International Immunopharmacology 90 (2021) 107172

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

Short communication

Chicken Egg Yolk Antibodies (IgYs) block the binding of multiple SARS-CoV-2 spike protein variants to human ACE2

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ARTICLE INFO

Keywords:
Chicken Egg Yolk Antibodies
IgY
SARS-CoV-2
Spike protein variants
Neutralizing agent

ABSTRACT

The SARS-CoV-2 virus is still spreading worldwide, and there is an urgent need to effectively prevent and control this pandemic. This study evaluated the potential efficacy of Egg Yolk Antibodies (IgY) as a neutralizing agent against the SARS-CoV-2. We investigated the neutralizing effect of anti-spike-S1 IgYs on the SARS-CoV-2 pseudovirus, as well as its inhibitory effect on the binding of the coronavirus spike protein mutants to human ACE2. Our results show that the anti-Spike-S1 IgYs showed significant neutralizing potency against SARS-CoV-2 pseudovirus, various spike protein mutants, and even SARS-CoV *in vitro*. It might be a feasible tool for the prevention and control of ongoing COVID-19.

1. Introduction

The ongoing COVID-19 pandemic caused by severe acute respiratory syndrome-coronavirus SARS-CoV-2, a novel strain of coronaviruses, has rapidly spread and evolved since the end of 2019 [1]. To date, SARS-CoV-2 accounts for more than 40 million infections and more than 1.1 million COVID-19 - related deaths worldwide. Worryingly, there are still no available vaccines or antiviral drugs against the SARS-CoV-2.

Previous studies have demonstrated that the spike (S) glycoprotein homotrimer on the surface of SARS-CoV-2 plays an essential role in human ACE2 receptor binding and virus invasion [2]. Therefore, neutralizing antibodies against SARS-CoV-2 spike glycoprotein present the most promising approach against COVID-19. Besides, several neutralizing antibodies that target the receptor binding domain (RBD) of SARS-CoV-2 have been isolated from convalescent patients [3]. Despite the advancements, the use of monoclonal antibodies in the treatment of COIVD-19 faces a wide range of safety threats that are yet to be addressed [4]. Besides, the high production cost and low yield might complicate the use of the neutralizing antibodies, especially in the developing world. Therefore, there is need to explore other strategies that might be more economically suitable and feasible in the fight against COVID-19 prevention and control.

The first report about Egg Yolk Antibodies (IgY) as a neutralizing agent against tetanus toxin was published in 1893 [5]. Three years later, Behring and S. Kitasato discovered the diphtheria antitoxin (the 1901 Nobel Prize in Physiology or Medicine). The use of IgYs did not gain clinical significance and wide application until the advent of the 3Rs principle that was first described by Russell and Burch in 1959, The IgYs gained more attention for their stable chemical properties, low cost, high yield, and improved animal welfare. More importantly, IgYs neither bind the human rheumatoid factors, nor activate the human complement system, which minimizes the risks of inflammation [6]. As a passive immune agent against viral and bacterial diseases, IgYs have the potential to make functional foods and new drugs. Several IgY formulations have been approved to treat goose plague, duck plague, and other diseases by China Veterinary Pharmacopoeia. IgY antibodies have

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https://doi.org/10.1016/j.intimp.2020.107172
Received 15 September 2020; Received in revised form 24 October 2020; Accepted 30 October 2020
Available online 3 November 2020

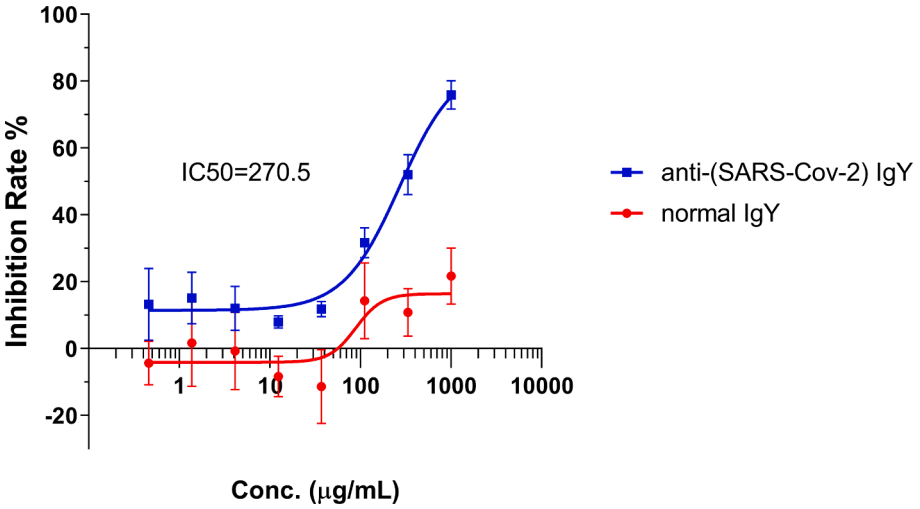

Fig. 1. Luminescence inhibition rate curve of the anti-(SARS-Cov-2) IgY (blue) and normal (control) IgY (red) from the pseudovirus neutralization assay. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

also been applied to combat human viral infections such as the respiratory syncytial virus (RSV), influenza virus, and Coxsackie virus. In one study, anti-SARS coronavirus IgYs were purified from chicken that were immunized with inactived SARS coronavirus, and the IgY antibodies were able to neutralize the SARS coronavirus both *in vitro* and *in vivo* [7].

Here, we purified anti-spike-S1 IgYs from hens that were immunized with the S1 domain of the SARS-CoV-2 spike protein and interrogated their ability to neutralize SARS-CoV-2 pseudovirus using Hela cells with overexpressed human ACE2. In addition, we used competition ELISA assays to validate the IgY's competitive binding to various SARS-CoV-2 Spike protein mutants, as well as the SARS-CoV Spike protein.

2. Materials and methods

2.1. Preparation and quantification of anti-S1 IgY

DNA sequence encoding S1 of SARS-CoV-2 Spike protein was codon-optimized and synthesized by GenScript USA, Inc (Supplementary Materials). The gene was then subcloned into pFastBac1 vector for Insect cell expression using Bac-to-Bac® Baculovirus system. The codon-optimized SARS-CoV-2 Spike-S1 was expressed in Sf9 insect cells using the baculovirus/insect cell expression system (Fig. S1). The purified recombinant SARS-CoV-2 S1 protein was mixed and emulsified with Freund's immune adjuvant in equal volume and then used as an immunogen. Each hen was injected (intramuscular) with 150 μg of the recombinant spike protein under the wings, once a week for 4 weeks, and then IgY was extracted and the titer evaluated. Here, we adopted an improved extraction as described by Sock HweeTan [8], with slight modification for subsequent processing. We removed lipids and lipoproteins, and then precipitated the supernatant with a final concentration of 15% cold ethanol, instead of ammonium sulfate. The purity of the extracted IgYs was more than 80%, without the ammonium sulfate residue and the process took less than 2 h (Fig. S2). Moreover, centrifugation could also be replaced with filtration, which makes the extraction process more suitable for large-scale industrial production. The extracted IgYs titer was quantified by indirect ELISA. Briefly, the ELISA plate wells were coated with the recombinant SARS-CoV-2 Spike-RBD protein expressed in HEK 293 cells, then serial dilutions of IgYs were added to the wells, and 1:10000 dilution of HRP-conjugated goat anti-IgY antibody was added.

2.2. Pseudovirus neutralization assay

The blocking potency of IgYs on the SARS-CoV-2 pseudovirus was evaluated by luciferase-generated luminescence. Here, Hela monoclonal cells with overexpressed ACE2 were infected with the lentivirus carrying SARS-CoV-2 spike protein and the luciferase reporter gene (GenScript Co., Nanjing, China). The IgYs' ability to neutralize the antigen was

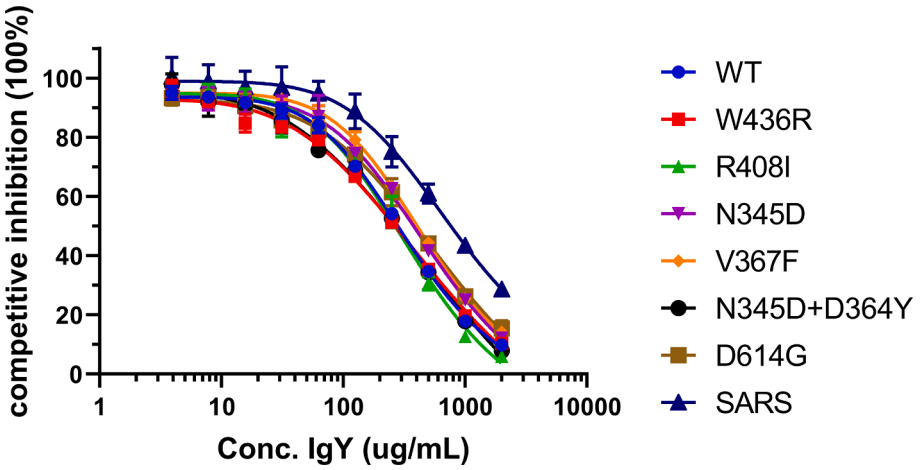

Fig. 2. Competitive inhibition of the eight coronavirus spike protein mutants as determined by competitive ELISA.

evaluated by performing the pseudovirus neutralization assay, as reported by the luciferase reporter gene (Supplementary Materials).

2.3. Competition ELISA

We used a competition ELISA to evaluate the ability of the IgYs to inhibit binding of eight different coronavirus spike protein mutants (including seven SARS-CoV-2 spike proteins and one SARS-CoV spike protein) to the human ACE2. The SARS-CoV-2 RBD or RBD mutants (Table S2) were incubated overnight at 4℃ in high bind 96 well plate. A serial dilution of purified the IgY and 0.3 ng/well Fc tagged human ACE2 (Cat. No. AC2-H5257, ACROBiosystems) were added into the coated plate and then incubated for 1 h at 37 ℃. HRP-conjugated anti-human Fc (1:20000) (Cat. No. 109-035-098, Jackson ImmunoResearch) was added as the secondary antibody. The OD450 were read by plate reader. All data were analyzed using GraphPad Prism 8.

3. Results

The results showed that the ELISA titer of IgYs reached 2 [10] after the third booster. The pseudovirus neutralization assay data showed that the IC50 values for the anti-(SARS-Cov-2) IgYs was 270.5 μg/mL, with maximum inhibition of 75.86%. On the other hand, the control IgYs had no obvious inhibitory effect, indicating that the anti-(SARS-Cov-2) IgYs had a neutralizing activity (Fig. 1). However, compared with the reported monoclonal antibodies, the IC50 value for the IgYs was relatively high. We associated this phenomenon with the fact that, like the other polyclonal antibodies, only about 10% of the IgYs specifically recognized SARS-CoV-2, and the proportion of IgYs with neutralizing activity was even lower. Whereas the IC50 for the polyclonal IgYs was high, theoretically, the IgYs should have multiple sites for the neutralizing activity.

Anti-(SARS-Cov-2) IgYs showed obvious competition with ACE2 in binding both the wild type SARS-Cov-2 (IC50 = 309.9 μg/mL) and SARS-Cov (IC50 = 617.9 μg/mL) spike proteins. Besides, IgYs also showed competitive binding to the six SARS-Cov-2 spike protein mutants [9] (W436R, R408I, N345D, V367F, N345D/D364Y, and the more dominant mutant D614G) [10] with an IC50 range of 324.0−490.9 μg/mL (Fig. 2).

4. Conclusion

In summary, the anti-Spike-S1 IgYs showed significant neutralizing potency against SARS-CoV-2 pseudovirus, various S mutants, and even SARS-CoV *in vitro*. However, the safety and efficacy of the IgYs still needs further interrogation in animal models.

At present, the SARS-CoV-2 virus is still spreading around the world, and there is much to be done to prevent and control the pandemic. The use of IgYs in aerosol or spray formulations on the respiratory tract, the oral cavity, and even the digestive tract may be a worthwhile strategy. It might prevent the invasion of the SARS-CoV-2 virus through the natural infection route. Long-term control of the SARS-CoV-2, however, will require a combination of active and passive immunization tools, drug therapy, and other preventive measures.

Funding

This work was supported by grants from the Natural Science Foundation of Jiangsu Province (Grants No BK20180224) and Natural Science Foundation for Young Scientists of Jiangsu Province (Grant No. BK20160362).

CRediT authorship contribution statement

Shuangshi Wei: Conceptualization, Methodology, Formal analysis, Writing - original draft, Funding acquisition. **Shengbao Duan:** Methodology, Writing - review & editing. **Xiaomei Liu:** Formal analysis, Writing - original draft. **Hongmei Wang:** Software. **Shaohua Ding:** Software. **Yezhou Chen:** Validation. **Jinsong Xie:** Software. **Jingjing Tian:** Validation. **Nong Yu:** Resources, Methodology, Investigation. **pingju Ge:** Methodology. **xinglin Zhang:** Investigation. **Xiaohong chen:** Validation. **Yong Li:** Conceptualization, Supervision, Funding acquisition. **Qinglin Meng:** Methodology, Funding acquisition.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.intimp.2020.107172.

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