HBc-hexon (Ser19-Pro82) and HBc-hexon (Gly932-Phe956) (displaying 70% and 40% protection, respectively). However, only 50% birds survived in the control groups (vaccinated with the commercially available vaccine or PBS) when challenged with a pathogenic FAdV-4 isolate. All birds injected with PBS (and not challenged) were alive (Table 1). Post-mortem examinations of dead birds revealed typical signs and symptoms of HPS such as an enlarged liver and accumulation of a transparent jelly-like or straw-colored fluid in the pericardium (Fig. 2). Histopathological examination of the hepatic parenchyma revealed necrosis, vacuolar degeneration and intranuclear inclusion bodies in the control challenged group. However, no such changes were present in the unchallenged control group. In the commercial vaccine group, few inclusion bodies were present along with necrotic changes. In the HBc-hexon (Asp348-Phe369) group, no inclusion bodies were present but mild vacuolar degeneration and necrotic changes were present. In HBc-hexon (Ser19-Pro82) and HBc-hexon (Gly932-Phe956) groups, these changes were present from mild to moderate intensity having cellular infiltration (Fig. S5). Though different HBc-fused hexon proteins offer protection against the virus to a varying degree, the antibody titres detected against all the three epitopes were similar as judged by ELISA (Fig. 1C), suggesting that only antibodies produced against HBc-fused hexon (Asp348-Phe369) confer protection against viral infection. These findings are in agreement with our bioinformatics analyses which predict Asp348-Phe369 to be a highly antigenic region of FAdV-4 hexon (Table S6). It is worth-noting that lower antibody titres of HBc-hexon epitopes than the commercial vaccine group are due to normalization of the antibody titres to HBc (containing no FAdV-4 hexon epitope; Fig. 1C). Furthermore, the antibody titres of HBc-epitopes determined by coating whole FAdV-4 reveal that

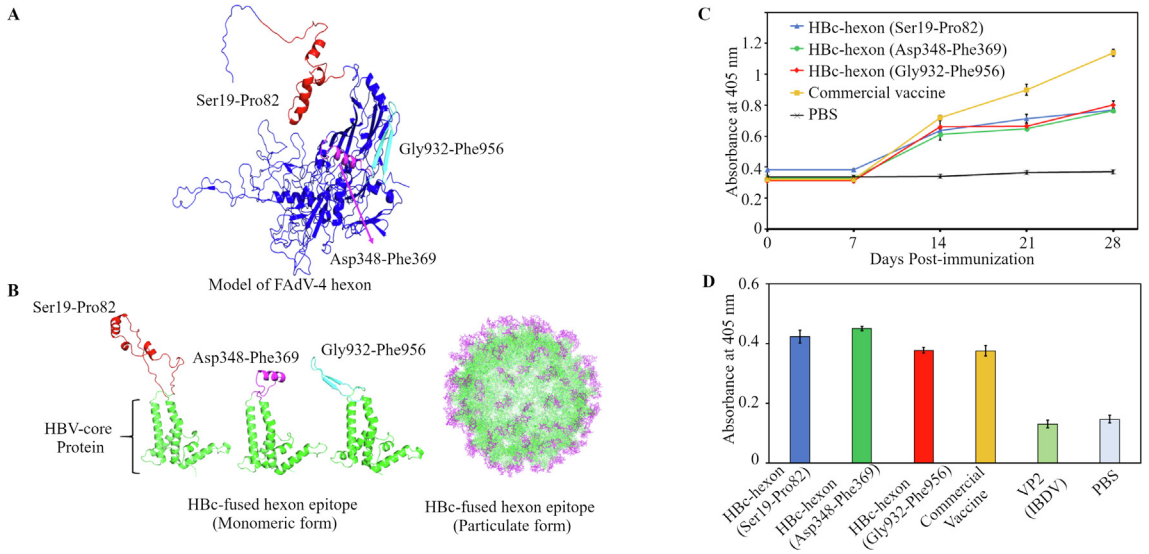

Fig. 1. Models of the FAdV-4 hexon protein, hexon epitopes fused to hepatitis B core protein (HBc) in monomeric and particulate forms, and antibody titres of the serum samples of chickens immunized with HBc-hexon epitopes and commercial vaccine. (A) The predicted structure of the monomeric form of FAdV-4 hexon computed using the hexon protein of FAdV-1 (PDB: 2iny) as a template is shown. In the model, predicted epitopic regions, Ser19-Pro82, Asp348-Phe369, and Gly932-Phe956, are shown in red, purple, and cyan colors, respectively. (B) Models of the epitopic regions of FAdV-4 hexon fused to HBc in the monomeric and particulate forms are depicted. The models were visualized using the PyMOL software (The PyMOL Molecular Graphics System, Version 1.3, Schrödinger, LLC). (C) The antibody titres of the serum samples collected from different groups of chickens injected with Montanide™ ISA71 VG-adjuvanted HBc-fused hexon epitopes and commercial vaccine are shown. Blood serum samples collected at the 7th, 14th, 21st and 28th day post-immunization show equivalent antibody titres in serum samples of chickens immunized with HBc-hexon-based vaccine candidates. The antibody titres in the HBc-hexon vaccinated groups have been normalized to antibody titres to HBc alone (with no FAdV-4 hexon). The commercial vaccine (Angara NIAB) used is a formalin-inactivated vaccine [12]. (D) The antibody titres of the serum samples collected (at the 28th day post-immunization) from different groups of chickens injected with Montanide™ ISA71 VG-adjuvanted HBc-fused hexon epitopes, commercial vaccine, and the VP2 capsid protein of infectious bursal disease virus (IBDV). ELISA assays were performed using purified FAdV-4 as a coating antigen. The antibody titres of HBc-fused hexon epitopes indicate that the immune response elicited is capable of recognizing the hexon epitopes (Ser19-Pro82, Asp348-Phe369, and Gly932-Phe956), suggesting that the hexon epitopes are surface-exposed. In panels (C) and (D), PBS (phosphate buffer saline) was used as a negative control, whereas error bars represent standard deviations from mean values of three independent measurements. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Post-challenge mortality and percent protection in broiler chickens after vaccination.

Treatment	Day of treatment/ injection	Age at challenge (day)	Mortality	Mortality period (days)	Protection (%)
HBc-hexon (Ser19-Pro82) (100 μg) + Montanide™ ISA71 VG-adjuvant (1:1) [Group A]	7th and 21st	28th	3/10	3 to 5	70
HBc-hexon (Asp348-Phe369) (100 μg) + Montanide™ ISA71 VG-adjuvant (1:1) [Group B]	7th and 21st	28th	1/10	4	90
HBc-hexon (Gly932-Phe956) (100 μg) + Montanide™ ISA71 VG-adjuvant (1:1) [Group C]	7th and 21st	28th	6/10	3 to 5	40
Commercial vaccine (Angara NIAB) (300 μL) [Group D]	7th and 21st	28th	5/10	3 to 5	50
PBS (pH 7.9) (500 μL) Challenged [Group E]	7th and 21st	28th	5/10	3 to 5	50
PBS (pH 7.9) (500 μL) Unchallenged [Group F]	7th and 21st	–	0/10	–	–

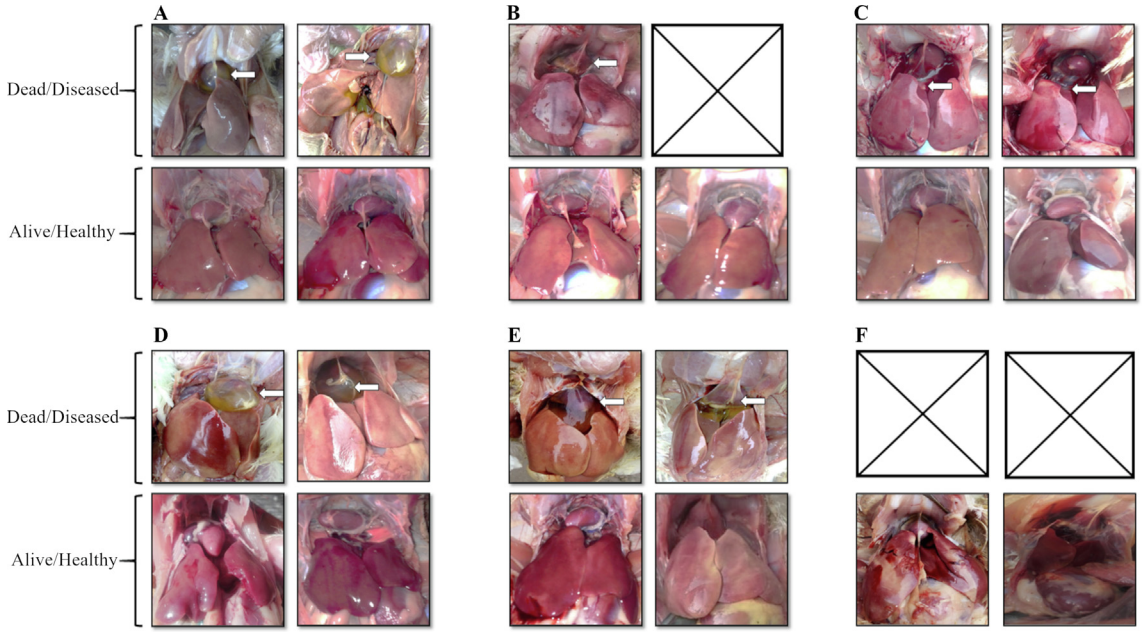

Fig. 2. Post-mortem findings of dead/diseased and alive/healthy chickens after challenge with pathogenic FAdV-4 isolate. Vaccination of chickens with Montanide™ ISA71 VG-adjuvanted HBc-hexon-based vaccine candidates revealed that HBc-hexon (Ser19-Pro82) provided 70% protection (A), HBc-hexon (Asp348-Phe369) conferred the best (90%) protection (B), and HBc-hexon (Gly932-Phe956) provided 40% protection (C) against a pathogenic FAdV-4 isolate. No disease symptoms were observed in healthy chickens after challenge with FAdV-4, whereas dead/diseased chickens showed typical signs and symptoms of the HPS disease such as an enlarged liver and accumulation of a transparent jelly-like or straw-colored fluid in the pericardium (indicated with arrows). Post-mortem findings of the birds injected with the commercial vaccine (positive control) (D) and PBS (negative control) (E) after challenge with FAdV-4. (F) The post-mortem findings of healthy birds injected with PBS that were not challenged with FAdV-4.

the immune response elicited is capable of recognizing hexon epitopes (Fig. 1D), suggesting that the hexon epitopes (Ser19-Pro82, Asp348-Phe369, and Gly932-Phe956) are surface-exposed.

4. Discussion

Adenoviruses cause infectious diseases in vertebrates [1]. Recent years have witnessed frequent outbreaks of industrially important adenoviral diseases which cannot be controlled using commercially available vaccines [16] necessitating the development of novel vaccines which are cost-effective, and induce long-term immunity against adenoviral infections. The genome of all adenoviruses encodes hexon which is a highly immunogenic major capsid protein [2]. To date, none of the hexon-based vaccines provides complete protection against adenoviral infections [5–7]. To this end, we analyzed the hexon protein of a representative adenovirus, FAdV-4, using bioinformatics tools and evaluated the protective efficacy of its epitopic regions in fusion with HBc as vaccine candidates for providing protection against HPS in chickens. As observed in other studies [11,17], all of the recombinant proteins were expressed in the form of inclusion bodies upon expression in the *E. coli* system in the current work, necessitating refolding of the desired proteins. The proteins were successfully refolded on-column by gradually decreasing the concentration of GndCl, yielding the recombinant proteins in sufficient quantities for performing immunization studies in chickens on a small scale.

Importantly, the immunological data presented here reveal that the hexon epitopes are exposed on the surface of FAdV-4, and can be recognized by the host immune system (Fig. 1D). Upon injection in chickens, two out of three HBc-fused epitopes (Ser19-Pro82 and Asp348-Phe369) offered better protection (70% and 90%, respectively) against FAdV-4 infection as compared to the commercial vaccine (50% protection). Lower protection offered by the commercial vaccine could be due to an inadequate vaccine composition or the use of improperly-killed viral strains [10]. It has been observed that formalin treatment often does not lead to complete inactivation of the viral strains or may cause alteration of epitopes which induce neutralizing antibodies [18,19], resulting in lower efficacy of formalin-inactivated vaccines. It is important to note that the loop-1 (L1) region (Met262-His528), of which Asp348-Phe369 is a part, provided 70% protection when used as a subunit vaccine in a previous report [5]. In another study, it has been documented

that the L1 region (Lys74-His367), which harbours Asp348-Phe369 and a part of Ser19-Pro82, provides 27% protection (73% mortality) when employed as a subunit vaccine [6]. In our work, better protection (90%) displayed by the epitopic region (Asp348-Phe369) could be due to a boosted immune response generated upon exposure of the epitopic region at MIR of HBc. Enhanced protection offered by the HBc-fused epitopic region (Asp348-Phe369) is in agreement with our in-silico analyses which suggest that the epitopic region (Asp348-Phe369) could be best presented to the immune system out of the three selected epitopic regions of FAdV-4 hexon (Table S6). Interestingly, structural comparison of FAdV-4 hexon with homologous capsid proteins reveals that regions corresponding to the potent epitopic region (Asp348-Phe369) share a similar conformation across the *Adenoviridae* family (Fig. S1), suggesting that the homologous regions of hexon proteins in other adenoviruses could also provide protection against respective adenoviral infections. In the current study, the third epitopic region (Gly932-Phe956) appeared to be toxic to some extent as the number of survived chickens was less than the negative control group (Table 1; Fig. S6). The analysis of the sequence of this epitopic region by the AllerTOP program [20] suggests that the region could have allergic properties (Table S6), corroborating our experimental findings and suggesting that the protection offered by the other two epitopic regions (Ser19-Pro82 and Asp348-Phe369) is not by chance. Though antibodies against HBc are also produced (Fig. S4) and the antibody titres of HBc-hexon epitopes are equivalent (Fig. 1), the difference in the protective efficacy of hexon epitopes could be due to the induction of different levels of neutralizing antibodies. Efforts are currently underway to upscale the production of the most promising vaccine candidate (HBc-fused Asp348-Phe369) for conducting field trials on a large scale.

5. Conclusions

In this work, we have identified a highly conserved epitopic region on the hexon capsid proteins of adenoviruses. Using hexon from a representative adenovirus, FAdV-4, we have demonstrated that the epitopic region (Asp348-Phe369) in the HBc-fused form provides better protection (up to 90%) against HPS in chickens as compared to the commercially available vaccine. Importantly, the sequence and the structural architecture of this potent epitopic region are similar to that of the corresponding regions in hexon proteins of other adenoviruses, suggesting that the homologous regions in hexon proteins could be employed as epitopic regions for development of vaccine candidates against respective adenoviral infections.

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Ethical statement

This study was conducted according to the guidelines and regulations of the Institutional Animal Care and Use Committee of Animal Sciences Division, Nuclear Institute for Agriculture and Biology, Faisalabad, Pakistan.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2021.05.023.

References

[1] Harrach B, Tarjan ZL, Benko M. Adenoviruses across the animal kingdom: a walk in the zoo. FEBS Lett 2019;593:3660–73. https://doi.org/10.1002/1873-3468.13687.
[2] Wohlfart C. Neutralization of adenoviruses: kinetics, stoichiometry, and mechanisms. J Virol 1988;62:2321–8. https://doi.org/10.1128/JVI.62.7.2321-2328.1988.
[3] Niczyporuk JS. Deep analysis of Loop L1 HVRs1-4 region of the hexon gene of adenovirus field strains isolated in Poland. PLoS ONE 2018;13:e0207668.
[4] Crawford-Miksza L, Schnurr DP. Analysis of 15 adenovirus hexon proteins reveals the location and structure of seven hypervariable regions containing serotype-specific residues. J Virol 1996;70:1836. , http://jvi.asm.org/content/70/3/1836.abstract.
[5] Wang X, Tang Q, Chu Z, Wang P, Luo C, Zhang Y, et al. Immune protection efficacy of FAdV-4 surface proteins fiber-1, fiber-2, hexon and penton base. Virus Res 2018;245:1–6. https://doi.org/10.1016/j.virusres.2017.12.003.
[6] Schachner A, Marek A, Jaskulska B, Bilic I, Hess M. Recombinant FAdV-4 fiber-2 protein protects chickens against hepatitis–hydropericardium syndrome (HHS). Vaccine. 2014;32:1086–92. https://doi.org/10.1016/j.vaccine.2013.12.056.
[7] Hu K, Fu M, Guan X, Zhang D, Deng X, Xiao Y, et al. Penton base induces better protective immune responses than fiber and hexon as a subunit vaccine candidate against adenoviruses. Vaccine 2018;36:4287–97. https://doi.org/10.1016/j.vaccine.2018.05.118.
[8] Roose K, De Baets S, Schepens B, Saelens X. Hepatitis B core-based virus-like particles to present heterologous epitopes. Expert Review of Vaccines. 2013;12:183–98. https://doi.org/10.1586/erv.12.150.
[9] Chen Z, Shi S, Qi B, Lin S, Chen C, Zhu C, et al. Hydropericardium syndrome caused by fowl adenovirus serotype 4 in replacement pullets. The. J Vet Med Sci 2019;81:245–51. https://doi.org/10.1292/jvms.18-0168.
[10] Aziz F, Tufail S, Shah MA, Salahuddin Shah M, Habib M, Mirza O, et al. In silico epitope prediction and immunogenic analysis for penton base epitope-focused vaccine against hydropericardium syndrome in chicken. Virus Res 2019;273:197750. https://doi.org/10.1016/j.virusres.2019.197750.
[11] Malik IR, Chen A, Brass A, Ahlen G, Rahman M, Sallberg M, et al. A bi-functional hepatitis B virus core antigen (HBcAg) chimera activates HBcAg-specific T cells and preS1-specific antibodies. Scand J Infect Dis 2012;44:55–9. https://doi.org/10.3109/00365548.2011.608711.
[12] Salahuddin M, Chaudhry T, Yaqoob N, Ashraf A, Khaliq S, Shah F. Immunogenicity of NIAB Angara vaccine in broilers. Int J Biol Biotech 2006;3:599–601.
[13] Wilson I, Gamble M. The hematoxylins and eosin. Theory and Practice of Histological Techniques. London: Churchill Livingston; 2002. p. 125–38.
[14] Doytchinova IA, Flower DR. VaxiJen: a server for prediction of protective antigens, tumour antigens and subunit vaccines. BMC Bioinf 2007;8:4. https://doi.org/10.1186/1471-2105-8-4.
[15] Ponomarenko J, Bui H-H, Li W, Fusseder N, Bourne PE, Sette A, et al. ElliPro: a new structure-based tool for the prediction of antibody epitopes. BMC Bioinf 2008;9:514. https://doi.org/10.1186/1471-2105-9-514.
[16] Pan Q, Yang Y, Gao Y, Qi X, Liu C, Zhang Y, et al. An inactivated novel genotype fowl adenovirus 4 protects chickens against the hydropericardium syndrome that recently emerged in China. Viruses. 2017;9:216. , https://www.mdpi.com/1999-4915/9/8/216.
[17] Xue L, Liu J, Wang Q, Zhang C, Xu L, Luo J, et al. Purification and assembling a fused capsid protein as an enterovirus 71 vaccine candidate from inclusion bodies to pentamer-based nanoparticles. Biochem Eng J 2017;117:139–46. https://doi.org/10.1016/j.bej.2016.10.009.
[18] Sanders B, Koldijk M, Schuitemaker H. Inactivated viral vaccines. In: Nunnally BK, Turula VE, Sitrin RD, editors. Vaccine Analysis: Strategies, Principles, and Control. Springer, Berlin Heidelberg: Berlin, Heidelberg; 2015. p. 45–80.
[19] Murphy BR, Walsh EE. Formalin-inactivated respiratory syncytial virus vaccine induces antibodies to the fusion glycoprotein that are deficient in fusion-inhibiting activity. J Clin Microbiol 1988;26:1595. , http://jcm.asm.org/content/26/8/1595.abstract.
[20] Dimitrov I, Bangov I, Flower DR, Doytchinova I. AllerTOP v.2-a server for in silico prediction of allergens. J Mol Model 2014;20:2278. https://doi.org/10.1007/s00894-014-2278-5.