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ABO incomplete antibodies for COVID-19

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ABSTRACT

Aim: To investigate whether the infectivity of severe acute respiratory syndrome coronavirus 2 was affected by covering and hiding blood group antigens with incomplete ABO antibodies.

Methods: Incomplete antibodies were produced with using animal and human monoclonal/polyclonal antiA and AntiB antibodies. The aforementioned antibodies were converted to incomplete ABO antibodies through the utilization our patented method. The aforementioned incomplete antibodies were then incubated with the coronavirus in cell cultures. Moreover toxicity and effect of these two type incomplete antibodies were calculated.

Results: Incomplete antibodies obtained from animals was highly toxic when encountered with severe acute respiratory syndrome coronavirus 2 in cell culture, the cells could not divide and the process could not be performed. However, the incomplete form of antibodies obtained from humans exhibited markedly reduced or even no toxicity when encountered with severe acute respiratory syndrome coronavirus 2. Even so, the study could be done with human originated incomplete antibodies, these incomplete antibodies were not effective against severe acute respiratory syndrome coronavirus 2, meaning they could not be used for treatment and prevention purposes for COVID-19.

Conclusion: Human-derived incomplete antibodies did not completely eliminate the COVID-19 virus from infecting the cell, but slightly reduced it. Incomplete antibodies of animal origin are toxic to the cell. This study has shown that human-derived incomplete antibodies cannot currently be used as a treatment option for COVID-19, but since they are not toxic to the cell, and further studies can be carried out. The results of this study need to be supported by immunosuppressed animal studies.

Keywords: Incomplete, antibody, ABO, blood group, COVID-19.

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1. Introduction

Coronavirus disease 2019 (COVID-19) emerged in China in late December 2019. Severe acute respiratory syndrome coronavirus 2 (Sars-CoV-2) infection caused a global epidemic all over the world and in our country [1-2]. The World Health Organization (WHO) declared a pandemic in March 2020 [3]. The pandemic

resulted in the collapse of healthcare systems worldwide.

During the study period when vaccine studies against Sars-CoV-2 were continuing. Early studies showed that COVID-19 was more severe in patients with blood group A. On the other hand, it was observed that patients with blood group 0 were less likely to contract COVID-19 or their symptoms were milder [4-7]. It has been established through prior research that Sars-CoV-2 acts by binding to the angiotensin-converting enzyme 2 (ACE2) receptor, which is situated in the nasopharynx and lungs [8]. Moreover, previous studies have shown that some natural antibodies present in plasma can also bind to ACE2 and that these antibodies prevent the virus from binding to ACE2 [9]. In previous studies, the lower incidence of COVID-19 in people with blood group 0 was explained by the S protein of the virus combining with the ACE2 receptor and preventing the virus from entering the cell by anti-A antibodies, and by the presence of fewer ACE2 receptors in individuals with blood group 0 [10- 11].

On the other hand, it is known that blood groups also have an antigen, and these ABO antigens bind to the receptors of some viruses such as Sars-CoV-2, causing the infection to progress more easily in the lung and nasopharynx [12]. We showed in our previous study that these blood group antigens can be eliminated by coating them with incomplete antibodies. We used incomplete blood group antibodies to eliminate these antigens. In our previous in invitro study, we showed that cross-reactions are prevented by eliminating blood group antigens with this method [13]. We planned a study to see if we could make blood group antibodies incomplete and coat blood group antigens and at least prevent COVID-19 transmission. In this study, we investigated whether the infectivity of Sars-CoV-2 was affected by covering and hiding blood group antigens with incomplete antibodies produced by our patented method (Ozen M, Patent number: TR 2016 01910 B).

2. Materials and methods

Production of incomplete antibodies from animals: We used PierceTMF(ab´)2 Preparation Kit to produce incomplete antibodies from murine monoclonal antibodies (LorneMonoclonal IgM ABO bloodgroupingreagants) according to previous technique [13]. In order to obtain incomplete antibodies from mice, commercial monoclonal antibodies containing Anti-A and Anti-B were used.

The Production of incomplete antibodies from human sources is as follows: To obtain incomplete antibodies of human origin, first complete antibodies were obtained from fresh frozen plasma. For this purpose, blood group 0, which contains both Anti-A and Anti-B, was selected. After we treat pepsin with FFP in acidotic pH we have produced incomplete monoclonal antibodies similar to previous technique [13].

Quality control of both incomplete antibodies were done with blood group crossmatch tests. Only effective incomplete antibodies were evaluated with SARS-CoV2 virus in the cell culture. The aforementioned crossmatch tests were previously elucidated [13].

The antiviral tests of this study were conducted in the Koç University İşBank Research Center for Infectious Diseases (KUISCID) laboratory.

For cell study; 100 ul: 1/100, 1/1000 and 1/10000 monoclonal antibody and 100 ul virus were incubated at 37 ^{0}C for an hour in CO_2. Then, 200 ul of virus monoclonal antibody mixture was added onto E6 vero cells. A 2x96 well plate was planted. CPE was monitored at 24 hours and at the fourth and fifth days. At the 24th hour, it was

stained with anti-SARS-CoV-2 spike ab and immunofluorescence (IF) imaging was performed. Negative, positive and Mock groups were added.

During the Covid period when our study was conducted, data losses occurred due to problems in computer systems. However, this study was conducted with recovered data.

The study was carried out with the permission of the Bayındır Sogutozu Hospital Clinical-Experimental Research Ethics Committee (approval number: 8/2020.K-8, date: 04.01.2020).

3. Results

In this study, we obtained two blood group antibodies. One of the antibodies is the incomplete form of monoclonal antibodies obtained from mice, the other is an incomplete form of an antibody obtained from humans. It was observed that the incomplete state of monoclonal antibodies obtained from animals was highly toxic when encountered with Sars-CoV-2 in cell culture, the cells could not divide and the process could not be performed (Figure1). In the second stage, it was observedthat the incomplete form of monoclonal antibodies obtained from humans had much less or even no toxicity when encountered with Sars-CoV-2 (Figure2). As a result, it was seen that the study could be done, but these incomplete antibodies were not effective against Sars-CoV-2, meaning they could not be used for treatment purposes (Figure 3).

Figure 1. Image of cell study with monoclonal antibodies obtained from animals.

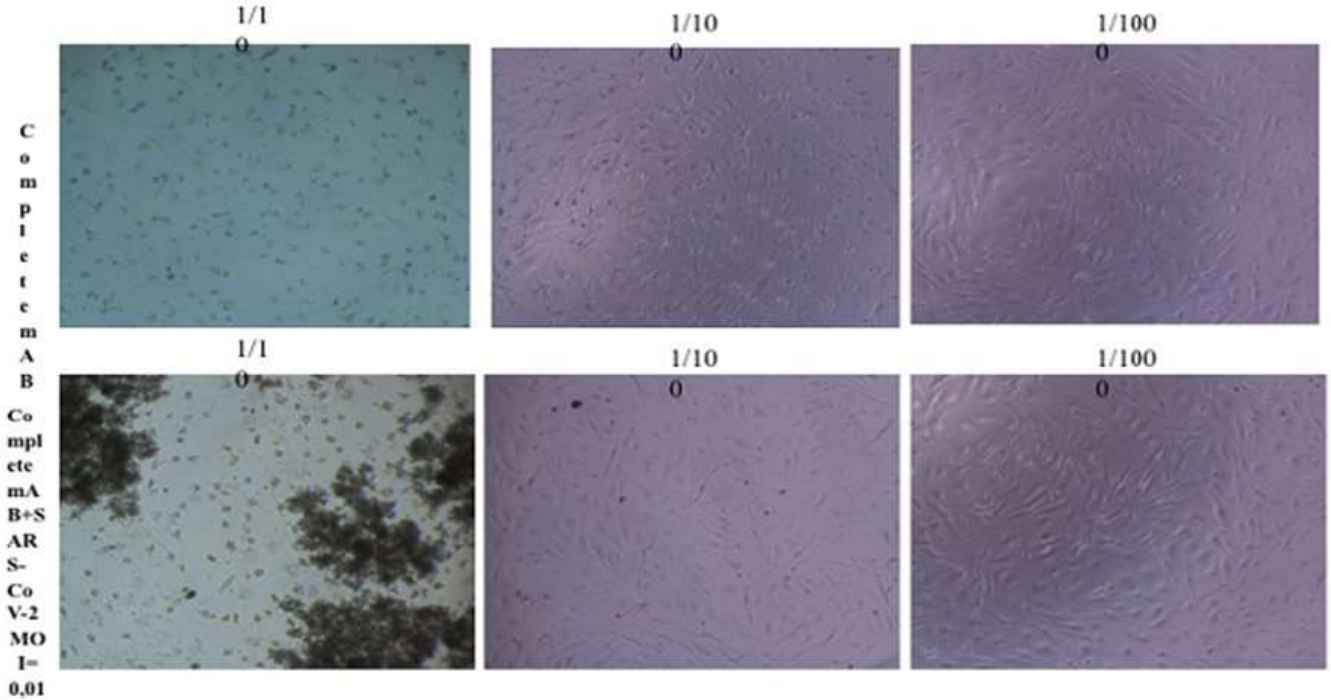
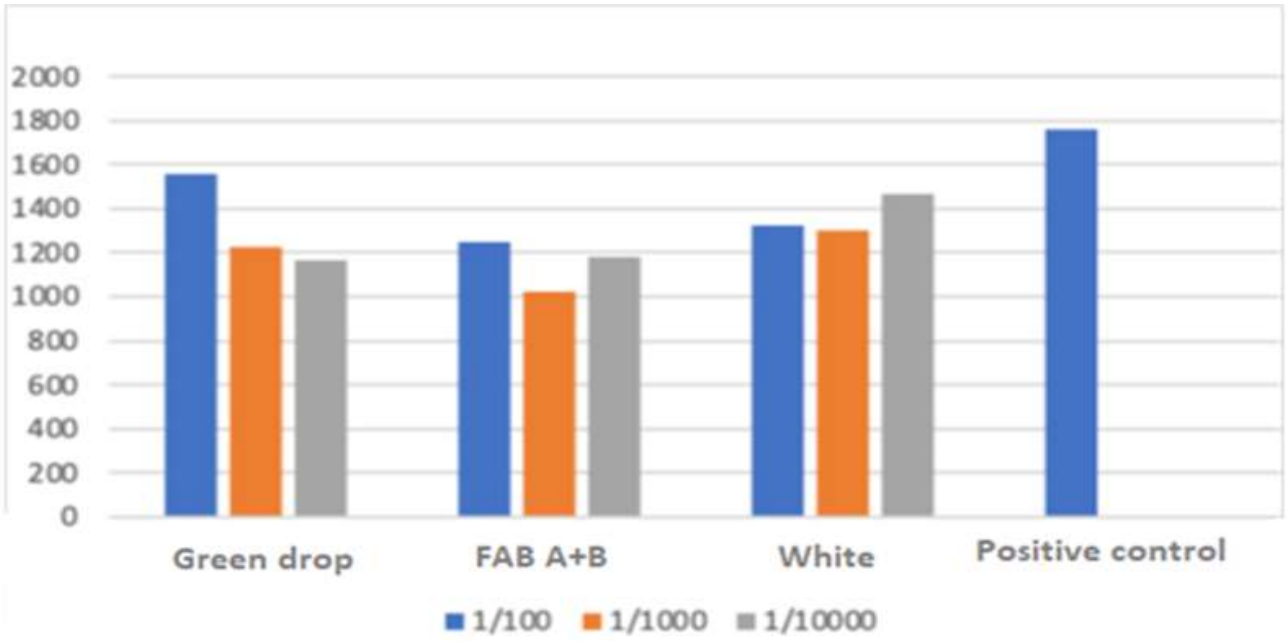


Figure 2. Image of cell study with monoclonal antibodies obtained from humans.



	1/100	1/1000	1/10000
Green drop	1556,5	1223	1162,5
FAB A+B	1245,5	1019	1182
White	1326	1298,5	1465,5
Positive control	1759		

Figure 3. Antiviral affectivity of human derived incomplete antibodies.

4. Discussion

The observation that Sars-CoV-2 has a milder course in individuals with blood group 0, particularly in those with blood group A, has been attributed to a number of different mechanisms. One of these is that the S protein of Sars-CoV-2 combines with the ACE2 receptor and prevents the virus from entering the cell by anti-A antibodies [14]. The other is that individuals with blood group 0 have fewer ACE2 receptors [15]. It has also been suggested that the mortality associated with thrombotic events during the course of COVID-19 is lower due to the fewer von Willebrand factor and factor VIII in blood group 0 [16].

Not only are ABO blood group antigens specific antigens found in the erythrocyte membrane, they are also expressed in airway and alveolar epithelial cells [17]. A previous study showed that blood group antigens serve as receptors for microorganisms in some infections [18]. In our study, we coated blood group antigens by using these incomplete monoclonal antibodies, which are still in the development stage. Thus, we aimed to slow down the transmission of coronavirus through these antigens expressed in airway epithelial cells and even its progression through these antigens synthesized in the alveolar epithelium.

Previous studies have shown that some infections are more severe in some blood groups

[18]. For example, Schistosomiasis infection is 3 times more common, especially in blood groups A and B, compared to blood group 0, and periportal fibrosis is 2 times more common [19-20]. It is known from previous studies that blood type 0 is protective against serious malaria infection [21]. Similarly, while COVID-19 progresses more severely in the presence of antigen A (blood group A), it is less mortal in the absence of antigen (blood group 0). Especially in people with blood groups A, B, AB, coating the blood group antigens using these incomplete antibodies can close the entrance to the body for some infections or make the infection less severe. The objective of this study was to identify an alternative treatment for COVID-19. It was observed that incomplete monoclonal antibodies obtained from animals were quite toxic in cell culture and therefore could not be used in other diseases. At least in this study, it was shown that human-derived incomplete antibodies can be used in other diseases because they are not toxic in cell culture. In this study, human incomplete antibodies did not eliminate the possibility of Sars-CoV-2 infecting cells, and the virus still continued to infect cells. Nevertheless, these incomplete antibodies slightly reduce the ability of the virus to infect these cells (by around 1/3), as seen in the positive control. Therefore, it appears that this approach may not be sufficient for use as a standalone treatment. Therefore, the study was terminated. Since monoclonal antibodies obtained from animals are toxic to the cell, it was not possible to investigate their effectiveness. In this study, it was predicted that incomplete monoclonal antibodies obtained from humans could not be used in standard animal studies due to species differences. On the other hand, since direct human studies cannot be carried out, instead of using standard animals, we can proceed with a specially designed immunosuppressed animal study. It is anticipated that human incomplete antibodies that bind blood group antigen may be employed in the future for the treatment of other diseases related to blood groups.

4.1. Study Limitations: The most important limitation of this study is that it is a cell culture study and does not reflect the functioning of the entire system. Animal studies are needed to support the study results. Furthermore, focusing solely on Sars-CoV-2 in this study limits the broader applicability of the findings to other viral infections.

4.2. Conclusion*:* As a result, we showed in this study that human-derived incomplete antibodies did not completely eliminate the covid virus from infecting the cell, but slightly reduced it. Monoclonal antibodies derived from animals have been shown to exert toxic effects on cells. This study has shown that human-derived monoclonal antibodies cannot currently be used as a treatment option for COVID-19, but since they are not toxic to the cell, further studies can be carried out. Consequently, human-derived incomplete antibodies may be suitable for use in the treatment of other diseases. The results of this study need to be supported by immunosuppressed animal studies.

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Conflict of Interest: *Mehmet Ozen has a patent (Patent number: TR 2016 01910 B). The other authors have no competing interests.*

Ethical Statement: *The study was carried out with the permission of the Bayındır Sogutozu Hospital Clinical-Experimental Research Ethics Committee (approval number: 8/2020.K-8, date: 04.01.2020).*

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