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Antibody-dependent SARS coronavirus infection is mediated by antibodies against spike proteins

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ABSTRACT

The severe acute respiratory syndrome coronavirus (SARS-CoV) still carries the potential for reemergence, therefore efforts are being made to create a vaccine as a prophylactic strategy for control and prevention. Antibody-dependent enhancement (ADE) is a mechanism through which dengue viruses, feline coronaviruses, and HIV viruses take advantage of anti-viral humoral immune responses to infect host target cells. Here we describe our observations of SARS-CoV using ADE to enhance the infectivity of a HL-CZ human promonocyte cell line. Quantitative-PCR and immunofluorescence staining results indicate that SARS-CoV is capable of replication in HL-CZ cells, and of displaying virus-induced cytopathic effects and increased levels of TNF-α, IL-4 and IL-6 two days post-infection. According to flow cytometry data, the HL-CZ cells also expressed angiotensin converting enzyme 2 (ACE2, a SARS-CoV receptor) and higher levels of the FcγRII receptor. We found that higher concentrations of anti-sera against SARS-CoV neutralized SARS-CoV infection, while highly diluted anti-sera significantly increased SARS-CoV infection and induced higher levels of apoptosis. Results from infectivity assays indicate that SARS-CoV ADE is primarily mediated by diluted antibodies against envelope spike proteins rather than nucleocapsid proteins. We also generated monoclonal antibodies against SARS-CoV spike proteins and observed that most of them promoted SARS-CoV infection. Combined, our results suggest that antibodies against SARS-CoV spike proteins may trigger ADE effects. The data raise new questions regarding a potential SARS-CoV vaccine, while shedding light on mechanisms involved in SARS pathogenesis.

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1. Introduction

Coronaviruses are enveloped viruses with positive stranded, capped, and poly-adenylated RNA genomes ranging between 28 and 32 kb [1]. Coronaviruses cause respiratory and enteric diseases in both humans and domesticated animals—for example, human coronaviruses 229E (HCoV-229E) and OC43 (HCoV-OC43) are known to cause mild upper respiratory tract infections. In 2003, a new severe acute respiratory syndrome coronavirus (SARS-CoV) was discovered during an outbreak of that disease [1], which infected more than 8000 individuals and killed more than 700, with most identified cases occurring in Asia [2]. Originally it was assumed that SARS-CoV is transmitted via respiratory droplets or direct contact [3]. However, some researchers now suggest that it can also be spread via airborne or other unknown processes [1,3].

The SARS-CoV genome consists of 28 putative open reading frames (ORFs) in 9 mRNA transcripts. ORF1a and ORF1b, which

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account for approximately two-thirds of the genome, both encode large polyproteins. The genome encodes four major structural proteins: spike (S), envelope (E), membrane (M), and nucleocapsid (N) [2]. Viral surface spike glycoproteins initiate SARS-CoV entry into cells by binding to angiotensin-converting enzyme 2 (ACE2) receptors, followed by conformational changes resulting in membrane fusion and genome delivery to the cytoplasm [4]. SARS-CoV S protein is a type I transmembrane protein consisting of distinct N-terminal (S1) and C-terminal (S2) domains that respectively mediate receptor binding and virus-cell fusion. S proteins are key targets for generating protective neutralizing antibodies against coronaviruses [2,5]. Minor alterations in the S protein can affect tissue, species tropism, and coronavirus virulence. The primary function of a nucleocapsid protein is to form helical ribonucleoprotein complexes with viral RNA (vRNA) [6]. In addition to comprising the core structure of the SARS-CoV virion, such complexes are involved in multiple functions such as transcription, replication, and virus packaging [2,6].

Several vaccine strategies under study target SARS-CoV spike genes or proteins to induce neutralizing and protective antibodies. However, a significant concern is that vaccinations against different kinds of coronaviruses have markedly different outcomes [7]—for example, anti-spike antibodies offer protection against mouse hepatitis and transmissible gastroenteritis, but boost feline-coronavirus (FCoV) infections [8]. An alternative virus entry process known as antibody dependent enhancement (ADE) has been observed in viruses such as dengue, yellow fever, HIV, and FCoV, among others [7]. Although the ADE mechanism is not completely understood, it is generally assumed that increased virus yields are due to infections of large numbers of susceptible cells. Such increases are mediated by immunoglobulin receptors such as Fc (FcRs) or complement receptors that facilitate the uptake of virus-antibody complexes into phagocytes, resulting in enhanced target cell infections [7]. However, it remains unclear whether SARS-CoV uses ADE to enhance infectivity.

We used SARS-CoV clinical isolates and pseudotyped viruses to determine whether ADE occurs during SARS-CoV infection. Our experiments involved a human promonocyte cell line, HL-CZ, that has not yet been reported for SARS-CoV infections. According to our data, HL-CZ cells express ACE2 and FcRII receptors, and can be infected by SARS-CoV. We also noted that diluted anti-sera against SARS-CoV promotes SARS-CoV infection, and that this phenomenon is significantly mediated by anti-S antibodies. Finally, only one of several monoclonal antibodies against S proteins generated for this project expressed neutralizing action; the others displayed mild-to-moderate ADE effects on HL-CZ cells.

2. Materials and methods

2.1. Ethics statement

Approval for obtaining sera from SARS-CoV patients was applied for and received from the Institutional Ethics Committee of National Yang-Ming University. All procedures were conducted according to committee rules.

2.2. Cell lines, viruses and anti-sera

Five cell lines were used: HL-CZ human promonocyte isolated from a leukemia patient [9]; HEK293T human kidney; Vero-E6 African Green Monkey kidney; Raji B; and THP-1 monocyte. SARS-CoV (TW1 strain, GenBank accession No. AY291451) was obtained from the Taiwan Centers for Disease Control (Taiwan CDC) [10]. SARS-CoV pseudo-typed virus particles carrying the SARS-CoV S protein were generated by co-transfecting HEK293T cells with pNL-Luc-E-R- and pcDNA3-S [4,11]. Anti-SARS CoV sera were collected from SARS-CoV-infected patients and confirmed at the Taiwan CDC labs.

2.3. Infectivity and neutralization assays

HL-CZ or Vero-E6 cells (1×10^5/well) were used in direct infection assays. SARS-CoV or pseudotyped viruses (MOI = 0.1) were incubated with cells for 2 h at 37 °C in serum-free medium and washed with PBS. Following incubation, 2 ml of RPMI or DMEM medium containing 10% serum and 1X (100 IU penicillin and 100 μg/mL streptomycin) antibiotics were added, followed by another incubation in 5% CO_2 at 37 °C for different lengths of time. Viral supernatants and infected cell lysates and were collected and subjected to quantitative RT-PCR analyses aimed at the ORFIb region [12]. Pseudotyped SARS-CoV particle quantities were determined in terms of luciferase activity. For neutralization assays, HL-CZ or Vero E6 cells were added to 96-well tissue culture plates (5×10^4 cells/well). SARS-CoV (3.125 $TCID_{50}$) or SARS-CoV pseudotyped viruses (100 ng of p24) were mixed with various mAbs or sera at different dilutions and incubated at 37 °C for 2 h before being added to HL-CZ or Vero E6 cells. Culture supernatants were collected 2–4 d post-infection; viral loads were measured by real-time RT-PCR.

2.4. Real-time RT-PCR

Viral RNA was extracted from culture supernatant using a QIAamp Viral RNA Mini Kit (Qiagen). The two real-time RT-PCR primer sets used to measure mRNAs were CDC-2 forward and reverse (nucleocapsid; U.S. CDC catalogue #KT0051) [11] and BNIoutS and BNIoutAS (ORF Ib) [12].

2.5. Flow cytometry and immunofluorescence staining

Cells were harvested and held for 30 min at 4 °C in PBS containing 1% FBS, followed by another 30 min of incubation at 4 °C with isotype-matched control or monoclonal antibodies (1 μg/ml) for each indicated human Fc receptor. Immunofluorescence staining procedures are described in [13].

2.6. SARS-CoV S and N protein preparation

A Taiwanese SARS-CoV isolate TW1 strain (GenBank accession No. AY291451) was used as a template to clone and amplify nucleocapsid and spike genes using plasmids expressed in the prokaryote system. Details and schemes are described in the Supplemental file; see especially S-Figs. 2 and 3 [3,11].

2.7. Generation of monoclonal or polyclonal antibodies against SARS-CoV S and N proteins

Our procedures for generating monoclonal or polyclonal antibodies are the same as those described in [14]. Briefly, BALB/c mice were immunized once and given two boosters of 25 μg GST-spike (GST-SIa, -SIb, and -SII) recombinant proteins in 0.25 ml of PBS emulsified with equal volumes of complete/incomplete Freund's adjuvant (Sigma–Aldrich, St. Louis, MO). ELISAs and Western blots were used to confirm antibody titers against these recombinant fusion proteins prior to harvesting splenocytes and fusing them with NS-1 myeloma cells. ELISAs and Western blots were also used to screen hybridoma culture supernatants for the GST-spike fusion protein. Clones producing specific antibodies were selected using the dilution method described in [15].

2.8. Apoptosis assays

HL-CZ cell apoptosis assays were performed using Annexin V staining.

2.9. Immunoblotting

HL-CZ or Vero-E6 cells were infected with SARS-CoV and incubated at 37 °C for 48–72 h. Culture supernatants were concentrated by ultracentrifugation at 25,000 rpm for 2.5 h and lysed in SDS–PAGE sample buffer. SDS–PAGE electrophoresis was performed according to the protocols given in [11,14].

2.10. Antibody-dependent enhancement assays

Assay details are as previously described [5,16,17]. Assays were performed using sera from normal and SARS-CoV-positive patients. Rabbit polyclonal or mouse monoclonal antibodies against S or N proteins were used to evaluate ADE. HL-CZ cells were infected with SARS-CoV or pseudotyped viruses in the presence of control and either anti-spike or anti-nucleocapsid antibodies (10- to 2000-fold dilution) and incubated at 37 °C for 48 h. Supernatants and cell lysates were collected and viral titers determined by quantitative RT-PCR or luminescence analyses.

2.11. Statistical analyses

All experiments were performed a minimum of three times. GraphPad Prism software was used for all statistical analyses. Statistical significance ($p < 0.05$) was calculated using unpaired Student's *t*-tests.

3. Results

3.1. Susceptibility of HL-CZ cells to SARS-CoV infection

Previous researchers have infected HL-CZ human promonocyte cells with HIV-1 and dengue viruses, among several others [9]. Our infectivity assay results indicate that HL-CZ can be infected by SARS-CoV (Fig. 1A). According to quantitative RT-PCR data, higher mRNA expression levels of TNF-α, IL-4 and IL-6 were detected in HL-CZ cells 2 d post-infection (Fig. 1B). Trace levels of IL-3 and

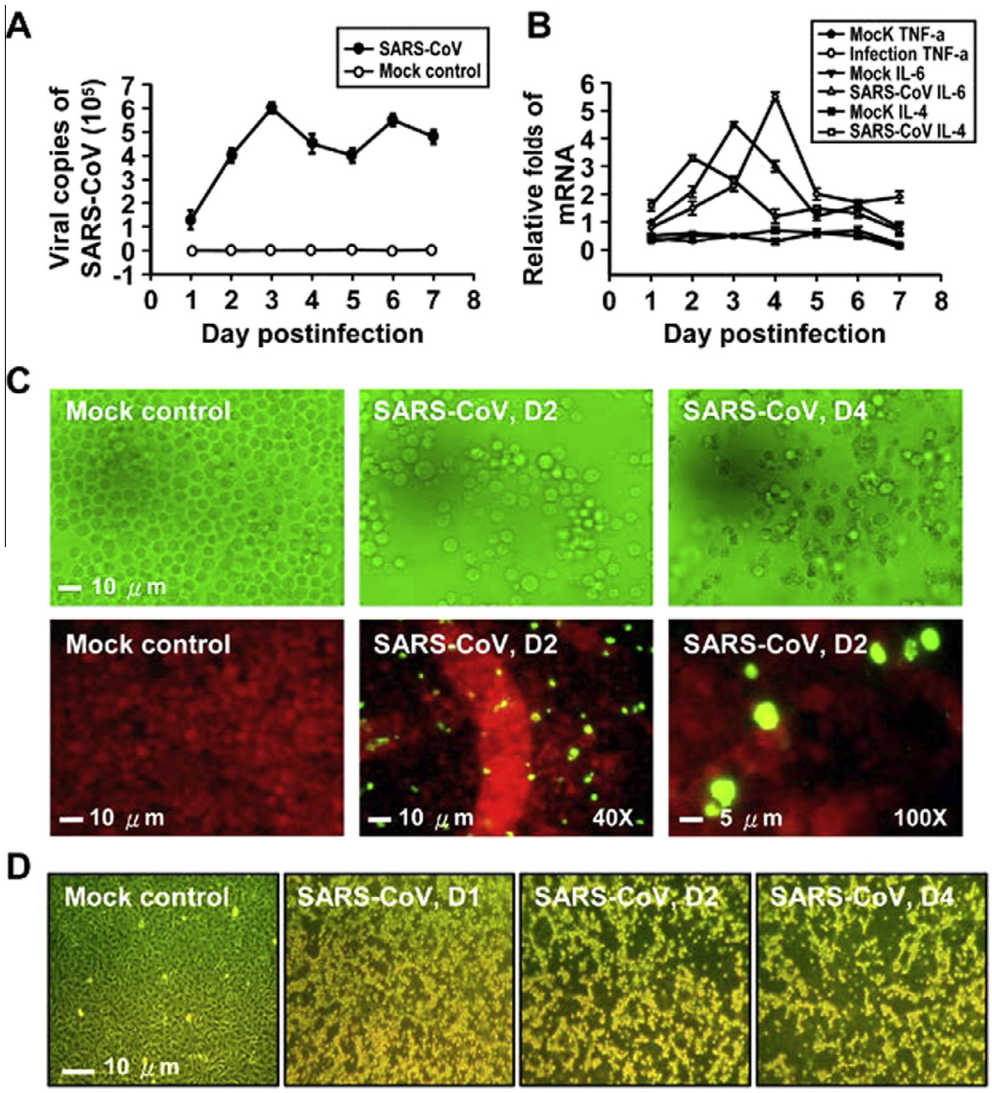

Fig. 1. HL-CZ cells are susceptible to SARS-CoV infection. After infecting cells with SARS-CoV, culture supernatant and cell lysates were collected for (A) SARS-CoV detection and (B) cellular cytokine mRNA expression. (C) Results from HL-CZ cytopathic effect observation and immunofluorescence staining 2 d post-infection. (D) Supernatants from mock and SARS-CoV-infected HL-CZ cells were used to infect Vero-E6 cells for 2 d. Results are from one representative experiment of the three performed.

IL-1β were also detected (data not shown). Light microscopy and immunofluorescence staining observations indicate (a) a cytopathic effect (CPE) 2 d post-infection (Fig. 1C) and (b) the presence of SARS-CoV in SARS-CoV-infected HL-CZ cells, detected by anti-sera collected from SARS-CoV-positive patients (Fig. 1C). Infectious SARS-CoV virus production in HL-CZ cells was confirmed by using supernatants from those cells to infect Vero-E6 cells, which are known to be susceptible to coronaviruses. We also observed a significant CPE in Vero-E6 cells on days 2 and 4 post-infection (Fig. 1D).

3.2. HL-CZ cells express angiotensin-converting enzyme 2 (ACE 2) and Fcγ receptors, and display antibody-dependent SARS-CoV infectivity

ACE2 is a functional SARS-CoV receptor that is abundant in human lung and small intestine epithelial cells [18]. Fc receptors (FcR) and complement receptors [7], which have been identified as alternatives for the cellular entry of certain viruses, have been shown to enhance antibody-dependent infectivity [7,17]. Our flow cytometry results indicate that the HL-CZ cells expressed both ACE2 receptors and higher levels of FcRII (CD32) (Fig. 2A).

We tried to determine if this antibody-dependent enhancement also occurred in SARS-CoV-infected HL-CZ cells. Our data indicate that treatment with anti-sera collected from SARS-CoV patients and diluted 1000- to 2000-fold resulted in increased virus infectivity and CPE compared to treatment with 10-fold diluted anti-sera (Fig. 2B). According to transmission electron microscopy images, higher quantities of SARS-CoV viral particles were observed in HL-CZ cells treated with more diluted anti-sera against SARS-CoV (2000-fold dilution) (Fig. 2C-3) compared to those treated with less diluted anti-sera (10-fold) (Fig. 2C-1) and less diluted normal control sera (10-fold) (Fig. 2C-2). We performed additional infectivity assays to confirm this observation, and found significantly higher levels of virus infection following treatment with 100- and 1000-fold diluted anti-sera compared to control sera from non-SARS-CoV patients at the same dilutions ($p < 0.05$) (Fig. 3A). We also found that highly diluted anti-sera against SARS-CoV significantly increased virus-induced apoptosis in HL-CZ cells compared to normal sera at the same dilutions ($p < 0.05$) (Fig. 3B).

3.3. Antibody-dependent SARS-CoV enhancement is mediated by anti-spike antibodies

Our next task was to determine which antibody promoted SARS-CoV infection. We noted that antibodies against S and N proteins were produced at high levels during SARS-CoV infections, and therefore used S- and N-protein-recognizing antibodies to detect early SARS-CoV infections. After generating mouse antibodies against S and N recombinant proteins (Supplemental Figs. 1–3),

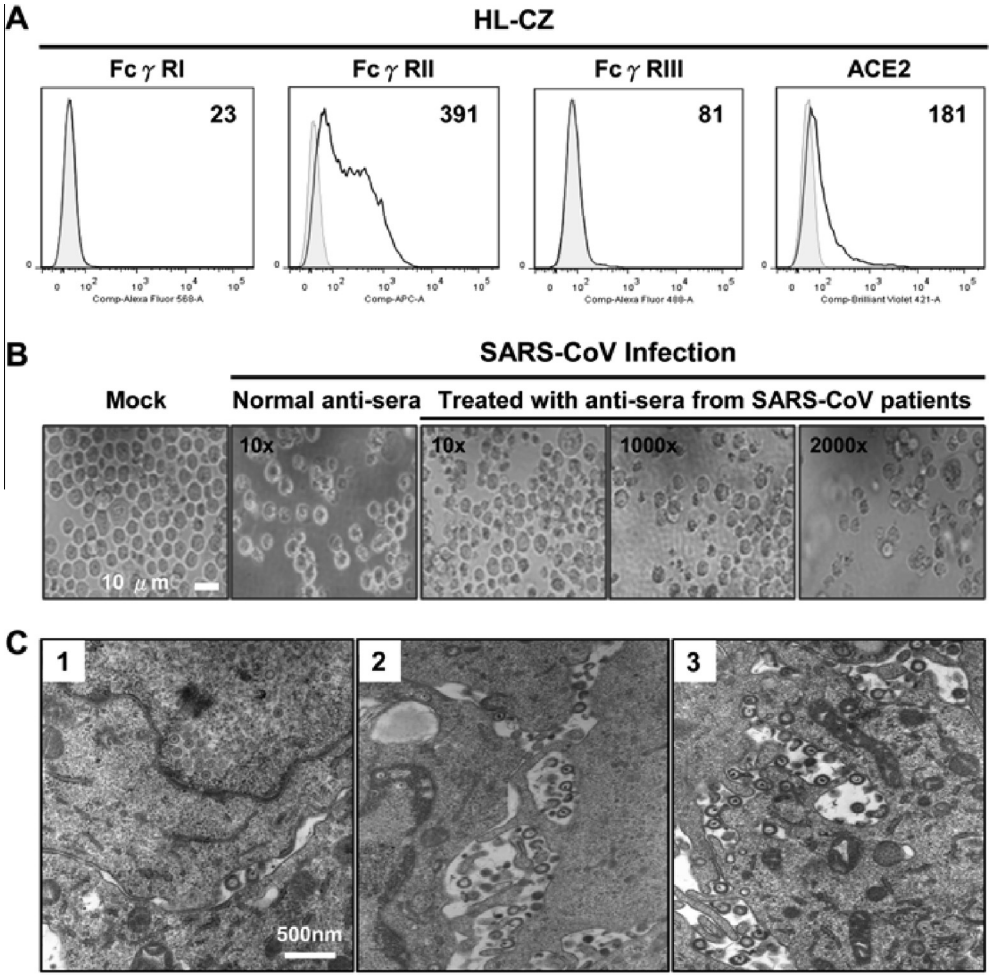

Fig. 2. HL-CZ cells express ACE2 and Fc receptors, and display antibody-dependent enhancement. (A) Results from flow cytometry analyses of Fc receptors and ACE2 expression in HL-CZ cells. (B) SARS-CoV or mock infections in the presence of various dilutions of anti-sera drawn from SARS-CoV patients. (C) Transmission electron microscopy images of HL-CZ cells infected with SARS-CoV in the presence of various dilutions of anti-sera against SARS-CoV. Images 1 and 3 indicate 10-, and 2000-fold dilutions of anti-sera against SARS-CoV, respectively. Image 2 indicates 10-fold dilutions of normal sera control treatment.

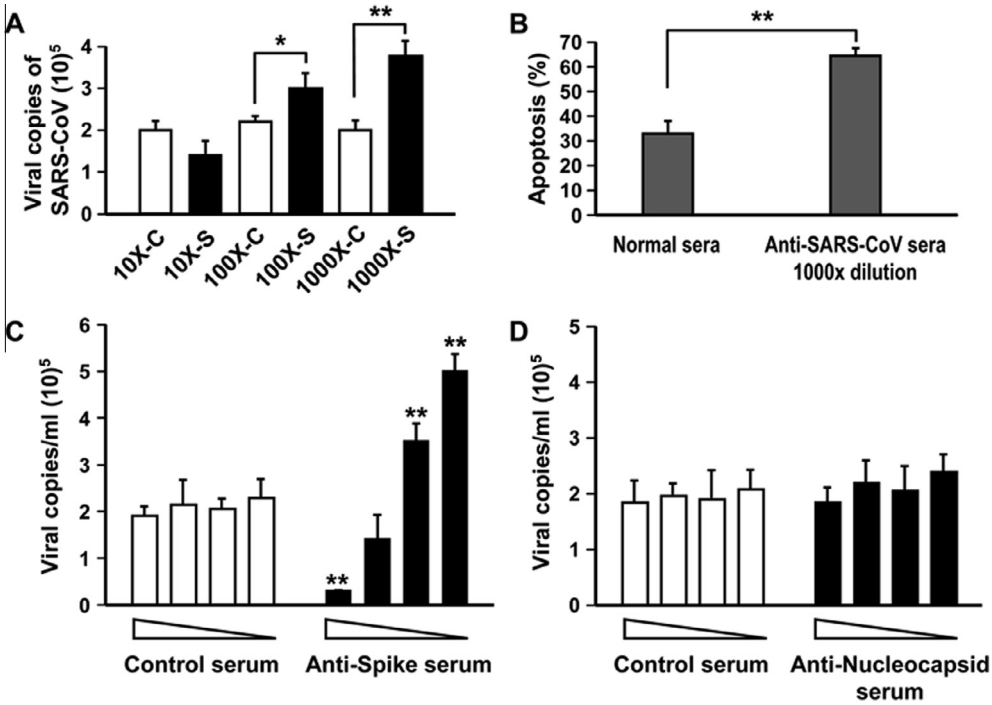

Fig. 3. Antibody-dependent enhancement of SARS-CoV infection is mediated by anti-spike protein antibodies. (A) Results from infectivity assays using HL-CZ cells infected with SARS-CoV and treated with various dilutions of anti-sera collected from SARS-CoV patients. (B) Apoptosis analyses of HL-CZ cells infected with SARS-CoV in the presence of 1000-fold diluted normal sera and anti-SARS-CoV sera (Annexin V staining). (C) HL-CZ cells infected with SARS-CoV in the presence of various dilutions (10-, 100-, 1000- or 2000-fold) of mouse anti-spike protein serum and isotype control mouse serum. (D) HL-CZ cells infected with SARS-CoV in the presence of different dilutions (10-, 100-, 1000- or 2000-fold) of mouse anti-nucleocapsid serum and isotype control mouse serum. C, normal sera (control); S, anti-SARS sera (sample). Shown are results from one representative experiment of three performed (*$p < 0.05$; **$p < 0.01$).

we performed infectivity assays using different mouse sera against S or N proteins at different dilutions. Assay results indicate that diluted anti-S mouse sera exhibited significantly greater SARS-CoV ADE effects on HL-CZ cells compared to diluted mouse control sera ($p < 0.05$) (Fig. 3C). Note that diluted anti-N mouse sera did not exert any SARS-CoV ADE effects on HL-CZ cells (Fig. 3D).

A series of monoclonal antibodies (mAbs) against spike proteins was generated to confirm this finding (Supplemental Figs. 1 and 3). S-protein epitopes recognized by the mAbs are shown in Fig. 4A. As shown, only SIb4 (epitope ^{435}NYNWKR^{440} in the SIb region) had neutralizing capabilities. Other monoclonal antibodies against S proteins exhibited mild-to-moderate enhancement effects on lentiviral particles carrying a luciferase gene pseudotyped with SARS envelope spike proteins (Fig. 4B). Infectivity assay results also indicate that the SIb4 mAb significantly inhibited the SARS-CoV infection of HL-CZ cells, while SIb2 promoted virus infection compared to the isotype control. According to microscopy observations, SIb2 promoted a significantly stronger CPE compared to SIb4 (which also displayed neutralizing capability) and the isotypic control (Fig. 4D).

4. Discussion

While the generation of protective antibodies against specific proteins is a useful strategy for microbial disease control, some immune responses to pathogens can have deleterious effects—for example, cytokine storms associated with avian influenza virus, and increased mortality from SARS-CoV infections. Cell infection via antibody-dependent enhancement has been observed for several viral diseases, including dengue virus, HIV-1, and feline infectious peritonitis virus (FIPV) [7,13]. In the present study we identified the susceptibility of an alternative cell line, HL-CZ, to SARS-CoV infection (Fig. 1). Specifically, we found that HL-CZ cells express ACE2 receptors and display a cytopathic effect induced by SARS-CoV. Two research teams have reported that ACE2 serves as a functional receptor for SARS-CoV infection, and that human peripheral monocytes and macrophages can be infected by SARS-CoV [19,20]. Our results suggest that HL-CZ can serve as an alternative cell line for SARS-CoV research—specifically, SARS-CoV-infected HL-CZ cells showed increased TNF-α, IL-4, and IL-6 secretions (Fig. 1B), but only trace amounts of IL-3 and IL-1β. According to the same two studies, human peripheral monocytes and macrophages can be infected by SARS-CoV. Another research group observed the up-regulation of IL-6 and TNF-α induced by the SARS-CoV spike protein in murine macrophages, and identified IL-6 and IL-8 as key SARS-CoV-induced epithelial cytokines [21]. Combined, these data suggest that SARS-CoV-induced IL-6 and TNF-α play roles in SARS pathogenesis, especially in terms of inflammation and high fever.

Currently there are no licensed vaccines against human coronaviruses. For animals, vaccines have been generated against several, including the transmissible gastroenteritis virus [22]. However, attempts to develop a feline coronavirus vaccine have failed due to ADE [8], which is mediated by receptors—most notably the Fc gamma receptor (FcγR), which facilitates antigen–antibody complex uptake by target cells. Our data indicate that HL-CZ cells express high levels of FcγRII. According to one report, FcγRII (CD32) plays a predominant role in mediating of SARS-CoV by using antibodies against different types of FcγRs on immune cells [17]. In that study, THP-1, Raji, and Daudi cells displayed ADE during SARS-CoV infection [17]. We found that FcyRII and ACE2 receptors were expressed in THP-1, Raji, and HL-CZ cells (Supplemental Fig. 4). Combined, these findings suggest that FcyRII is important for ADE mediation in some types of immune cells during SARS-CoV infection.

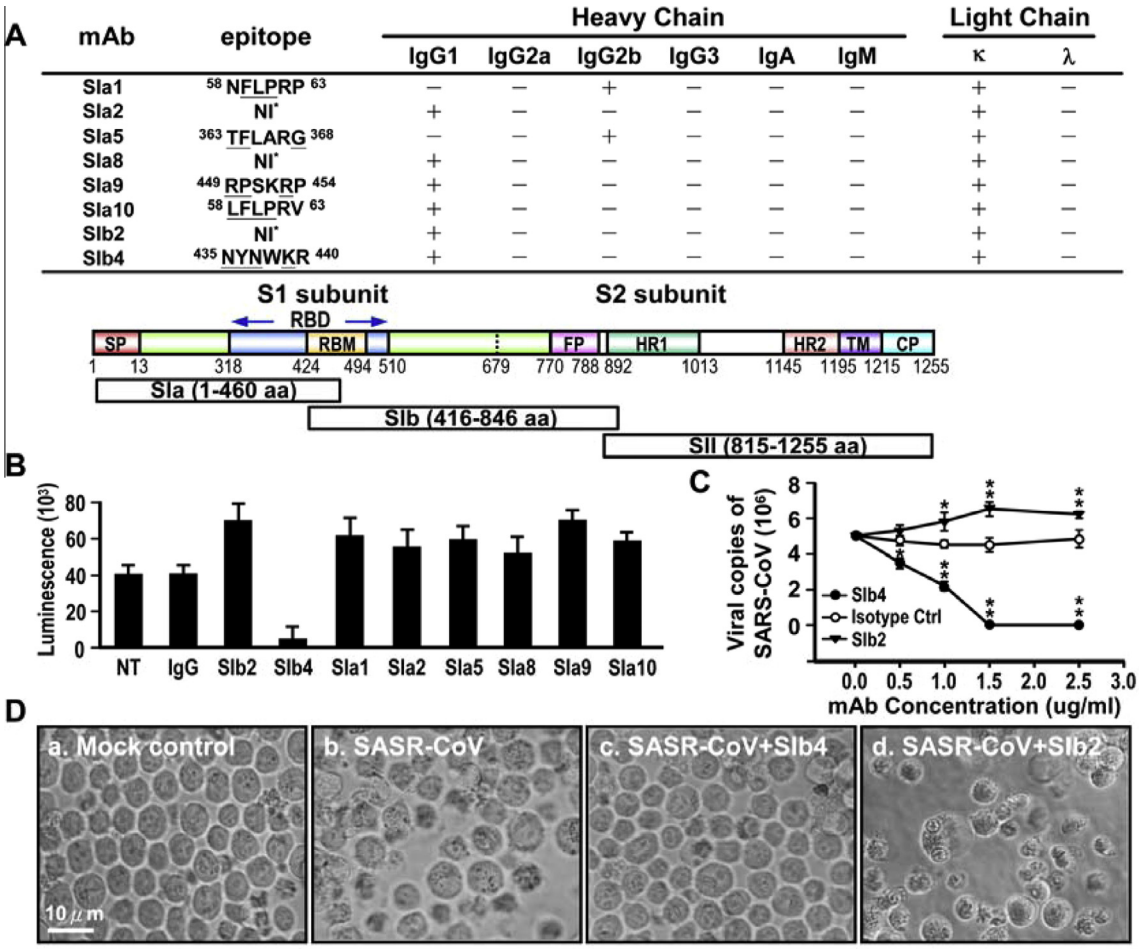

Fig. 4. Monoclonal antibodies against the SIa regions of spike proteins show antibody-dependent enhancement capability. (A) Isotype and epitope mapping of monoclonal antibodies against SARS-CoV spike proteins (NI*, not identified; bold, conservative amino acid residues). Lower part, fragment scheme of spike proteins used to generate monoclonal antibodies. (B) Evaluations of ADE effects of SARS-CoV-pseudotyped virus infections of HL-CZ cells in the presence of the monoclonal antibodies listed in (A). (C) Infectivity assays were performed using SARS-CoV-infected HL-CZ cells treated with SIb4, SIb2, or isotype control antibodies. (D) Cytopathic effects observed in HL-CZ cells treated with SIb4, SIb2, or isotype control antibodies. Presented results are from one representative experiment of three performed (*$p < 0.05$; **$p < 0.01$).

Some antibodies are capable of increasing virus replication efficiency by either boosting the uptake of infectious antibody-virus complexes, or by increasing the synthesis of viral proteins and nucleic acid [23]. There has been at least one report indicating that compared to untreated viruses, HIV-1 replication speeds up when viruses are pretreated with HIV-1 antibodies [24]. According to another report, a higher FIPV infection rate in macrophages occurred when anti-FIPV antibodies were present [25].

We found that higher concentrations of anti-sera collected from SARS-CoV-infected patients (10- to 100-fold dilution) displayed greater viral neutralization capability compared to more diluted concentrations (1000- to 2000-fold), which facilitated SARS-CoV infection and induced higher levels of virus-induced apoptosis (Fig. 3). We found that this phenomenon occurred via ADE and was mediated by anti-S antibodies, but not by anti-N antibodies. At least two studies have shown that SARS-CoV infection induces abundant amounts of antibodies against S and N proteins [2,6]. Spike proteins, especially those in the ACE-2 binding region (318–510 aa), are capable of producing SARS-CoV-neutralizing antibodies; nucleocapsid proteins induce cytotoxic T lymphocyte (CTL) immune responses that are specifically SARS-CoV-protective [26]. However, another research team has suggested that SARS-CoV subunit vaccines may induce neutralization and/or partial ADE effects via B lineage cells [16]. In a separate report describing similar results, the researchers suggested that vaccine-induced anti-spike antibodies against trimeric S proteins may mediate the ADE of SARS-CoV-pseudotyped virus entry into FcR-expressing cells [27]. Our anti-spike monoclonal antibody data indicate that most of those against SIa regions (1–460 aa) displayed the ADE effect on both SARS-CoV-pseudotyped (Fig. 4B) and real viruses (Fig. 4C), and that only one monoclonal antibody against SIb regions (416–486 aa) showed viral neutralizing capability. The associated mechanisms require further investigation.

In this study we identified an alternative susceptible cell line, HL-CZ, for future SARS-CoV research, and found that ADE associated with SARS-CoV infection is primarily mediated by anti-spike antibodies. It is our hope that this information will be useful in the development of a human SARS-CoV vaccine, as well as in research involving immune-mediated infections tied to SARS pathogenesis.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.07.090.

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Supplements

Materials and Methods

Preparation of SARS-CoV spike and nucleocapsid proteins

A Taiwanese SARS-CoV isolate TW1 strain (GenBank accession AY291451) was used as a template with a single-tube RT-PCR (Stratagene, La Jolla, CA) to amplify SARS-CoV nucleocapsid and spike genes (S-Figs. 2 and 3) [3, 11]. Primer pairs were N gene: NF, 5'-GCCGAATTCATGTCTGATAATGGACCCCA-3' and NR, 5'-GCGCGT CGACGTTATGCTGAGT TGAATCA-3'; SIa gene (aa residues 1-460 of the S protein): S1F, 5'-GCGGAATTCATGTTTATTTTCTTATTATTTCTTAC-3' and S1R, 5'-GCG CTCGAGGAAAGGCACATT AGATATGTCT-3'; SIb gene (aa residues 416-846 of the S protein): S2F, 5'-GCGGAATTCATGGGT TGTGTCCTTGCTTGGA-3' and S2R, 5'-GCGCTCGAGCAGAGGTGGCAACACTGTA AGT-3'; SII gene (aa residues 815-1255 of the S protein): S3F, 5'-GCGGAATTCATGAAG CAATATGGCGAATGC-3' and S3R, 5'-GCGC TCGAGTGTGTAATGTAATT TGACACCC-3'. PCR products were subcloned into pGEX-4T-1 (Amersham Biosciences, NJ) or pET-29a vectors (Norvogen). Recombinant proteins (RP) were induced into BL21 cells with isopropyl-β-D-thiogalactopyranoside (IPTG) (Sigma-Aldrich). For induction, the optic densities of BL21 cells containing the pGEX-NP, pGEX-SIa, pGEX-Sib or pGEX-SII plasmids were 0.6-0.7. Following IPTG induction at 37°C for 2.5 h, bacterial cells were collected by centrifugation at 6,000 rpm at 4°C for 15 min. Cell pellets were washed twice with ice-cold phosphate buffered saline (PBS pH 7.4) (140 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4), resuspended in 20 ml PBS, French pressed, centrifuged at 16,000 x g for 30 min at 4ºC, and resuspended in 5 ml 8M urea. Resulting

supernatant was applied to gels using a SDS-polyacrylamide gel process. Following overnight dialysis against PBS, proteins were concentrated with a Centricon Plus-10 concentrator (Millipore, MA, USA). RP concentrations were measured with a Pierce bicinchoninic acid protein assay reagent (Pierce, IL); purity was analyzed using samples with a 12.5% sodium dodecyl sulfate-polyacrylamide mini-gel (Bio-Rad Laboratories, CA).

Figure legends:

Figure 1. Construction of plasmids containing specific regions of the SARS-CoV spike (S) gene. We cloned the S gene in a pGEX system for prokaryotic expression. Gene fragments were generated by one-tube RT-PCR. For cloning to the pGEX system, each PCR product was gel-purified, digested with EcoRI and XhoI, and ligated into a pGEX-4T-1 vector. Each construct was sequenced using a dye terminator cycle sequencing core kit on a DNA sequencer.

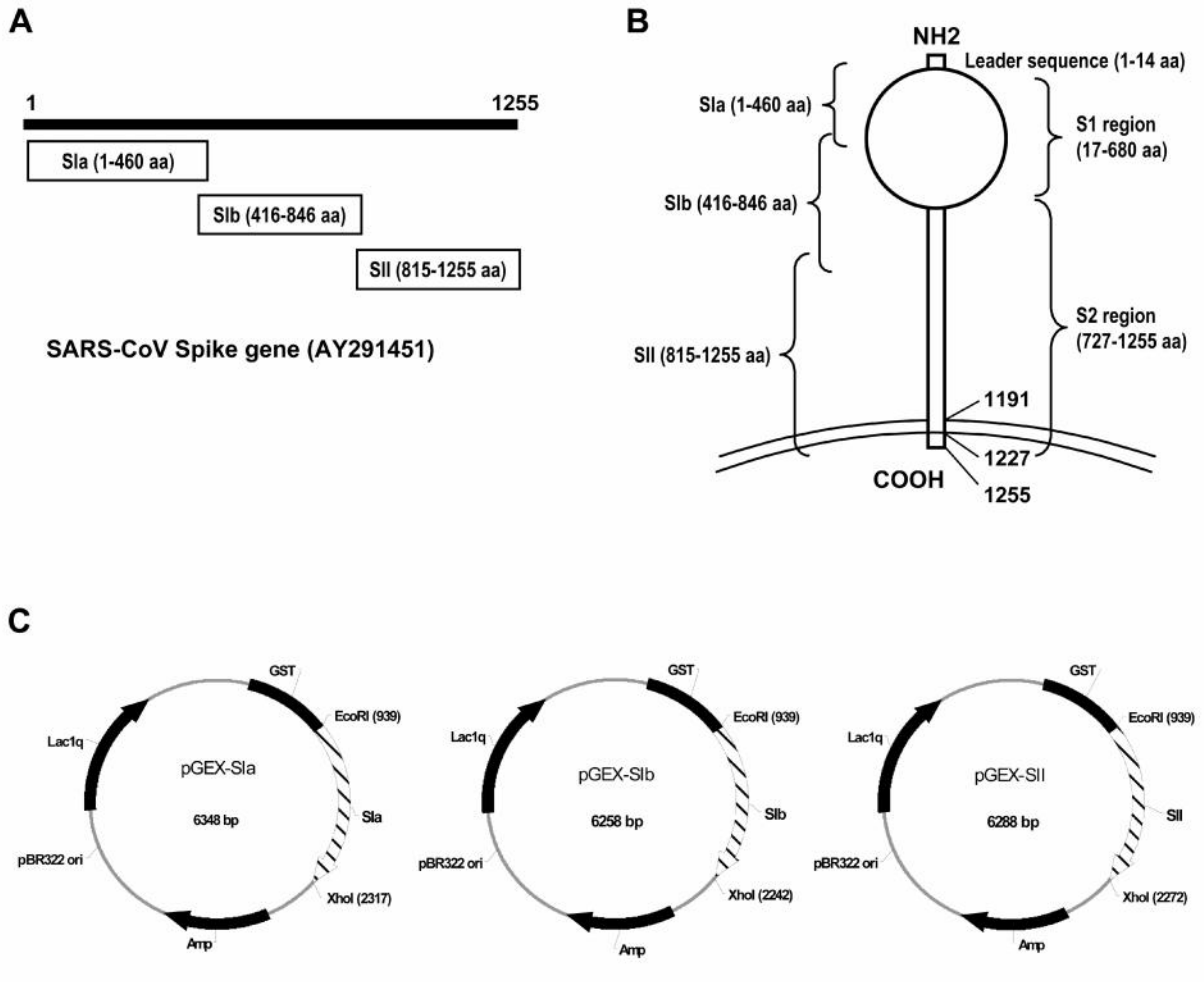
Figure 2. Construction of plasmids containing specific regions of the SARS-CoV nucleocapsid (N) gene. We cloned the N gene in a pGEX system for prokaryotic expression. Gene fragments were generated by one-tube RT-PCR. For cloning to the pGEX system, each PCR product was gel-purified, digested with EcoRI and XhoI, and ligated into a pGEX-4T-1 vector. Each construct was sequenced using a dye terminator cycle sequencing core kit on a DNA sequencer.

Figure 3. Antibody reactivity to SARS-CoV spike (S) and nucleocapsid (N) proteins using immunoblotting assays. Shown are results from immunoblotting assays of S and N recombinant proteins (arrows) detected by anti-S or anti-N mouse anti-sera (lane 2), SARS-positive sera (lane 1), or mouse control sera (lane 3).

Figure 4. FACS analyses of ACE2 and Fc Receptors. Raji and THP-1 cells were stained with mouse monoclonal antibodies against human CD16, CD32, CD64 and isotype controls. Cells were washed and incubated for 30 min at 4°C with

FITC-conjugated goat anti-mouse antibodies. Stained cells were analyzed by LSRII flow cytometry. Results shown are from one representative experiment out of three performed.

S-Figure 1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1
1255
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SIb (416-846 aa)
SII (815-1255 aa)
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B
NH2
Leader sequence (1-14 aa)
SIa (1-460 aa)
S1 region
(17-680 aa)
SIb (416-846 aa)
S2 region
(727-1255 aa)
SII (815-1255 aa)
1191
1227
COOH
1255
C
GST
EcoRI (939)
Lac1q
pGEX-SIa
6348 bp
SIa
pBR322 ori
XhoI (2317)
Amp
GST
EcoRI (939)
Lac1q
pGEX-SIb
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SIb
pBR322 ori
XhoI (2242)
Amp
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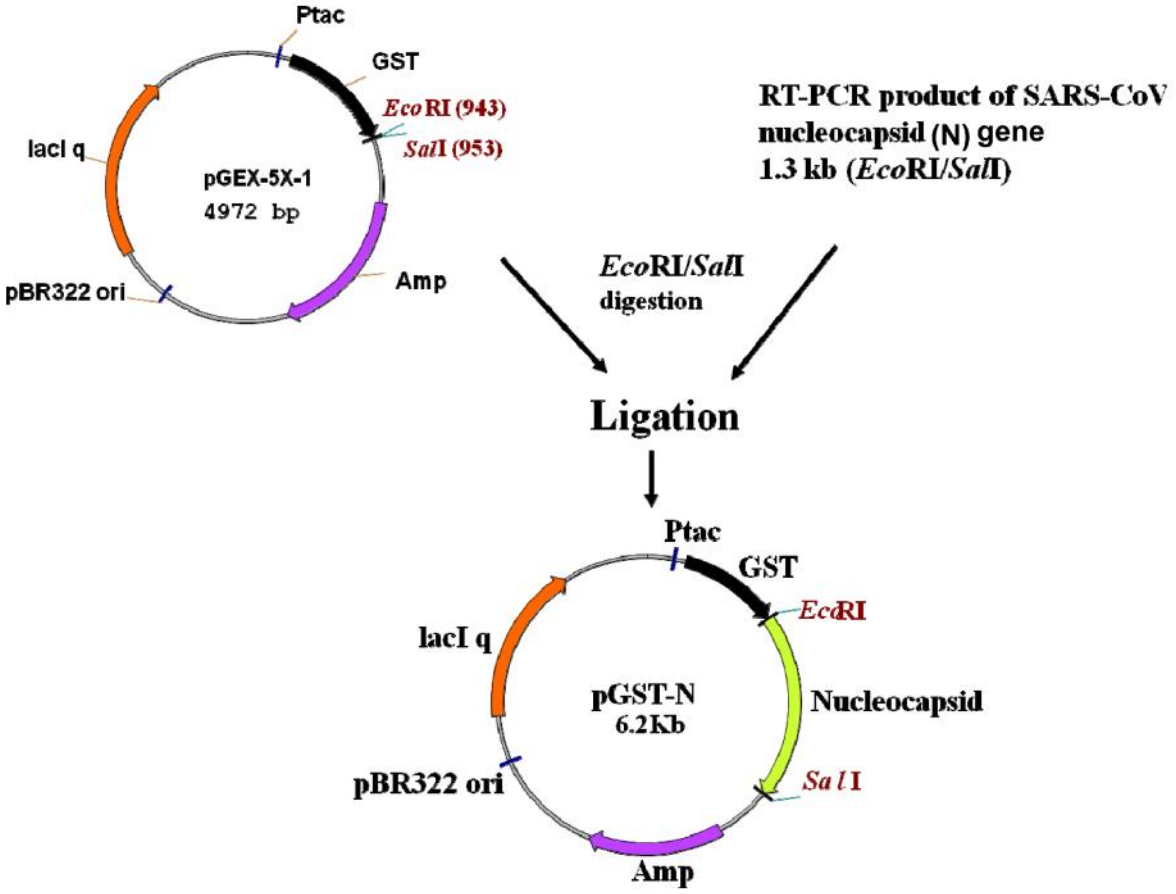
XhoI (2272)
Amp

S-Figure 2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EcoRI (943)
SalI (953)
lacI q
pGEX-5X-1
4972 bp
pBR322 ori
Amp
RT-PCR product of SARS-CoV
nucleocapsid (N) gene
1.3 kb (EcoRI/SalI)
EcoRI/SalI
digestion
Ligation
Ptac
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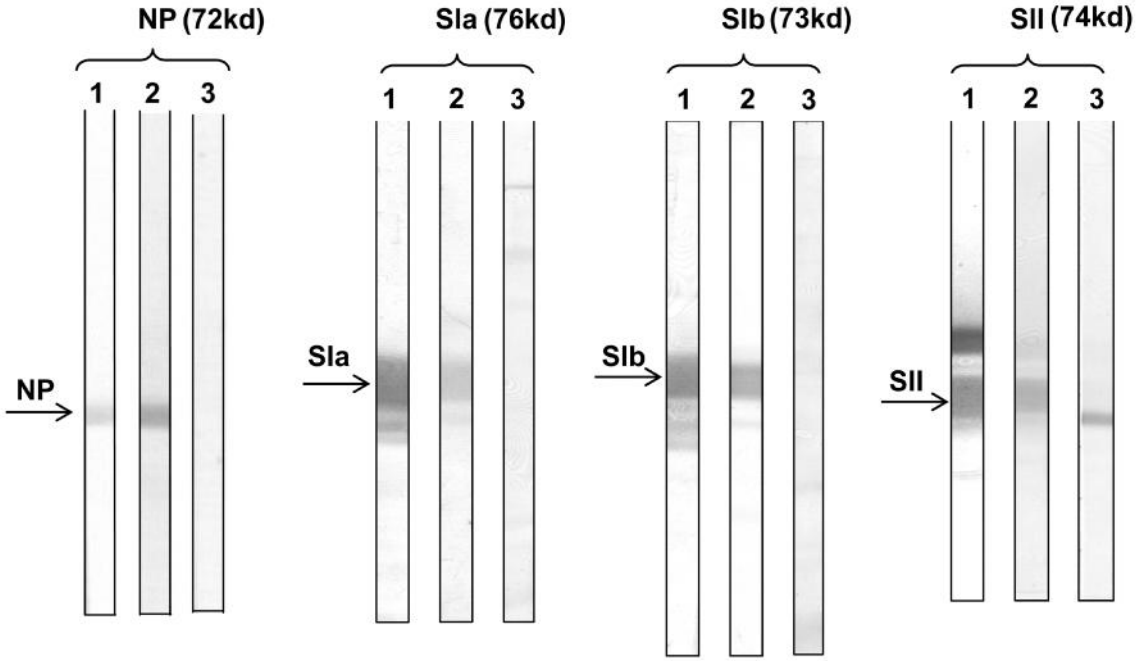
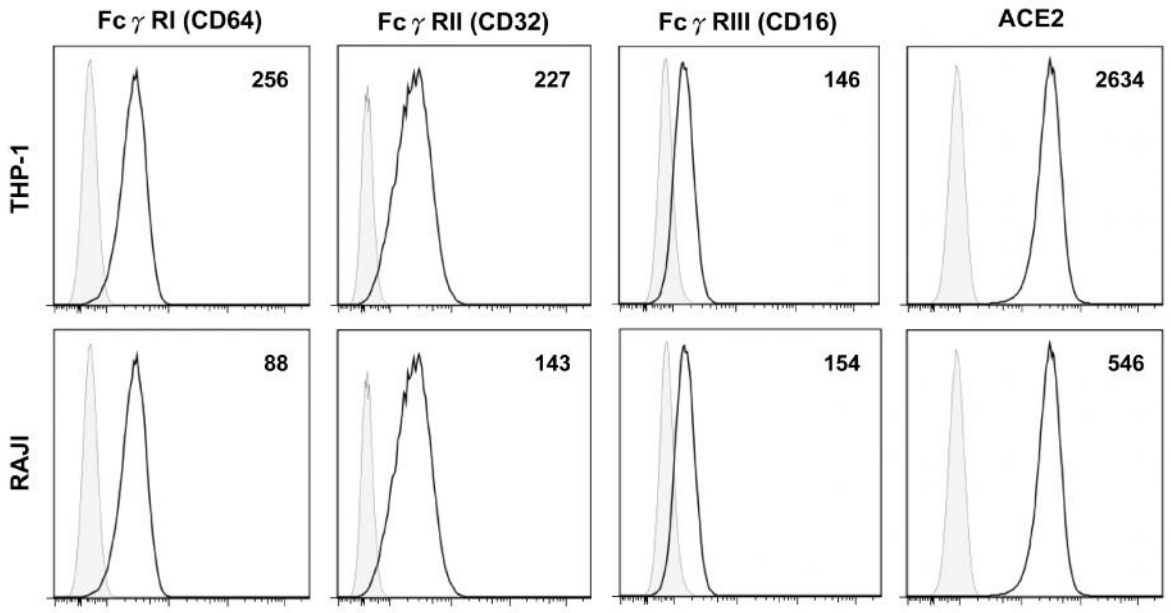
6.2Kb
Nucleocapsid
pBR322 ori
Sal I
Amp

S-Figure 3


NP (72kd)
1
2
3
NP
SIa (76kd)
1
2
3
SIa
SIb (73kd)
1
2
3
SIb
SII (74kd)
1
2
3
SII

S-Figure 4


Fc γ RI (CD64)
Fc γ RII (CD32)
Fc γ RIII (CD16)
ACE2
THP-1
RAJI
256
227
146
2634
88
143
154
546