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SARS CoV subunit vaccine: antibody-mediated neutralisation and enhancement

Introduction

Public health measures successfully contained outbreaks of the severe acute respiratory syndrome coronavirus (SARS-CoV), which infected more than 8000 people worldwide with a mortality of about 10%. However, concerns over future recurrences remain. Continuous efforts have been made to develop safe vaccine strategies against SARS-CoV.

Among the four major structural SARS-CoV proteins, the Spike envelope glycoprotein (S) is the most significant SARS-CoV neutralising and protective antigen. The binding of the Spike protein to its receptor Angiotensin Converting Enzyme 2 (ACE2) is responsible for SARS-CoV entry into cells. Vaccine strategies aiming at blocking/restricting infection by SARS-CoV mainly focus on targeting the Spike viral glycoprotein. Nonetheless, such strategy poses a singular dilemma for coronaviruses, as previous vaccination protocols have highlighted the possibility of immune-mediated enhancement of the disease.^{1,2}

Immune-mediated mechanisms, particularly antibody-dependent enhancement (ADE) have been exploited by a variety of viruses, including dengue virus, Feline Coronavirus (FCoV), and HIV, as alternative strategies to infect host cells.^{2,3} Besides interaction between viral protein and host receptor, these viruses can enter cells through binding of virus/immune-complexes to Fc-receptor (FcR), complement-receptor or by inducing conformational change in envelope glycoproteins that are required for virus-cell membrane fusion.³ In the case of FCoV infection, immunisation of cats with recombinant vaccinia virus preparations expressing the FCoV Spike protein resulted in the induction of S-specific antibodies responsible for an enhanced susceptibility to infectious challenge.¹ The enhanced infection of macrophages following antibody-mediated entry of feline coronavirus is responsible for the occurrence of deadly feline infectious peritonitis.

We investigated whether a recombinant native full length Spike-protein trimer (triSpike) of SARS-CoV was able to elicit a neutralising and protective immune response. Furthermore, we explored the capacity of vaccine-induced anti-Spike immune-serum to mediate ADE of SARS-CoV infection. We adopted reasonable safety concerns regarding the use of SARS-CoV vaccine in humans, and explored new ways to investigate SARS pathogenesis.

Methods

This study was conducted from 17 July 2006 to 16 June 2008.

Cell lines, expression vectors

VeroE6 (African green monkey kidney epithelial cells), K-562 (human chronic myelogenous leukaemia cells), U-937 (human histiocytic lymphoma cells), THP-1 (human acute monocytic leukaemia cells), SUP-T1 (human lymphoblastic leukaemia T lymphoblast), MOLT-3 (human acute lymphoblastic leukaemia T lymphoblast), MT4/R5 (Human T cell lymphoblast expressing CCR5), Raji (Burkitt's lymphoma B lymphoblast), Daudi (Burkitt's lymphoma B lymphoblast), ST486 (Burkitt's lymphoma B lymphoblast lacking expression

Key Messages

1. A SARS vaccine was produced based on recombinant native full-length Spike-protein trimers (triSpike) and efficient establishment of a vaccination procedure in rodents.
2. Antibody-mediated enhancement of SARS-CoV infection with anti-SARS-CoV Spike immune-serum was observed in vitro.
3. Antibody-mediated infection of SARS-CoV triggers entry into human haematopoietic cells via an FcγR-dependent and ACE2-, pH-, cysteine-protease-independent pathways.
4. The antibody-mediated enhancement phenomenon is not a mandatory component of the humoral immune response elicited by SARS vaccines, as pure neutralising antibody only could be obtained.
5. Occurrence of immune-mediated enhancement of SARS-CoV infection raises safety concerns regarding the use of SARS-CoV vaccine in humans and enables new ways to investigate SARS pathogenesis (tropism and immune response deregulation).

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of Fc γ R), 721.221 (EBV-transfected human B cells), NK-92 (malignant non-Hodgkin's lymphoma NK cell), P388D1 (murine macrophage-like lymphoblast), J774A.1 (murine monocyte/macrophage cells) were cultured in accordance with ATCC guidelines. Optimised DNA and Semliki Forest Virus expression vectors encoding SARS-CoV S-protein fused to a C-terminal FLAG-sequence have been described.⁴

Immunisation with inactivated SARS-CoV or recombinant Spike protein

For evaluation of ADE of triSpike-elicited serum, BALB/c mice 6 to 8 weeks old (n=4 per group) were immunised intraperitoneally with 2 μ g of purified triSpike in presence or absence of 1 mg of aluminium hydroxide gel on days 0 and 28. Animals in the control group received PBS with 1 mg of aluminium hydroxide gel on the same days. Blood samples were collected by saphenous vein bleeding on days -1, 27 and 55 post-immunisation (in accordance with local guidelines), and sera were prepared and heat-inactivated.

For comparison of SARS-CoV vaccines, BALB/c mice (n=4 per group) were immunised intraperitoneally with either 2 μ g of recombinant codon-optimised SARS-CoV Spike full-length (Opt. Spike Flag; O.S.F; aa1-1255), 2 μ g of recombinant codon-optimised SARS-CoV Spike ectodomain (Opt. SpikeECD Flag; O.SECD.F; aa1-1184), 2 μ g of recombinant codon-optimised SARS-CoV Spike subunit 1 (Opt. S1 Flag; O.S1.F; aa1-757), or 10 μ g of recombinant SARS-CoV Spike protein truncated early after the transmembrane domain (Soluble Spike Flag; Ssol.F; aa1-1193). An additional group immunised with 2 μ g (Spike-equivalent) of γ -irradiated SARS-CoV virion was also included as well as a mock control group injected with saline solution (PBS). Two immunisations were performed in presence of 1 mg of aluminium hydroxide gel at 3-week intervals, and sera were collected on days -1, 27, and 55 post-immunisation. Blood samples were harvested and handled as described above.

SARS-CoV pseudotype particles

Recombinant SARS-CoVpp lentiviral vectors expressing a luciferase reporter gene were produced from HEK293T cells as described elsewhere using 10 μ g of plasmid pNL4.3.Luc R-E-pro- and 10 μ g of plasmid pCDNA-S-FLAG encoding codon-optimised SARS-CoV S protein.⁴ For ADE assays, 25 μ L of serial two-fold dilutions of heat-inactivated mouse sera were incubated for 1 h at 37°C with 25 μ L of pseudovirus (50 ng p24). 50 μ L of Raji cells, at 2 \times 10⁶ cell/mL previously washed three times with serum-free RPMI, were added to the antibody-SARS-CoVpp mixture in a 96-well plate. After adsorption for 1 h at 37°C, 100 μ L of RPMI 1640 containing 5% FCS were added. Medium was renewed 16 h later and cells were incubated for an additional 48 h, washed in PBS, lysed and luciferase activity measured for 10 sec in a MicroBeta Jet Counter (Perkin Elmer) according to the instructions provided by the supplier (Promega). For ADE blocking assays, Raji cells were pre-incubated for 15 min at 4°C with 10 μ g/mL of

murine monoclonal antibody directed against human CD32 (Fc γ R2, BD Pharmingen) or goat polyclonal antibody directed against human ACE2 (R&D Systems) prior to infection with SARS-CoVpp.

Lysosomotropic agent and protease inhibitors

Cells were pre-incubated with the indicated amounts of either ammonium chloride (NH₄Cl) for 1 h, E-64d or Cathepsin L inhibitor (Cat L Inh) (Calbiochem) for 3 h prior to infection. Pseudoviruses (with or without serum) were mixed with the same concentrations of reagents in tubes and added to cells. After 5 h (E-64d or Cat L Inh) or 7 h (NH₄Cl), viruses were removed and replaced with fresh medium without drug. Cells were assayed for luciferase activity 60 to 65 h after infection.

Results

Production of a subunit SARS vaccine

We developed a SARS vaccine candidate (triSpike) based on recombinant native full-length Spike-protein trimers (the envelope glycoprotein involved in SARS-CoV entry into host cells). Our vaccine protocol elicited an in vivo neutralising and protective immune response in rodents.⁴ In vitro Spike-specific serum blocked binding of the Spike protein to the ACE2 receptor and neutralised SARS-CoV infection of permissive cells.⁴

Investigation of immune-mediated enhancement of SARS-CoV infection

By using SARS-CoV Spike-pseudotyped viral particle (SARS-CoVpp),⁴ we analysed the capacity of triSpike-elicited sera to trigger ADE^{2,3} of viral infection in vitro. The experiments exhibited opposite pattern according to cell types, while complement-inactivated sera from immunised animals still inhibit SARS-CoVpp entry in prototypic permissive cell lines, these sera induced virus penetration in human monocytic and lymphoblastic (B lineage) cell lines (Fig 1). Immune-mediated enhancement of infection was not restricted only to SARS-CoVpp, but also drove infection of human Raji B cells by live SARS-CoV (strain HK39849).

Unravelling molecular and biochemical pathways of antibody-dependent enhancement of SARS-CoV infection

To highlight differences, if any, in the ACE2⁵ and FcR-mediated entry pathways, we compared the effect of treatments by a lysosomotropic agent and protease inhibitors. Blockade of the acidification of the endosome, and annihilation of the cysteine protease activity did not abrogate ADE of SARS-CoVpp infection (Fig 2). In combination with the results of the investigation of the molecular pathway involved during the ADE process, entry into human haematopoietic cells occurred via an Fc γ R-dependent⁴ and ACE2-, pH-, cysteine-protease-independent⁴ pathways illustrating that ADE of virus infection is a novel cell entry mechanism of SARS-CoV (Fig 2).

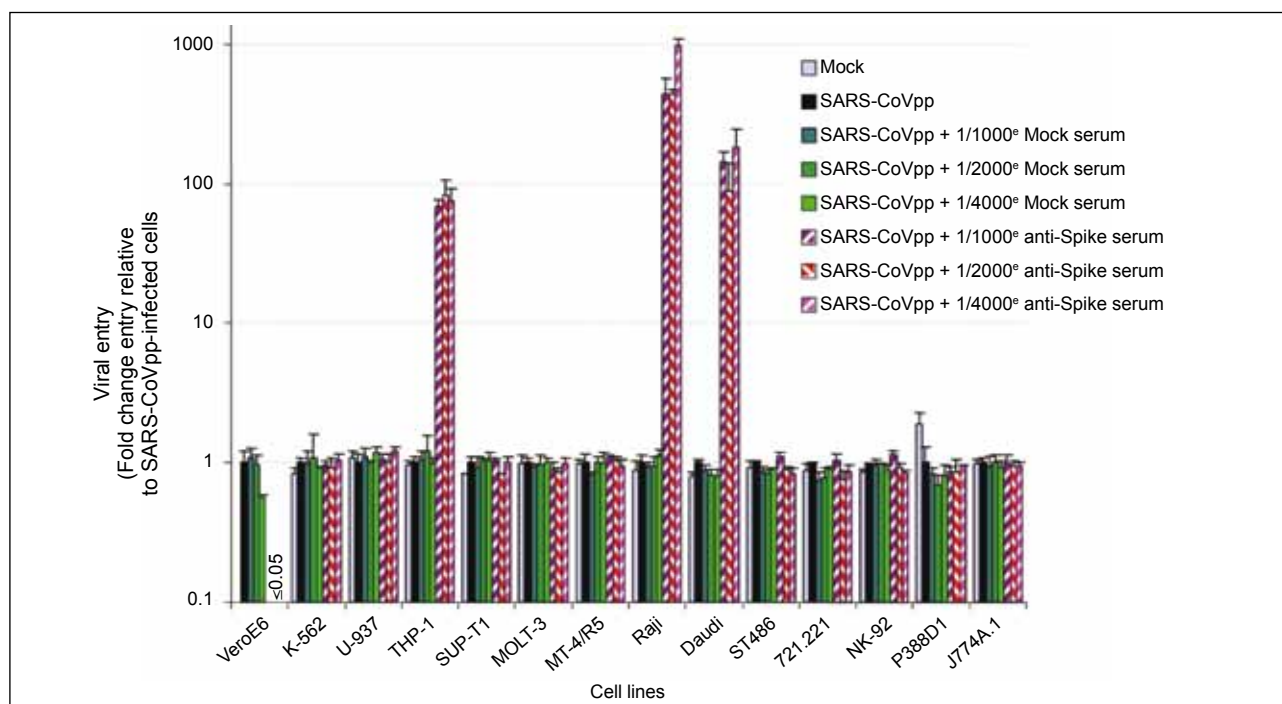


Fig 1. Susceptibility of haematopoietic cell lines to infection by SARS-CoV Spike pseudoparticles in presence of immune-serum SARS-CoVpp were incubated in presence or absence of different dilutions (eg 1/1000, 1/2000, and 1/4000) of serum from PBS/Alum-immunised (plain bars) or 2 μ g triSpike/Alum-immunised (hatched bars) BALB/c mice for 1 h prior to addition to the indicated cells. Three days post infection, 1 volume of luciferase substrate reagent was added to wells and luminescence was measured (Figure amended with permission from the *American Society for Microbiology* from FIG. 1A of the original publication⁹)

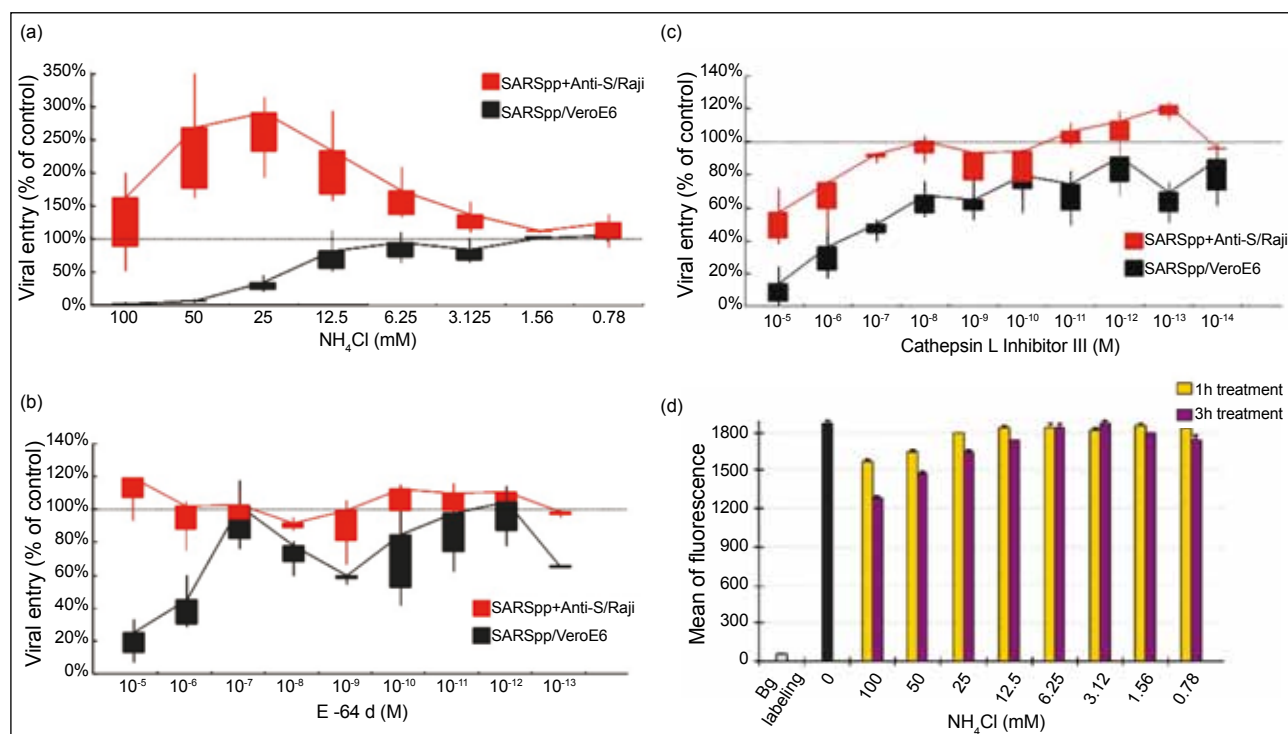


Fig 2. Antibody-mediated entry of SARS-CoVpp is independent of acidic pH and cysteine-protease activity of the endosomal/lysosomal compartment

Comparison of pH and protease requirements for ACE2- versus antibody-mediated entry of SARS-CoVpp. Prior to infection, VeroE6 and Raji cells were preincubated with indicated concentration of (a) ammonium chloride (NH_4Cl) for 1 h or (b) broad cysteine proteases inhibitor (E-64d) or (c) Cathepsin L inhibitor (Cat L Inh) for 3 h. SARS-CoVpp \pm 1/2000 anti-Spike serum (containing the indicated concentration of chemicals) was then added to the cells. (d) Cell surface expression of hCD32 protein on Raji cells following NH_4Cl treatment. Raji cells were incubated with indicated concentrations of NH_4Cl for 1, 3, and 5 h (not shown). Cells were then labelled with anti-hCD32 antibody and subjected to flow cytometry (Figure amended with permission from the *American Society for Microbiology* from FIG. 7 of the original publication⁹)

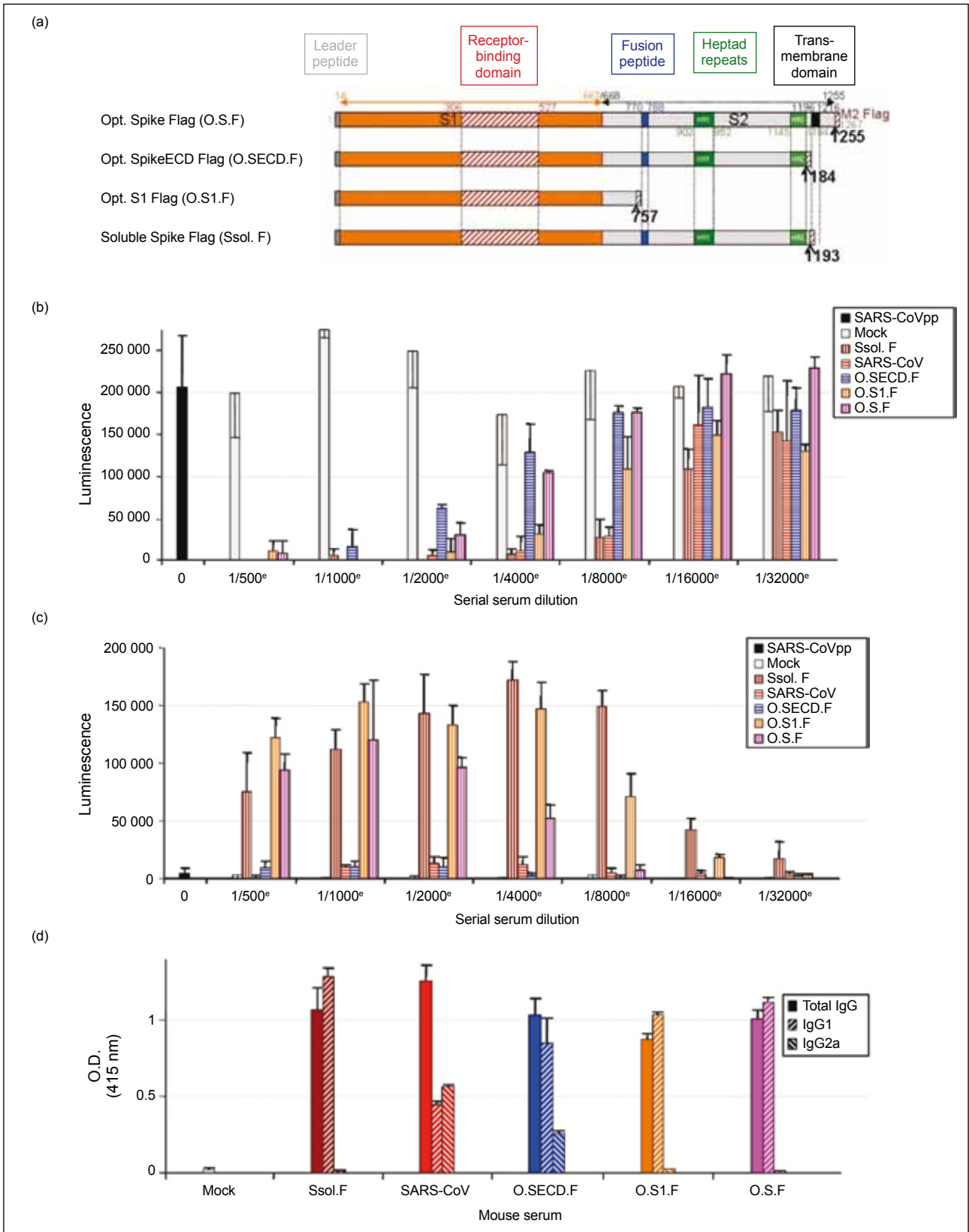


Fig 3. Effect of different vaccine formulation on SARS-CoV antibody-mediated enhancement of viral entry
 Schematic illustration of different forms of recombinant SARS-CoV Spike used for vaccination of BALB/c mice. (a) Positions of the different regions are indicated according to Swissprot accession no. P59594. Neutralising versus enhancing abilities of mouse sera raised after immunisation with different SARS vaccine candidates are shown. SARS-CoVpp were incubated in presence or absence of serial dilutions of pooled serum from four vaccinated BALB/c mice as indicated for 1 h prior to addition to the (b) VeroE6 or (c) Raji cells. Three days post-infection luminescence was measured. (d) The binding activity toward recombinant SARS-CoV Spike protein in 1/2000 dilution of pooled serum from vaccinated mice was estimated by enzyme-linked immune-absorbent assay (Figure amended with permission from the *American Society for Microbiology* from FIG. 8 of the original publication⁶)

SARS vaccine candidates and antibody-dependent enhancement of SARS-CoV infection

By comparing neutralising versus enhancing potency of different SARS vaccine candidates (subunit vaccines and whole inactivated virion), distinct patterns of ADE were highlighted, despite highly similar abilities of the immune-sera to neutralise infection of ACE2-expressing cells (Fig 3). Noticeably, two out of the five tested immunogens (Spike ectodomain only and whole inactivated virion) displayed relatively high neutralising titres without triggering SARS-CoVpp entry into immune cells. Preliminary results showed differences in the nature of humoral responses elicited by the five immunogens, as indicated by presence or absence of IgG2a in non-enhancing and enhancing sera, respectively (Fig 3).

Discussion

Currently, there is no licensed vaccine against a human coronavirus. Nonetheless, vaccines against some animal coronaviruses have been generated. Other vaccines are difficult to produce owing to immune enhancement of infection.^{1,2}

Our quest to produce an efficient vaccine against SARS-CoV has shed light on the incidence of opposite outcomes (ie neutralising and enhancing) depending on the patterns of expression of Fc receptor by the host cells. Notably, occurrence of antibody-mediated infection seems fairly complex as the expression of a particular class of FcR is not sufficient to indubitably predict the occurrence of ADE. Indeed, only background levels of SARS-CoVpp transduction were observed with several immune cells (human K-562, MT4-R5, 721.221, and murine P388D1, J774A.1 cell lines) despite their expression of the same Fc γ R (Fc γ RII) as THP-1, Raji, and Daudi cells (Fig 1). The reasons for this phenomenon remain unclear, but the involvement of FcR subfamilies, such as activating and inhibiting isoforms (ITAM- and ITIM-bearing FcR, respectively) and/or allelic variants, ie FcR γ IIA-H131 and FcR γ IIA-R131, is highly speculative.

Our studies on the biochemical requirements along the antibody-mediated infection have proved this pathway singularly distinct from the natural (ie ACE2) entry mechanism. In fact, ADE of SARS-CoVpp entry into human haematopoietic cells occurred via an ACE2-, pH- and cysteine-protease-independent route, illustrating that antibody-mediated infection is a novel cell entry mechanism of SARS-CoV.

Investigation of the neutralising versus enhancing potency of immune-sera elicited by different SARS vaccine candidates have highlighted distinct patterns of ADE, despite highly similar abilities to neutralise SARS-CoV infection. Further studies of the mechanisms underlying ADE of SARS-CoV infection are needed, particularly

to unravel which viral epitope(s) or immunoglobulin isotype(s) are responsible for the enhanced infection.

Because of antibody-mediated infection of SARS-CoV, we hypothesised that the ADE phenomenon might participate in SARS pathogenesis. Indeed, infection by SARS-CoV is not confined to the lungs, but also involves other organs, most importantly cells of the immune system. Direct infection of haematopoietic cells by SARS-CoV may provide a partial explanation for the widespread destruction of the lymphoid tissue and the cytokinic deregulation in many SARS patients. Nonetheless, it is not clear how SARS-CoV gets a foothold into the immune cells as they do not express the putative SARS-CoV receptor ACE-2. Antibody-dependent enhancement of SARS-CoV infection, in addition to other alternative entry pathways (involving C-type lectin), may provide SARS-CoV versatility in entry routes allowing it to broaden its target options.

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