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BRIEF REPORT

Investigating endothelial cell transduction and hexon: platelet factor 4 binding of ChAdOx1 in the context of vaccine-induced thrombotic thrombocytopenia

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Abstract

Background: Vaccines against SARS-CoV-2 have been essential in controlling COVID-19–related mortality and have saved millions of lives. Adenoviral (Ad)-based vaccines have been an integral part of this vaccine campaign, with licensed vaccines based on the simian Y25 isolate (Vaxzevria; AstraZeneca) and human Ad type 26 (Jcovden; Janssen) widely adopted. As part of the largest global vaccination program ever undertaken, cases of vaccine-induced thrombotic thrombocytopenia have been described in approximately 1:200 000 vaccinees administered with Ad-based SARS-CoV-2 vaccines.

Objectives: The mechanism underpinning these adverse events remains to be completely delineated but is characterized by elevated autoantibodies against platelet factor 4 (PF4), which, when complexed with PF4, cluster and bind FcγRIIa on platelets and induce thrombus formation. Here, we investigated the ability of ChAdOx1 to transduce and activate endothelial cells (ECs).

Methods: Using protein sequence alignment tools and *in vitro* transduction assays, the ability of ChAdOx1 to infect ECs was assessed. Furthermore, the ability of ChAdOx1 infection to activate ECs was determined. Finally, using surface plasmon resonance, we assessed the electrostatic interactions between the ChAdOx1 hexon and PF4.

Results: Despite lacking the primary cell entry receptor, coxsackie and adenovirus receptor, ChAdOx1 efficiently transduced ECs in a coxsackie and adenovirus receptor-independent manner. This transduction did not result in EC activation. Purified hexon protein from ChAdOx1 preparations did, however, bind PF4 with a similar affinity to that previously reported for the whole ChAdOx1 capsid.

Conclusion: These data confirm the need to develop non-PF4 binding Ad capsids and assess their potential to mitigate adverse events associated with vaccine-induced thrombotic thrombocytopenia.

KEYWORDS
adenovirus, endothelial cell, PF4, vaccine, VITT

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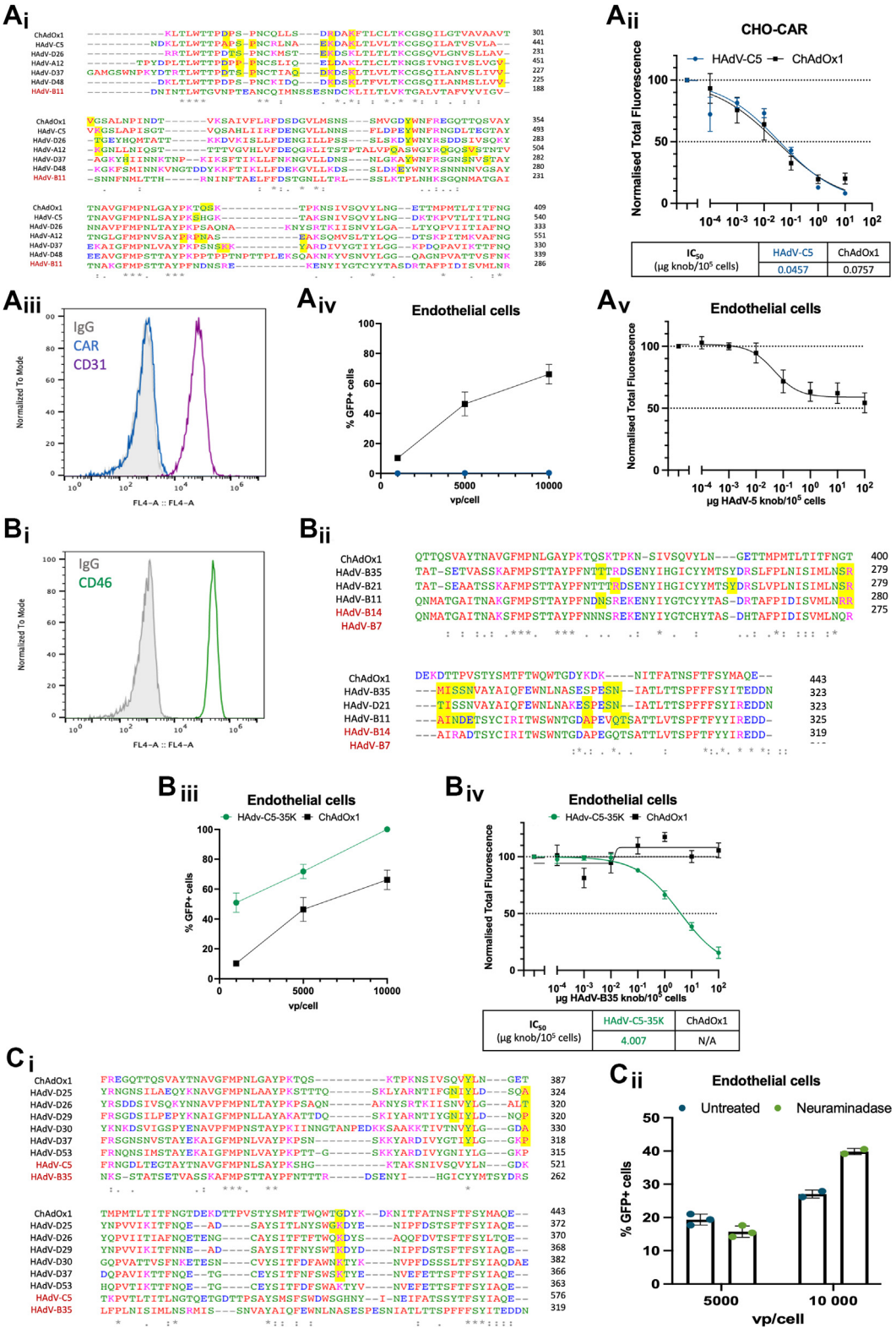

FIGURE 1 ChAdOx1 infects CAR-negative endothelial cells. (Ai) Clustal Omega sequence alignment of the ChAdOx1 fiber knob protein with other known CAR-binding HAdV fiber knob sequences [6]. Known CAR-binding residues are highlighted in yellow and demonstrate that ChAdOx1 shares many interacting residues with other CAR-binding adenoviruses. HAdV-B11 does not bind CAR. (Aii) Competitive inhibition studies in Chinese hamster ovary (CHO)-CAR cells, which express CAR but no other known primary HAdV receptors. Increasing concentrations of recombinant HAdV-C5 knob protein inhibited infection of the CHO-CAR cells by HAdV-C5-GFP and ChAdOx1-GFP, suggesting that ChAdOX1 uses CAR as a primary cell entry receptor. (Aiii) Immortalized human vascular endothelial cells are positive for CD31, a marker of endothelial cells, and negative for CAR. (Aiv) Transduction assays with HAdV-C5 demonstrated no infection, even at 10 000 vp/cell, yet ChAdOx1-GFP demonstrated infection of endothelial cells, suggesting the use of an unidentified cell entry receptor in the absence of CAR. (Av) Transduction of endothelial cells with ChAdOx1 was partially inhibited by HAdV-C5 fiber knob protein, suggesting that the predominant mechanism by which ChAdOx1 is infecting endothelial cells is independent of HAdV-C5. (Bi) Endothelial cells are positive for CD46, a primary

1 | INTRODUCTION

The ChAdOx1 nCoV-19 vaccine (Vaxzevria; AstraZeneca), derived from chimpanzee adenovirus Y25 [1], is credited with saving millions of lives during the COVID-19 pandemic. However, vaccine-induced thrombotic thrombocytopenia (VITT) was identified as an ultrarare but serious adverse effect of adenoviral (Ad) vector-based COVID-19 vaccines [2]. VITT resembles heparin-induced thrombocytopenia, a severe complication caused by the formation of antibodies against platelet factor 4 (PF4). Patients present with severe thrombocytopenia and thrombosis at uncommon sites, including the cerebral venous sinus, 5 to 24 days post-vaccination [3]. Existing evidence suggests PF4 forms an electrostatic interaction with the negatively charged ChAdOx1 viral capsid within the interhexon space, inducing autoimmune anti-PF4 responses and production of anti-PF4 antibodies [4]. These antibodies may then form PF4 immune complexes, which bind Fc receptors on platelets and neutrophils, resulting in activation and thrombus formation [5].

VITT patients present with signs of endothelial activation, yet role of the endothelium in VITT has not been described [6]. Investigations of ChAdOx1 vector-host interactions are critical for improving our understanding of how these rare adverse events occur; therefore, we assessed the direct effects of ChAdOx1 infection on endothelial cells (ECs). We demonstrate that ChAdOx1 transduces ECs despite the absence of it's primary entry receptor coxsackie and adenovirus receptor (CAR), but that this does not induce EC activation, suggesting that direct infection of ECs by ChAdOx1 does not contribute to EC activation in VITT patients.

Using surface plasmon resonance (SPR), we confirm PF4 binding to purified ChAdOx1 hexon protein, further supporting the finding that VITT pathogenesis may result from electrostatic interactions between PF4 and ChAdOx1.

2 | MATERIALS AND METHODS

2.1 | Cell lines

Chinese hamster ovary (CHO)-CAR and CHO-BC1 cells expressing CAR and CD46, respectively, and immortalized human vascular ECs (HUVECs) were maintained in Dulbecco's Modified Eagle Medium-F12 medium (Gibco) supplemented with 10% fetal bovine serum, 1% L-glutamine, and 2% penicillin and streptomycin.

2.2 | Viruses

ChAdOx1-green fluorescent protein (GFP), ChAdOx1-S, human adenovirus type 5 (HAdV-C5), HAdV-C5-26K, and HAdV-C5-35K were propagated and purified using the CsCl gradient method [4].

2.3 | Cell receptor staining

Cells were stained for receptors using primary CAR RmcB (Millipore, 05-644; 1:500), CD46 (GeneTex, MEM-258; 1:100), and CD31 (BioLegend, 303101; 1:100) antibodies and Alexa Fluor 647-conjugated secondary antibody (Thermo Fisher Scientific, A21237; 1:1000), then fixed in 4% paraformaldehyde, and staining was detected using a BD Accuri C6 cytometer. Analysis was performed in FlowJo.

2.4 | Fiber knob protein alignment

Sequences for HAdV fiber knob proteins were obtained from National Centre for Biotechnology Information and aligned using Clustal Omega [7].

2.5 | Generation of recombinant fiber knob proteins

Recombinant fiber knob proteins were produced as previously described [4].

2.6 | Competitive inhibition assays with recombinant fiber knob proteins

Competitive inhibition assays with recombinant fiber knob proteins were performed in CHO-CAR cells and ECs, as previously described [4].

cell entry receptor for some species of B and D adenoviruses. (Bii) ChAdOx1 fiber knob protein sequences were aligned with other known CD46-binding HAdVs using Clustal Omega [7]. ChAdOx1 shared no CD46 binding residues, suggesting ChAdOx1 is not using CD46 as a cell entry receptor. HAdV-B14/B7 does not bind CD46. (Biii) Competitive inhibition studies in CHO-BC1 cells, which express CD46 but no other known primary HAdV receptors. Transduction of CHO-BC1 cells with HAdV-C5-35K-GFP (pseudotyped HAdV-C5 with HAdv-B35 knob protein) was inhibited by preincubation of the cells with recombinant HAdV-B35 fiber knob protein (Half maximal inhibitory concentration (IC_{50}) 0.375 μg/10^5 cells). (Biv) HAdV-C5-35K and ChAdOx1 transduced 100% and 46% of endothelial cells, respectively. (Bv) Transduction of endothelial cells with HAdV-C5-35K-GFP could be inhibited by preincubation with recombinant HAdV-B35 fiber knob protein (IC_{50} = 1.043 μg/10^5 cells), whereas ChAdOx1 transduction was not inhibited, suggesting ChAdOx1 does not use CD46 to enter cells. (Ci) Using Clustal Omega sequence alignment [7], the sequence of the ChAdOx1 fiber knob protein was compared with other known sialic acid-binding species of D HAdVs. Sialic acid interacting residues are shown in yellow boxes. ChAdOx1 shared a tyrosine residue (Y381) in the sialic acid-binding regions identified in HAdV-D26, HAdV-D29, HAdV-D30, HAdV-D37, and HAdV-D53. (Cii) Pretreatment of endothelial cells with neuraminidase did not decrease ChAdOx1 transduction at 5000 and 10 000 vp/cell, suggesting ChAdOx1 uses another cell entry mechanism. CAR, coxsackie and adenovirus receptor; GFP, green fluorescent protein; HAdV, human adenovirus; IC_{50}, half maximal inhibitory concentration; IgG, immunoglobulin G; N/A, not available; VP, virus particle.

2.7 | Viral transduction assay

A total of 2.5×10^4 cells/well were transduced with viruses in serum-free medium for 3 hours. Virus was removed and replaced with complete medium for 45 hours. For GFP transduction, cells were washed, fixed, and analyzed as described [4]. Luciferase transduction was analyzed using Luciferase Assay System Kit (Promega), normalized to protein concentration. Luciferase was quantified using a BioTek Cytation 4 plate reader (Agilent Technologies).

2.8 | Neuraminidase assay

Neuraminidase transduction assays were performed as above, with seeded cells treated with 50 mU/mL neuraminidase A for 1 hour at 37 °C before transduction for 1 hour on ice.

2.9 | Thrombosis assay

ECs were transduced as above. Supernatants were collected 6, 12, 24, and 48 hours postinfection, centrifuged to remove debris, and EC production of factor (F)IX, interleukin (IL)-6, IL-8, plasminogen activator inhibitor-1, P-selectin, P-selectin glycoprotein ligand, sCD40L, and tissue factor was quantified using the LEGENDplex Human Thrombosis Panel (BioLegend) as per manufacturer's instructions.

2.10 | Hexon purification

Adenovirus hexons were propagated and purified using the CsCl gradient method [4] with the modification of extracting the top band containing the empty capsid rather than the lower band containing complete virions. Anion exchange was performed using an ÄKTA fast protein liquid chromatography (Cytiva) and 5 mL HiTrap Q FF columns (Cytiva). Using a 5 mL loop, the extracted band was loaded onto the column, and 4 column volumes of buffer A (20 mM Hepes, pH 7.4) were run across the column. Hexons were eluted using a linear gradient over approximately 10 column volumes of buffer B (20 mM Hepes, pH 7.4, 1 M NaCl). Fractions were collected, and those corresponding to hexon-sized peaks were run on a gel and stained with Coomassie blue (Thermo Fisher Scientific) to identify the fractions containing hexon protein. Fractions containing hexons were then concentrated with a 50 kDa molecular weight cut off (Amicon) centrifugal filter to a final volume of 1 mL, and size exclusion chromatography was performed with a Superose increase 10/300 GL size exclusion chromatography column (Cytvia) on an ÄKTA fast protein liquid chromatography (Cytvia). The column was equilibrated with gel filtration buffer (20 mM Hepes, pH 7.4, 150 mM NaCl), and the concentrated fractions were loaded using a 1 mL loop. Buffer was then run at 0.5 mL/min, and samples were collected from 10 to 25 mL, corresponding to the hexon peak.

2.11 | SPR

All SPR experiments were performed at 25 °C in 10 mM phosphate buffer, pH 7.4, 140 mM NaCl, and 0.27 mM KCl running buffer. Data were collected with a Biacore T200 instrument (Cytiva Life Sciences) at a rate of 1 Hz. Hexons were coupled to the CM5 sensor chip by amine coupling reactions with an immobilization density of approximately 1000 resonance units. PF4 was serially diluted in running buffer (0.125 mM, 0.25 mM, 0.5 mM, 1 mM, and 2 mM) and then injected onto the surfaces. Blank samples contained only running buffer. After each cycle, the biosensor surface was regenerated with a 60-second pulse of 10 mM Tris-glycine (pH 1.5) at a flow rate of 30 μL/min.

2.12 | Statistical analysis

Results are presented as mean ± SD. Half maximal inhibitory concentration (IC_{50}) curves were calculated and fitted by nonlinear regression. All statistics and graphs were generated using GraphPad Prism.

3 | RESULTS AND DISCUSSION

3.1 | Determining the mechanism by which ChAdOx1 infects CAR-negative ECs

The primary virus-cell interaction during infection is mediated by the adenovirus fiber knob, where CAR is a high-affinity ChAdOx1 receptor [4]. Alignment of ChAdOx1 fiber knob with other CAR-binding adenoviruses [8–10] identified shared contacts (Figure 1A_i). The use of CAR as a high-affinity ChAdOx1 fiber knob receptor was confirmed through competitive inhibition assays with HAdV-C5 and ChAdOx1, demonstrating an IC_{50} of 0.0457 and 0.0757 μg/10^5 cells, respectively (Figure 1A_{ii}). Cross-species utilization of CAR has been reported previously for canine adenoviruses [11].

CAR regulates mechanotransduction, including maintenance and modulation of vascular function in ECs [12]. CAR expression was undetectable on ECs (Figure 1A_{iii}), and HAdV-C5 could not transduce these cells, even at 10 000 vp/cell (Figure 1A_{iv}). However, transduction assays with ChAdOx1.GFP demonstrated efficient transduction, suggesting use of an unidentified cell entry receptor (Figure 1A_{iv}). Competitive inhibition using HAdV-C5 fiber knob demonstrated that the HAdV-C5 fiber knob protein does not effectively block ChAdOx1 infection (Figure 1A_v). ECs downregulate CAR expression in the presence of inflammatory cytokines, suggesting a mechanism by which ECs can decrease Ad infection at sites of inflammation [13]. Therefore, the ability of ChAdOx1 to infect ECs in the absence of CAR suggests that direct infection of ECs could contribute to EC activation in VITT.

CD46 is a primary attachment receptor for species B adenoviruses. EC CD46 expression was high (98.6% positive cells; median fluorescence intensity, 6534; Figure 1B_i); however, ChAdOx1 fiber knob does not share CD46 binding residues for species B

adenoviruses [10,14,15] (Figure 1B$_{ii}$). Competitive inhibition assays in ECs using HAdV-C5 adenovirus pseudotyped with the fiber knob from HAdV-B35 (HAdV-C5-35K) and ChAdOx1 demonstrated HAdV-B35K inhibits EC transduction of HAdV-C5-35K (IC_{50} = 4.007 μg/10^5 cells) but not ChAdOx1 (Figure 1B$_{iii,\ iv}$), suggesting ChAdOx1 does not engage CD46 to transduce ECs (Figure 1B$_v$).

Sialic acids (SAs) are primary receptors for HAdV-Ds [16]. ChAdOx1 fiber knob sequences were aligned with Clustal Omega (Figure 1C$_i$). Y381 (ChAdOx1), conserved across all sequences, is a key interacting residue for SA binding across HAdV-Ds; in addition, T386 was shared with HAdV-D26. However, a key lysine residue of the SA binding pocket shared by HAdV-Ds is glycine (G420) in ChAdOx1. To investigate ChAdOx1's ability to engage SA, neuraminidase was used to remove cellular SA [16]. In ECs, neuraminidase treatment did not decrease ChAdOx1 transduction at either viral concentration tested, suggesting ChAdOx1 does not use SA to enter cells (Figure 1C$_{ii}$).

The ability of ChAdOx1 to transduce ECs suggests a dual tropism where ChAdOx1 can transduce cells in the absence of CAR. Dual tropisms have been demonstrated in other HAdVs, such as the use of both CAR and SA by HAdV-Ds [16,17]. Although this mechanism is still unclear, ChAdOx1 may utilize integrins to infect CAR-negative ECs. Following attachment of HAdVs to cellular receptors, $\alpha_v\beta3$ and $\alpha_v\beta_5$ integrins

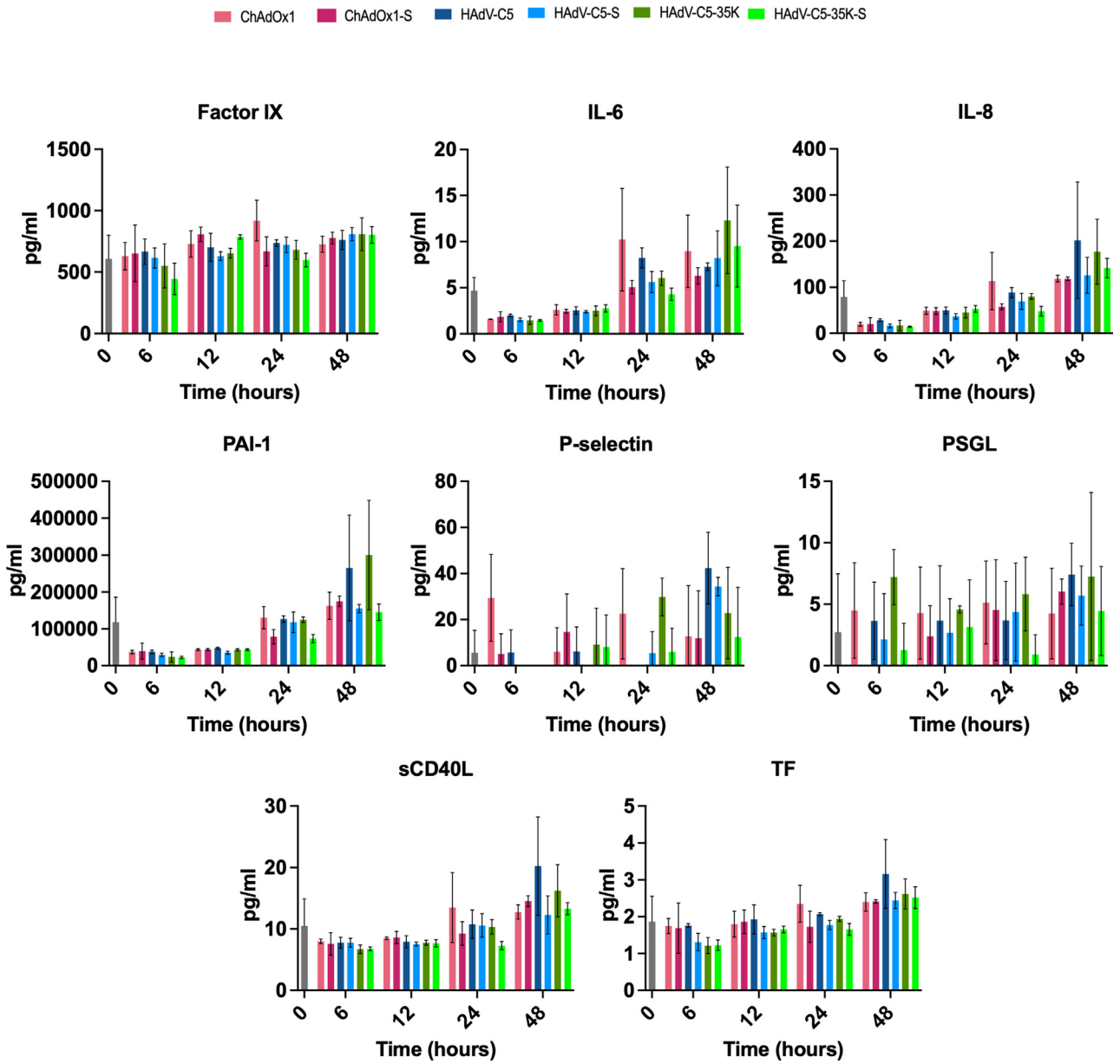

FIGURE 2 ChAdOx1 infection does not induce endothelial cell (EC) activation. Activated ECs release procoagulants that contribute to thrombosis formation. ECs were infected with ChAdOx1, ChAdOx1-spike(S), HAdV-C5, HAdV-C5-S, HAdV-C5-35K, and HAdV-C5-35K-S, and the supernatants from infected cells were collected and analyzed for EC thrombosis markers using the LEGENDplex Human Thrombosis Panel. Infection with all viruses did not significantly increase EC production of any of these markers compared with uninfected cells (0 hours: gray bar), suggesting that EC activation in vaccine-induced thrombotic thrombocytopenia is ChAdOx1- and spike protein-independent. HAdV-C5, human adenovirus type 5; IL, interleukin; PAI-1, plasminogen activator inhibitor-1; PSGL, P-selectin glycoprotein ligand; TF, tissue factor.

facilitate viral uptake through interaction of the penton base RGD motif. $\alpha_v\beta_3$ integrin is necessary for efficient infection of epithelial cells by HAdV-D26 [18], suggesting ChAdOx1 infection may similarly be integrin-mediated. Alternatively, ChAdOx1 could engage heparan sulfate proteoglycans; HAdV-C2, HAdV-C5, HAdV-B3, and HAdV-B35 all demonstrate low-affinity interactions with heparan sulfate proteoglycans, functioning as alternative receptors for infection [19,20]. Furthermore, other direct mechanisms of interaction exist between the ChAdOx1 capsid and the cell surface, such as the hexon-CD46 interaction demonstrated in HAdV-D56 [21]. Investigation into the mechanism utilized by ChAdOx1 to transduce ECs is required, and whether this interaction would be sufficient for productive infection of ECs *in vivo*.

3.2 | ChAdOx1 does not induce EC activation

During endothelial dysfunction, ECs become activated, releasing procoagulants that contribute to thrombosis generation. EC activation has been demonstrated in VITT patients, and addition of VITT patient serum to ECs increased P-selectin expression and platelet adhesion, suggesting EC activation contributes to VITT pathogenesis [22]. Furthermore, viral infection induces EC activation [23]. The ability of ChAdOx1 to induce EC activation was quantified by measurement of prothrombotic factors in the serum of transduced ECs (Figure 2). Transduction with ChAdOx1, HAdV-C5, and HAdV-C5-B35K did not significantly increase EC production of FIX, IL-6, IL-8, plasminogen activator inhibitor-1, P-selectin, P-selectin glycoprotein ligand, sCD40L, and tissue factor compared with uninfected cells. Furthermore, expression of SARS-CoV-2 spike protein did not increase EC activation, further supporting that the activated EC phenotype in VITT patients is an indirect effect of ChAdOx1. The inability of direct ChAdOx1 infection of ECs to induce thromboinflammation was further demonstrated in a recent paper, which showed that ChAdOx1 infection of HUVECs prior to blood perfusion did not increase platelet, fibrin, or neutrophil accumulation [22].

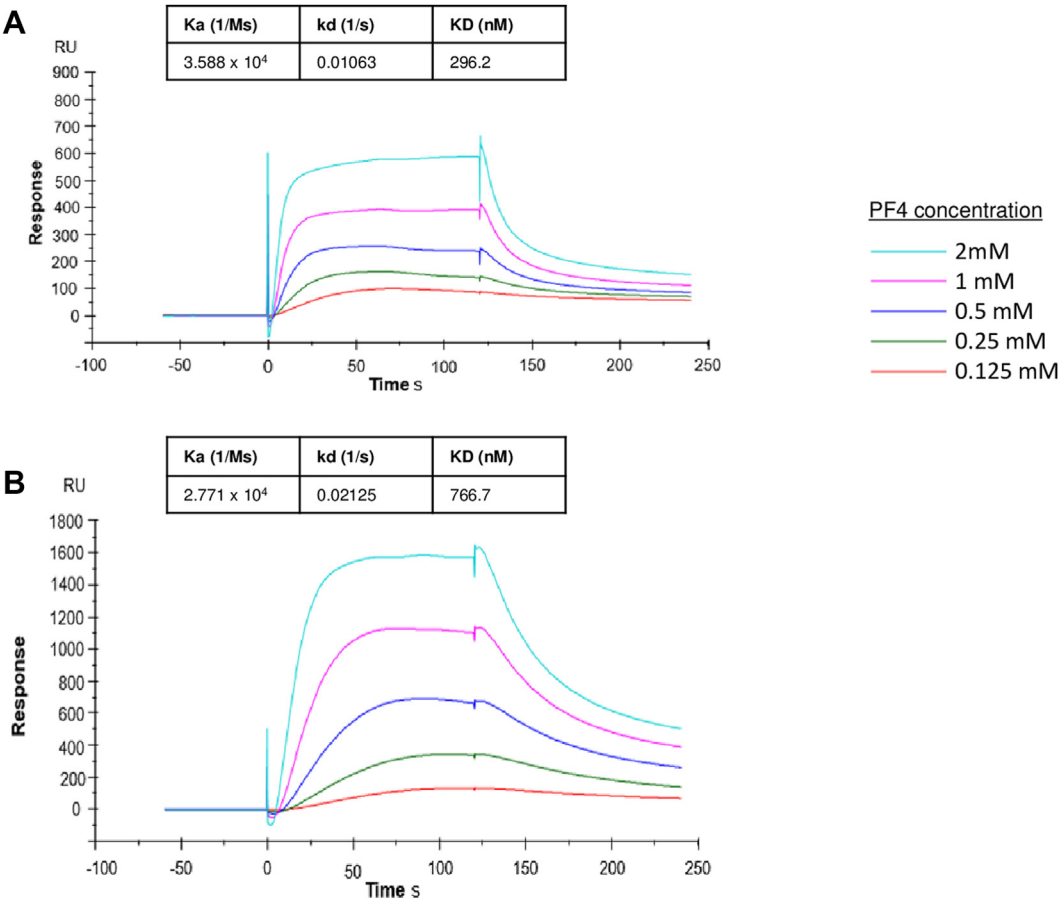

FIGURE 3 Adenovirus hexon proteins bind platelet factor 4 (PF4). Surface plasmon resonance analysis using PF4 at increasing concentrations over immobilized (A) ChAdOx1 and (B) HAdV-C5 hexon proteins demonstrates that ChAdOx1 hexon has a greater affinity for PF4 than HAdV-C5, as previously reported for pure ChAdOx1 and Vaxzervia. This further supports that electrostatic interactions between PF4 and the ChAdOx1 viral capsid contribute to vaccine-induced thrombotic thrombocytopenia pathogenesis. However, the orientation of purified hexon protein may expose regions that would not be surface-exposed on intact virions, suggesting such interactions could be nonspecific, charge-dependent interactions. HAdV-C5, human adenovirus type 5; Ka, association constant; kd, 1/s dissociation rate constant; KD, dissociation constant.

3.3 | Adenovirus hexon proteins bind PF4

Using SPR, we previously demonstrated that PF4 binds with nanomolar affinity to pure ChAdOx1 (dissociation constant [KD], 616 nM) and Vaxzevria preparations (KD, 514 nM), as well as to purified HAdV-D26, the platform of the Johnson and Johnson SARS-CoV-2 vaccine, Jcovden [4]. These similar affinities confirm the interaction between the virus and PF4 rather than cell line-derived impurities in the vaccine [24]. A recent publication cast doubt on these findings, indicating that low pH acid washing during regeneration cycles caused increased PF4 binding, suggestive of low pH compromising Ad capsid stability, exposing negatively charged DNA, and promoting electrostatic binding of positively charged PF4 protein to viral DNA [25]. To address this concern, we generated purified preparations of Ad hexon protein and performed SPR studies to assess direct PF4-hexon protein interactions in the absence of viral DNA. SPR analysis using immobilized purified hexon protein from ChAdOx1 demonstrated a greater affinity (KD, 296.2 nM) for PF4 than that observed for both purified ChAdOx1 and Vaxzevria (Figure 3A) and higher affinity than for HAdV-C5 hexon (KD, 766.7 nM). The orientation of purified hexon protein exposes regions that would otherwise not be surface-exposed on intact virions, suggesting such interactions could be nonspecific, charge-dependent interactions. This 2.2-fold increase in affinity is within a similar range but is unlikely to be statistically different. Collectively, these data support direct electrostatic PF4 interactions with the ChAdOx1 viral capsid as the initiating event for VITT pathogenesis.

4 | CONCLUSIONS

The ChAdOx1 nCoV-19 vaccine is safe and efficacious in protecting against symptomatic COVID-19 infection. Despite ultrarare occurrence of VITT, Ad vector-based vaccines remain vital worldwide. This study demonstrates that ChAdOx1 transduces CAR-negative ECs, but this is unlikely to contribute to the pathogenesis of VITT. Furthermore, the occurrence of VITT symptoms 5 to 24 days postvaccination is consistent with anti-PF4 memory B cell responses. While HUVECs are a common cell line for EC research, we do recognize the limitations of using cells from 1 vascular bed, and further investigations with different EC types, particularly those derived from the splanchnic veins and the cerebral venous sinus, would be favorable. Furthermore, we determine clear binding kinetics of PF4 to the purified ChAdOx1 hexon protein, confirming the interactions detected between PF4 and Ad vaccine vectors are not the result of low pH capsid destabilization, resulting in exposure of viral DNA. To develop safer Ad vaccines for the future, the focus should be on modification of the vector to prevent PF4 binding or identification of adenoviruses that do not bind PF4.

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AUTHOR CONTRIBUTIONS

C.L. designed and conducted experiments, analyzed data, and wrote the manuscript; L.F. designed and conducted experiments, analyzed data, and reviewed the manuscript; E.A.S. generated recombinant fiber knob proteins and reviewed and edited the manuscript; R.M.M. conducted experiments and reviewed and edited the manuscript; A.L.P. designed experiments, acquired funding, supervised the study, and wrote the manuscript.

DECLARATION OF COMPETING INTERESTS

A.L.P. is founder and Chief Scientific Officer of Trocept Therapeutics Ltd. All other authors declare no conflict of interest.

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REFERENCES

[1] Voysey M, Clemens SAC, Madhi SA, Weckx LY, Folegatti PM, Aley PK, Angus B, Baillie VL, Barnabas SL, Bhorat QE, Bibi S, Briner C, Cicconi P, Collins AM, Colin-Jones R, Cutland CL, Darton TC, Dheda K, Duncan CJA, Emary KRW, et al. Safety and efficacy of the ChAdOx1 nCoV-19 vaccine (AZD1222) against SARS-CoV-2: an interim analysis of four randomized controlled trials in Brazil, South Africa, and the UK. *Lancet*. 2021;397:99–111.

[2] Roytenberg R, García–Sastre A, Li W. Vaccine-induced immune thrombotic thrombocytopenia: what do we know hitherto? *Front Med (Lausanne)*. 2023;10:1155727. https://doi.org/10.3389/fmed.2023.1155727

[3] Makris M, Pavord S, Lester W, Scully M, Hunt B. Vaccine-induced immune thrombocytopenia and thrombosis (VITT). *Res Pract Thromb Haemost*. 2021;5:e12529. https://doi.org/10.1002/rth2.12529

[4] Baker AT, Boyd RJ, Sarkar D, Teijeira-Crespo A, Chan CK, Bates E, Waraich K, Vant J, Wilson E, Truong CD, Lipka–Lloyd M, Fromme P, Vermaas J, Williams D, Machiesky L, Heurich M, Nagalo BM, Coughlan L, Umlauf S, Chiu PL, et al. ChAdOx1 interacts with CAR and PF4 with implications for thrombosis with thrombocytopenia syndrome. *Sci Adv*. 2021;7:eabl8213. https://doi.org/10.1126/sciadv.abl8213

[5] Buka RJ, Montague SJ, Moran LA, Martin EM, Slater A, Watson SP, Nicolson PLR. PF4 activates the C-Mpl-Jak2 pathway in platelets. *Blood*. 2024;143:64–9.

[6] De Michele M, Piscopo P, Crestini A, Rivabene R, D'Amati G, Leopizzi M, Stefanini L, Pulcinelli F, Chistolini A, Iacobucci M, Schiavo OG, Berto I, Nicolini E, Petraglia L, Di Mascio MT, Toni D. Vaccine-induced immune thrombotic thrombocytopenia: a possible pathogenic role of ChAdOx1 nCoV-19 vaccine-encoded soluble SARS-CoV-2 spike protein. *Haematologica*. 2022;107:1687–92.

[7] Madeira F, Pearce M, Tivey ARN, Basutkar P, Lee J, Edbali O, Madhusoodanan N, Kolesnikov A, Lopez R. Search and sequence

analysis tools services from EMBL-EBI in 2022. *Nucleic Acids Res*. 2022;50:W276–9.

[8] Bewley MC, Springer K, Zhang YB, Freimuth P, Flanagan JM. Structural analysis of the mechanism of adenovirus binding to its human cellular receptor, CAR. *Science*. 1999;286:1579–83.

[9] Seiradake E, Lortat–Jacob H, Billet O, Kremer EJ, Cusack S. Structural and mutational analysis of human Ad37 and canine adenovirus 2 fiber heads in complex with the D1 domain of coxsackie and adenovirus receptor. *J Biol Chem*. 2006;281:33704–16.

[10] Baker AT, Greenshields–Watson A, Coughlan L, Davies JA, Uusi–Kerttula H, Cole DK, Rizkallah PJ, Parker AL. Diversity within the adenovirus fiber knob hypervariable loops influences primary receptor interactions. *Nat Commun*. 2019;10:741.

[11] Soudais C, Boutin S, Hong SS, Chillon M, Danos O, Bergelson JM, Boulanger P, Kremer EJ. Canine adenovirus type 2 attachment and internalization: coxsackievirus-adenovirus receptor, alternative receptors, and an RGD-independent pathway. *J Virol*. 2000;74:10639–49.

[12] Chung J, Kim KH, An SH, Lee S, Lim BK, Kang SW, Kwon K. Coxsackievirus and adenovirus receptor mediates the responses of endothelial cells to fluid shear stress. *Exp Mol Med*. 2019;51:1–15.

[13] Vincent T, Pettersson RF, Crystal RG, Leopold PL. Cytokine-mediated downregulation of coxsackievirus-adenovirus receptor in endothelial cells. *J Virol*. 2004;78:8047–58.

[14] Persson BD, Reiter DM, Marttila M, Mei YF, Casasnovas JM, Arnberg N, Stehle T. Adenovirus type 11 binding alters the conformation of its receptor CD46. *Nat Struct Mol Biol*. 2007;14:164–6.

[15] Cupelli K, Müller S, Persson BD, Jost M, Arnberg N, Stehle T. Structure of adenovirus type 21 knob in complex with CD46 reveals key differences in receptor contacts among species B adenoviruses. *J Virol*. 2010;84:3189–200.

[16] Mundy RM, Baker AT, Bates EA, Cunliffe TG, Teijeira–Crespo A, Moses E, Rizkallah PJ, Parker AL. Broad sialic acid usage amongst species D human adenovirus. *Npj Viruses*. 2023;1:1.

[17] Baker AT, Mundy RM, Davies JA, Rizkallah PJ, Parker AL. Human adenovirus type 26 uses sialic acid-bearing glycans as a primary cell entry receptor. *Sci Adv*. 2019;5:eaax3567. https://doi.org/10.1126/sciadv.aax3567

[18] Nestić D, Uil TG, Ma J, Roy S, Vellinga J, Baker AH, Custers J, Majhen D. αvβ3 integrin is required for efficient infection of epithelial cells with human adenovirus type 26. *J Virol*. 2018;93:e01474–18.

[19] Dechecchi MC, Melotti P, Bonizzato A, Santacatterina M, Chilosi M, Cabrini G. Heparan sulfate glycosaminoglycans are receptors sufficient to mediate the initial binding of adenovirus types 2 and 5. *J Virol*. 2001;75:8772–80.

[20] Tuve S, Wang H, Jacobs JD, Yumul RC, Smith DF, Lieber A. Role of cellular heparan sulfate proteoglycans in infection of human adenovirus serotype 3 and 35. *PLOS Pathog*. 2008;4:e1000189. https://doi.org/10.1371/journal.ppat.1000189

[21] Persson BD, John L, Rafie K, Strebl M, Frängsmyr L, Ballmann MZ, Mindler K, Havenga M, Lemckert A, Stehle T, Carlson LA, Arnberg N. Human species D adenovirus hexon capsid protein mediates cell entry through a direct interaction with CD46. *Proc Natl Acad Sci U S A*. 2021;118:e2020732118. https://doi.org/10.1073/pnas.2020732118

[22] Dupuy A, Liu X, Kong Y, Qi M, Perdomo J, Fenwick J, Tieng J, Johnston B, Shi QS, Larance M, Zhang Y, Ju LA, Coleman P, Gamble J, Gardiner EE, Poncz M, Tran H, Chen V, Passam FH. Endothelial cell activation enhances thromboinflammation in vaccine-induced immune thrombotic thrombocytopenia. *Blood Adv*. 2025;9:2891–906.

[23] Fosse JH, Haraldsen G, Falk K, Edelmann R. Endothelial cells in emerging viral infections. *Front Cardiovasc Med*. 2021;8:619690. https://doi.org/10.3389/fcvm.2021.619690

[24] Greinacher A, Selleng K, Palankar R, Wesche J, Handtke S, Wolff M, Aurich K, Lalk M, Methling K, Völker U, Hentschker C, Michalik S, Steil L, Reder A, Schönborn L, Beer M, Franzke K, Büttner A, Fehse B, Stavrou EX, et al. Insights in ChAdOx1 nCoV-19 vaccine-induced immune thrombotic thrombocytopenia. *Blood*. 2021;138:2256–68.

[25] van der Neut Kolfschoten M, Inganäs H, Perez–Peinado C, Calado da Silva Freire J, Melchers JM, van Dijk N, Przeradzka M, Kourkouta E, van Manen D, Vellinga J, Custers J, Bos R. Biophysical studies do not reveal direct interactions between human PF4 and Ad26.COV2.S vaccine. *J Thromb Haemost*. 2024;22:1046–55.