SARS-CoV-2 neutralizing human antibodies protect against lower respiratory tract disease in a hamster model

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Summary:

Prophylactic treatment with a highly neutralizing MAb or convalescent plasma protects against SARS-CoV-2 infection in the lower but not upper respiratory tract.

Abstract

Effective clinical intervention strategies for COVID-19 are urgently needed. Although several clinical trials have evaluated the use of convalescent plasma containing virus-neutralizing antibodies, the levels of neutralizing antibodies are usually not assessed and the effectiveness has not been proven. We show that hamsters treated prophylactically with a 1:2560 titer of human convalescent plasma or a 1:5260 titer of monoclonal antibody were protected against weight loss, had a significant reduction of virus replication in the lungs and showed reduced pneumonia . Interestingly, this protective effect was lost with a titer of 1:320 of convalescent plasma. These data highlight the importance of screening plasma donors for high levels of neutralizing antibodies.

Our data show that prophylactic administration of high levels of neutralizing antibody, either monoclonal or from convalescent plasma, prevent severe SARS-CoV-2 pneumonia in a hamster model, and could be used as an alternative or complementary to other antiviral treatments for COVID-19.

Keywords: SARS-CoV-2, convalescent plasma, monoclonal antibody, hamster, pneumonia

MAIN TEXT

Introduction

On 31 December 2019, the World Health Organization (WHO) was informed of a cluster of cases of pneumonia of unknown cause in Wuhan City, Hubei Province of China [1]. Subsequently a novel coronavirus (SARS-CoV-2), was identified and as of April 12, 2021, WHO reported 135 million cases of SARS-CoV-2 infection worldwide, with 3 million deaths. SARS-CoV-2 infection is characterized by a range of symptoms, including fever, cough, dyspnea and myalgia [2]. In severe cases, SARS-CoV-2 infection can be complicated by acute respiratory distress syndrome leading to respiratory insufficiency and multi-organ failure [3]. An effective treatment is a high priority as SARS-CoV-2 continues to circulate in many regions, and there is a risk of additional future waves of infection. To date, WHO reported at least 166 vaccine candidates being in different stages of development while other efforts include the development of neutralizing antibodies for prevention and/or treatment of SARS-CoV-2 infection. Early during the outbreak, the usefulness of convalescent plasma transfusion was considered for treatment of severe cases [4]. Several large clinical trials have now been initiated to evaluate the efficacy and safety of convalescent plasma treatment of SARS-CoV-2 patients [5]. Data on the outcomes of these trials have been limited and to date, preliminary results from only a few small cohorts and randomized clinical trials have been published [6-10]. Overall, these data show that convalescent plasma treatment is safe and suggest that it can reduce disease if given early enough and with sufficient levels of antibodies [11]. Therefore, virus-neutralizing antibodies can be used prophylactically to prevent infection in high-risk cases, such as vulnerable individuals with underlying medical conditions which may not be vaccinated, health care providers, and individuals with exposure to confirmed cases of COVID-19.

Although preclinical research indicated a limited protective effect of hamster serum when given to hamsters infected with SARS-CoV-2 early in the disease course [12, 13], effects of human plasma have not been analyzed in this animal model. Importantly, data on the level of neutralizing antibodies that are required to provide a clinically meaningful protective effect are not available.

In addition to convalescent plasma, different human monoclonal antibodies (MAb) against SARS-CoV-2 have been identified and characterized, for prophylactic and therapeutic use [14-18]. We previously determined that MAb 47D11 efficiently neutralizes both SARS-CoV and SARS-CoV-2 *in vitro* [19]. In the present study, we used this MAb and two doses of human convalescent plasma, representing a high and median neutralizing antibody concentration, to evaluate the efficacy of prophylactic antibody treatment in a hamster model of moderate to severe SARS-CoV-2 pneumonia.

Materials and Methods

Viruses and cells

SARS-CoV-2 (isolate BetaCoV/Munich/BavPat1/2020) was obtained from a clinical case in Germany diagnosed after returning from China and propagated on Vero E6 cells as previously described [20]. All work was performed in a Class II Biosafety Cabinet under BSL-3 conditions at the Erasmus Medical Center (MC).

MAbs and convalescent plasma

We previously identified MAb 47D11 which efficiently neutralizes SARS-CoV-2 *in vitro* [19]. The irrelevant isotype control antibody used in this study was characterized previously[32].

Convalescent plasma was collected from donors who had a RT-PCR confirmed SARS-CoV-2 infection and were asymptomatic for at least 14 days [21]. Of all donors tested, only plasma

with neutralizing antibodies confirmed by a SARS-CoV-2 plaque reduction neutralization test (PRNT) and a $PRNT_{50}$ titer of at least 1:1280 was used. Equal volumes of plasma from 6 donors was pooled and used for prophylactic treatment in hamsters (High dose). In addition, the pooled plasma was diluted 10-fold in PBS (Median dose). Normal human plasma from a healthy donor was used as a control.

Animal procedures SARS-CoV-2

Animals were handled in an ABSL3 biocontainment laboratory (Supplementary Methods). Female Syrian golden hamsters (*Mesocricetus auratus*; 6-week-old hamsters from Janvier, France) were anesthetized by chamber induction (5 liters 100% O2/min and 3 to 5% isoflurane). 24-hour prior to inoculation with virus, groups of 8 animals were treated with either 3mg of MAb in 1mL or 500 µl human convalescent plasma via the intraperitoneal route.

Animals were inoculated with 10^5 TCID50 of SARS-CoV-2 or PBS (mock controls) in a 100 µl volume via the intranasal route. Animals were monitored for general health status and behavior daily and were weighed regularly for the duration of the study (up to 22 days post inoculation; day p.i.). Nasal washes, throat swabs and rectal swabs were collected under isoflurane anesthesia during the study. Groups of 4 animals were euthanized on day 4 or day 22 p.i., and serum samples, as well as lung, and nasal turbinates, were removed for virus detection and histopathology.

Serological Analysis

To test for SARS-CoV-2 antibodies, hamster serum samples were collected at days 4 and 22 p.i.. Serum samples were tested for SARS-CoV-2 antibodies using in-house spike S1 and nucleocapsid protein (N) ELISA or plaque reduction neutralization test using authentic SARS-CoV-2 as previously described [22] (Supplementary Methods). The serum neutralization titer is the highest dilution resulting in an infection reduction of >50% (PRNT50).

Virus detection

Samples from nasal turbinates and lungs were collected post mortem for virus detection by RT-qPCR and virus isolation as previously described [20]. The SARS-CoV-2 RT-qPCR was performed and quantified as copy numbers as previously published [23].

Histopathology and immunohistochemistry

For histological examination lung and nasal turbinates were collected. Tissues for light-microscope examination were fixed in 10% neutral-buffered formalin, embedded in paraffin, and 3 µm sections were stained with haematoxylin and eosin. Sections of all tissue samples were examined for SARS-CoV-2 antigen expression by immunohistochemistry as previously described [20] (Supplementary Methods).

Statistical analysis.

Statistical analyses were performed using GraphPad Prism 5 software (La Jolla, CA, USA). Each specific test is indicated in the figure legends. P values of ≤ 0.05 were considered significant. All data are presented as means ± standard error of the mean (SEM).

Results

Characteristics of neutralizing antibodies

We pooled 6 convalescent plasma samples from PCR-confirmed COVID-19 patients. The samples were selected based on a minimum neutralizing antibody titer of 1:1280 ($PRNT_{50}$; **Supplementary Table 1**). The neutralizing antibody titer of the pooled plasma as well as the diluted pooled plasma were determined to be 1:2560 (high dose) and 1:320 (median dose) respectively (**Supplementary Table 1**). Only ten of 115 convalescent plasma donors previously tested had a titer of 1:2560 or higher while the 1:320 titer of the diluted plasma was just above the median titer of 1:160 of all donors tested [21].

In addition, we used human MAb 47D11 directed against SARS-CoV, which cross-reacts with SARS-CoV-2 and targets a conserved epitope in the S1 domain, previously shown to neutralize SARS-CoV-2 with an IC_{50} of 0.57 µg/ml [19]. At a concentration of 3mg/mL the human MAb 47D11 preparation had an equivalent neutralizing antibody titer of 1:5260.

Neutralizing antibodies protect against body weight loss from SARS-CoV-2 infection

To date, the Syrian golden hamster is the only animal species in which experimental SARS-CoV-2 infection results in moderate to severe pneumonia, with clinical signs, as well as shedding of virus [12, 13, 24]. Therefore, the prophylactic potential of the 47D11 MAb and convalescent human plasma was evaluated in this hamster model. Twenty-four hours prior to inoculation with SARS-CoV-2, animals were treated with MAb 47D11 or human convalescent plasma from COVID-19 patients. Volumes of human plasma treatment were chosen to mimic the application in humans. Animals were treated via intraperitoneal administration with either 3 mg MAb in 1mL (equivalent of a $PRNT_{50}$ of 1:5260) or 500 µl human convalescent plasma (comparable to 300mL

of convalescent plasma treatment in an adult human, based on % of total blood volume in hamster versus human) containing either high ($PRNT_{50}$ 1:2560) or median ($PRNT_{50}$ 1:320) levels of SARS-CoV-2 neutralizing antibodies. Unfortunately, due to technical restrictions blood could not be obtained on day 0 to determine the circulating neutralizing antibody titer. There were three control groups, consisting of hamsters that were not treated prior to SARS-CoV-2 inoculation, and hamsters that were treated either with an irrelevant isotype control MAb or with normal healthy human plasma (not containing neutralizing antibodies to SARS-CoV-2; **Supplementary Table 1**) 24 hr before SARS-CoV-2 inoculation.

In line with earlier studies [12, 13], experimental SARS-CoV-2 inoculation via the intranasal route resulted in a transient but significant weight loss in untreated animals as early as 3 days p.i., approaching 20% weight loss by day 5 p.i. and normalizing by day 10 p.i. (**Figure 1A**). No other overt clinical signs were observed. Prophylactic treatment with MAb 47D11 or a high dose of convalescent plasma protected animals against significant weight loss between day 4-10 p.i., compared to controls (**Figure 1A**). In contrast, prophylactic treatment with the diluted convalescent plasma, control plasma, or control MAb did not protect against significant weight loss, with animals approaching 20% weight loss by day 5 p.i..

Minimal effect of prophylactic antibody treatment on SARS-CoV-2 shedding

SARS-CoV-2 inoculation of hamsters resulted in detection of viral RNA in throat swabs from all groups for up to 10 days p.i., with peak shedding between days 2 and 6 p.i. (**Figure 1B)**. In addition, viral RNA was detected in nasal washes for up to 10 days p.i. (**Figure 1C**). While animals were protected against weight loss following prophylactic treatment with either MAb 47D11 or high dose convalescent plasma, no significant reduction of viral RNA in throat swabs or nasal

washes was observed. Despite high levels of viral RNA in nasal washes for several days p.i., infectious virus could only be isolated on day 2 p.i. (**Figure 1D**) and no infectious virus could be detected in throat swabs. Interestingly, while no significant effect of treatment was found on viral RNA detection, both prophylactic treatment with MAb 47D11 and high dose convalescent plasma resulted in significant reduction of 1-2 logs in infectious virus in nasal washes on day 2 p.i. ($p<0.05$, ANOVA; **Figure 1D**).

Low levels of viral RNA were detected in rectal swabs on day 2 p.i. and occasionally at very low levels on other days in individual animals (data not shown). There was no significant difference in virus detection in rectal swabs between treated and control groups and no infectious virus was detected.

Prophylactic antibody treatment reduced SARS-CoV-2 replication in the lower respiratory tract

Virus replication in the lungs and nasal turbinates was examined on day 4 p.i. (**Figure 1E-H**). In the lungs, prophylactic treatment with MAb 47D11 or plasma with high neutralizing antibodies resulted in significant reduction of viral loads (both viral RNA, $p<0.01$ and infectious virus, $p<0.05$, ANOVA) (**Figure 1E and F**). In contrast, these prophylactic treatments did not result in a significant reduction of viral load in the nasal turbinates (**Figure 1G and H**).

Prophylactic antibody treatment reduces histopathological changes in the respiratory tract following SARS-CoV-2 infection

At necropsy on day 4 p.i., control treated hamsters had single or multiple foci of pulmonary consolidation, visible as well-delimited, dark red areas, and covering 50-90% of the lung surface (**Figure 2**). No gross lesions were observed in any of the animals treated with either MAb 47D11

or high dose of convalescent plasma. Lungs from animals treated with the diluted plasma, or control plasma/ control MAb showed similar lesions to untreated animals.

All animals, including the MAb 47D11 and high dose convalescent plasma groups, showed acute necrotizing and seropurulent rhinitis in the nasal cavity (**Supplemental Figure 1**). It was centered on the olfactory mucosa, where it was marked and locally extensive. It was characterized by edema in the lumen mixed with sloughed epithelial cells, neutrophils, and cell debris, and by the presence of a moderate number of neutrophils in the epithelium and underlying lamina propria. Many cells in the olfactory epithelium in all animals expressed SARS-CoV-2 antigen.

The main observation in the lungs of the non-treated animals and the animals treated with diluted convalescent plasma, control plasma, or control MAb was multifocal or coalescing diffuse alveolar damage, which was characterized by loss of histological architecture of the lung parenchyma, edema, fibrin, sloughed epithelial cells, cell debris, neutrophils, mononuclear cells, and erythrocytes (**Supplementary Figure 2**). By immunohistochemistry, many type I pneumocytes and fewer type II pneumocytes at the edges of the lesions expressed virus antigen. Prophylactic treatment with the 47D11 MAb resulted in a significant reduction of inflammation in the lungs ($p<0.01$, ANOVA; **Figure 3 and 4A**) and viral antigen expression in the lungs ($p<0.05$, ANOVA; **Figure 3 and 4B**). Although a reduction in inflammation and viral antigen was observed in the lungs of animals treated with high dose convalescent plasma, this was not statistically significant as compared to controls.

Following SARS-CoV-2 inoculation, all animals seroconverted by day 22 regardless of the treatment regimen (**Supplementary Table 2**). There was no significant difference in SARS-CoV-2 specific IgG titers among treatment groups with IgG titers of 1:12.800.

Discussion

Several studies have identified and characterized neutralizing antibodies against SARS-CoV-2 as a potential component of protective immunity [15, 19, 25-29]. However, to date, few studies have focused on evaluating the efficacy of antibodies to protect or prevent against SARS-CoV-2 infection or disease *in vivo*. Those studies focused mainly on clinical signs and infection in the lungs and demonstrated mixed results with reduction in virus replication but no protection against pulmonary lesions [13, 15], complicating the interpretation of data.

This study shows that prophylactic treatment with neutralizing antibodies prevents SARS-CoV-2 induced pneumonia in a hamster model. Animals prophylactically treated with a high dose of neutralizing antibodies 24 hours prior to challenge were protected against significant weight loss, did not show any gross lesions in their lungs and prophylactic treatment resulted in a very substantial reduction in lung inflammation and virus replication in the lungs.

In agreement with recent studies, we show that prophylactic treatment with neutralizing antibodies can protect against disease following SARS-CoV-2 infection [12-15]. While hamsters infected with SARS-CoV-2 showed no overt respiratory signs, they lose significant weight similar to what has been reported previously [13, 24]. Animals treated with high titers of neutralizing antibodies were protected against significant weight loss, did not show any gross lung lesions and had significantly less histological lesions and associated virus antigen expression in the lungs. Previous studies using convalescent hamster serum and MAbs showed that prophylactic treatment decreased virus replication in lungs similar to our findings, however the hamster serum did not protect against lung pathology [12, 13, 24]. This is likely due to the fact that a lower dose of neutralizing antibodies was used (1:427) than was efficacious in this study (1 ml of MAb with titer 1:5260, or 0.5 ml of convalescent plasma with titer 1:2560).

Using convalescent plasma with lower neutralizing antibody titers (0.5 ml of convalescent plasma with titer 1:320), but still comparable to the median neutralizing titer found in patients recovered from COVID-19 [21], the protective efficacy was completely annulled. From our study the minimal protective neutralizing antibody titer in 0,5mL human plasma is between 1:320 and 1:2560. However, extrapolation to the human setting should be done with caution and studies on the levels and kinetics of neutralizing antibodies observed in humans after treatment with convalescent plasma are needed. In the current study we inoculated animals with a high dose of virus and by a method that, ensures delivery of virus in the lower respiratory tract. While this results in a robust model of SARS-CoV-2 pneumonia, humans will most likely be exposed to a much lower level of virus. Nevertheless, these data highlight the importance of pre-screening convalescent plasma from donors prior to use for convalescent plasma treatment. Indeed, levels of neutralizing antibodies vary substantially between individuals with a recent study showing a median titer of 1:160 in convalescent plasma in 115 donors and 22% had a titer of 1:40 or lower [21]. The lower titers are more typically observed after mild or asymptomatic COVID-19 cases [22]; those that may actually act as plasma donor.

While prophylactic treatment resulted in protection against disease and reduced SARS-CoV-2 replication in the lungs, only a limited effect was found in the upper respiratory tract. The effect of antibody treatment on SARS-CoV-2 replication in the nose is generally not assessed in most studies but in two recent studies in the hamster model it was shown to be incomplete [14-18]. Previous studies with influenza virus have shown that serum IgG can diffuse into alveolar lining fluid, thus protecting the lung parenchyma against virus infection [30]. In contrast, the concentration of IgG on the surface of nasal mucosa is much lower. This suggests that treatment may protect against disease in the lungs but not virus transmission from the nose, since as little as 10 infectious virus particles can result in infection in hamsters [31]. Recent studies have

shown that SARS-CoV-2 can transmit between animals via both direct contact and air [13, 24, 32]. Similar to our study, infectious virus was only detected in nasal washes early during infection and the period in which virus could be transmitted to naïve animals correlated with the presence of infectious virus [24]. All animals treated with the MAb and convalescent plasma seroconverted, therefore, antibody based prevention of COVID-19 did not prevent the development of humoral immunity after SARS-CoV-2 exposure.

To date, the efficacy of prophylactic antibody treatment has not been evaluated in humans. Prophylactic treatment may be particularly interesting for high-risk cases, such as vulnerable individuals with underlying medical conditions which prevent them from getting vaccinated, health care providers, and individuals with exposure to confirmed cases of COVID-19. Several studies have reported on the possible efficacy and safety of therapeutic treatment with convalescent plasma in both small cohorts as well as a clinical trial, with variable and inconclusive results [7-10, 33, 34]. The main results from the small cohorts suggest a clinical benefit with improved survival and reduced virus loads [11]; however, given the limited information and lack of controls in some studies, adequately powered, randomized controlled trials are needed. Recently, two randomized clinical trials were prematurely terminated and did not result in a shorter time to clinical improvement [21, 33]. The effect of treatment may be limited due to use of convalescent plasma with low levels of neutralizing antibodies of at least 1:40 to 1:80. Furthermore, a recent study showed that most COVID-19 patients already have neutralizing antibody titers of 1:60 or higher at hospital admission [21], supporting our findings that only treatment with high levels of neutralizing antibodies may have a protective effect. In addition, in most studies, only severe cases of SARS-CoV-2 infection were included at the time when patients were admitted to a hospital for severe disease. At that time, the therapeutic window for antibody treatment may have passed since many patients with severe disease are

already resolving the virus infection in the lung while the observed severe disease is primarily due to an aberrant host response rather than virus infection. Hence for effective treatment, the timing and dosing of administered neutralizing antibodies is likely critical.

These challenges can be addressed by using purified and concentrated plasma derived antibodies or (combinations) of recombinantly produced MAbs. MAbs with desired properties can be selected from the immune repertoire of e.g. infected or immunized individuals with respect to binding affinity, potency and breath of neutralization. Moreover, antibody engineering allows to tweak the Fc-mediated immune effector functions and to improve MAb pharmacokinetics. However, the development of neutralization escape mutants remains a concern when using a single monoclonal antibody [26].

In conclusion, our data show that prophylactic treatment with a highly neutralizing MAb or convalescent plasma not only protects against weight loss and reduces virus replication in the lungs, it also limits histopathological changes in the lungs. In addition, we show that while prophylactic treatment may prevent disease, animals still become infected and shed virus, indicating that transmission will not be blocked. These data highlight the importance to include virus shedding, replication in lungs as well as clinical and pathological determinants of disease in evaluating the efficacy of antibody treatment. In contrast, while treatment with convalescent plasma with high neutralizing titers was protective, this effect was completely annulled when using the median neutralizing antibody dose found in recovered patients [21]. It is therefore crucial to select convalescent plasma from donors with high levels of neutralizing antibody.

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Figures

Figure 1. Effect of prophylactic neutralizing antibody treatment on weight loss and virus replication following SARS-CoV-2 infection in hamsters. A. Body weights of hamsters treated with antibodies were measured at indicated days after inoculation with SARS-CoV-2. SARS-CoV-2 viral RNA (B, C, E and G) or infectious virus (D, F and H) was detected in throat (B), nasal washes (C and D), lung (E and F) and nasal turbinates (G and H). The mean % of starting weight, the mean copy number or the mean infectious titer is shown, error bars represent the standard error of mean. n = 4. ** = $P<0.01$ and * = $P<0.05$, ANOVA compared to SARS-CoV-2 inoculated, untreated animals.

Figure 2. Gross pathological examination of the lungs of SARS-CoV-2 infected hamsters. Foci (arrowheads) of pulmonary consolidation in untreated SARS-CoV-2 infected animals (**A**) and animals treated with control MAb (**B**) or median dose plasma (**C**). Mock infected animals showed no gross pathological lesions (D). Protection against pulmonary lesions in hamsters treated with MAb 47D11 (**E**) and high dose plasma (**F**), similar to mock infected animals (**B**). Images are from representative animals of each treatment group.

Figure 3. Effect of preventive treatment with MAb or high dose convalescent plasma on severity of pneumonia and level of virus antigen expression in lung parenchyma of hamsters after challenge with SARS-CoV-2. Comparison of extent of histopathological changes (HE) and virus antigen expression (IHC) at four days after SARS-CoV-2 inoculation at low (2X) magnification (two left columns) and high (20X) magnification (two right columns) in hamsters treated 24 hours before virus inoculation with neutralizing antibodies (second, third and fourth rows) compared to no treatment before SARS-CoV-2 inoculation (first row) and sham inoculation (fifth row).

Figure 4. Quantitative assessment of histopathological changes and virus antigen expression in the lungs of SARS-CoV-2 infected hamsters treated with MAb or convalescent plasma. Percentage of inflamed lung tissue (A) and percentage of lung tissue expressing SARS-CoV-2 antigen (B) estimated by microscopic examination in different groups of hamsters at four days after SARS-CoV-2 inoculation. Individual (symbols) and mean (horizontal lines) percentages are shown. Error bars represent the standard error of mean. n = 4. ** = $P<0.01$ and * = $P<0.05$, 2-way ANOVA.

Figure 1

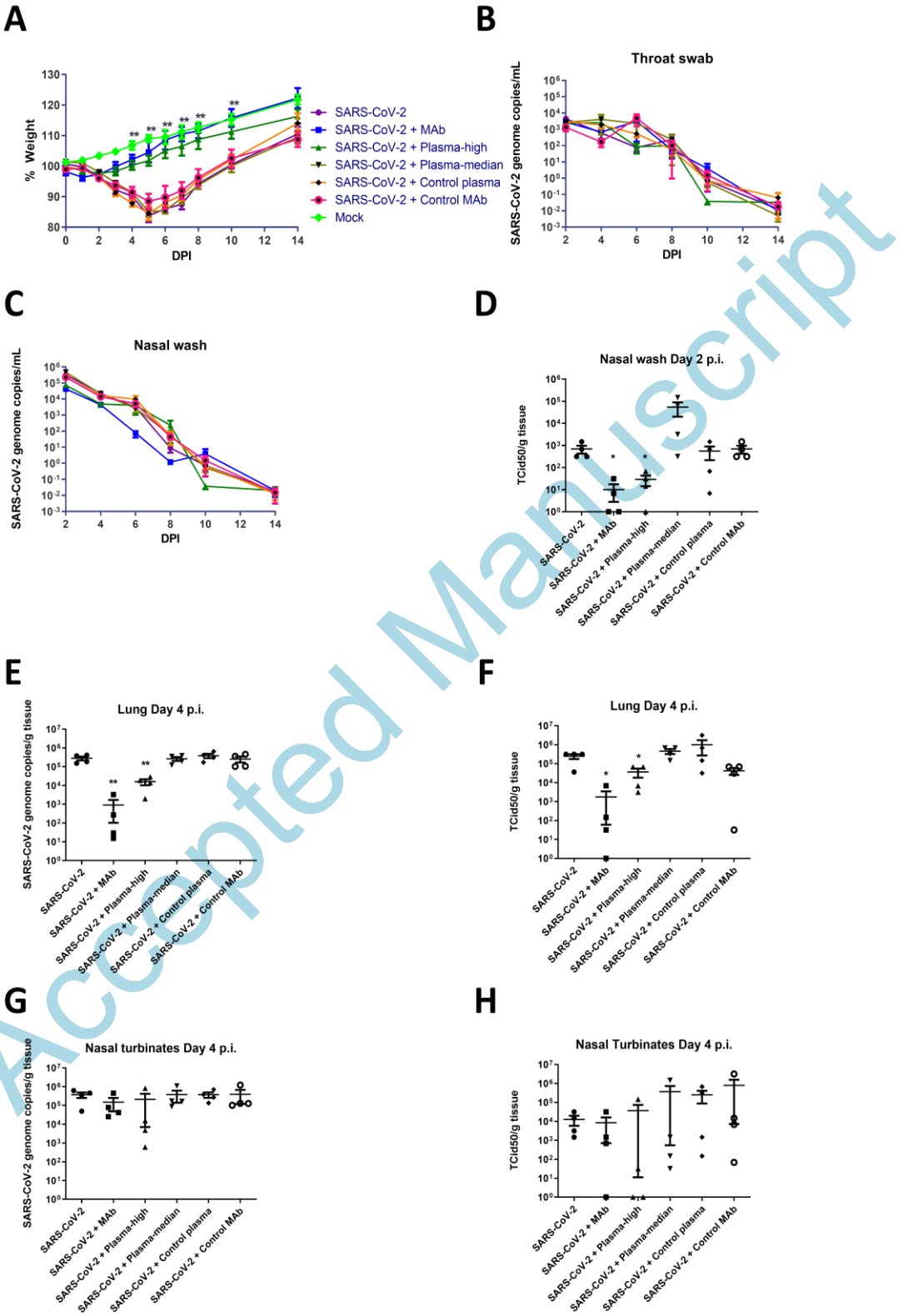

Figure 2

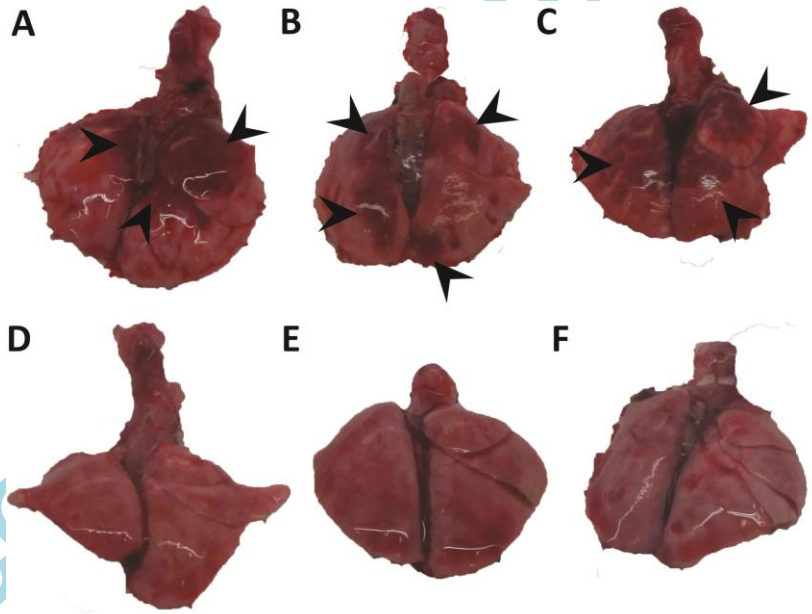

Figure 3

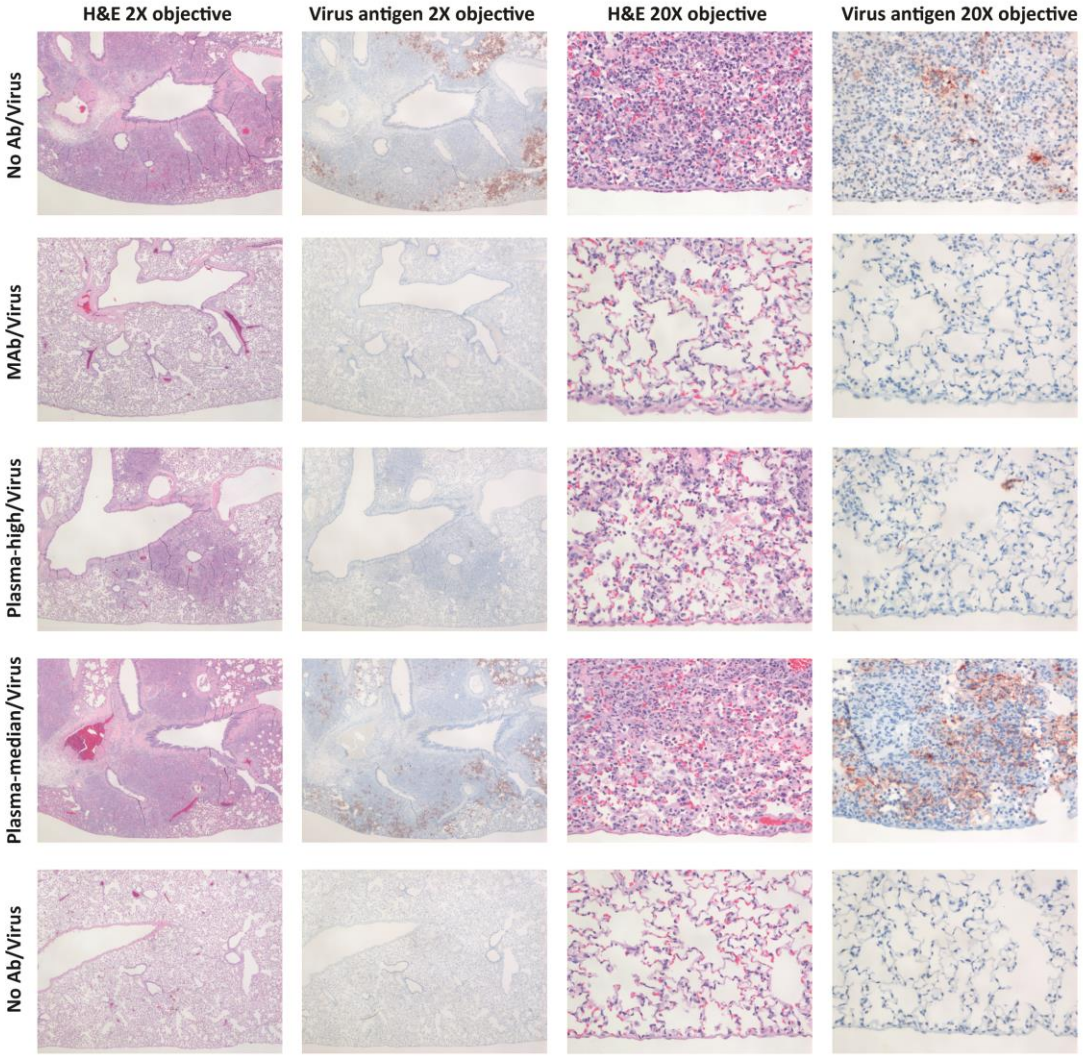

Figure 4