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MODIFIED ANTI-IDIOTYPE IMMUNIZATION/EXPERIMENTAL STUDY

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ABSTRACT

Coronavirus is a single-stranded RNA positive (+ssRNA) virus from the Nidovirales, Coronaviridae family and Orthocoronavirinae subfamily. The coronavirus that was first isolated from the lower respiratory tract of patients with pneumonia in Wuhan, China is a new type of coronavirus (SARS-CoV-2) belonging to the β genus and subgenus sarbe. It is different from zoonotic MERS-CoV and SARS-CoV. The S proteins on its surface form a rod-shaped structure and, as antigenic proteins, are the main targets in the typing of the virus. It has been reported that SARS-CoV-2 S-protein had a strong interaction with human angiotensin-converting enzyme 2 (ACE2) molecules, leading to the infection of humans with the virus by firstly using the S-protein-ACE2 binding pathway. Today, the world is in a fight against the pandemic and scientists are looking for effective treatments and safe vaccines. Currently, there are no vaccines or medicines for its treatment and the WHO prioritized the vaccine studies for the virus. Although there have been reports of promising results with the antimicrobials used against other disease agents to treat infected individuals for a short period, there hasn't been a report of exact results in the literature. This study investigates vaccine activity in the immunization trials by producing idiotype antibodies through stages following the injection of antibodies that were collected from individuals who recovered from the disease into laboratory animals. Anti-idiotype antibodies (Ab2) are produced by re-administering the antibodies that were prepared in a laboratory animal against a specific agent (antigen) to a different laboratory animal. They can be used in vaccination and their use can allow protective immunity. The second antibodies (Ab2) have the same internal image as the epitope of the antigen given to the animals and stimulate the synthesis of protective antibodies. We detected antibodies in the blood samples that were collected from individuals who were treated for the infection or had an inapparent infection using the Coronavirus disease (Covid-19) IgM/IgG Antibody Rapid Test (Colloidal Gold) (Beijing Hotgen Biotech Co. Ltd., China) kit. We developed antibodies from rabbits, rats and mice (Ab2) against the antibodies obtained from human plasma (Ab1). We determined that the second animal group developed immunization against the Ab2s produced in the laboratory animals. In conclusion, considering the risk of directly preparing a vaccine from the Covid-19 agent with a very high rate of infection and spread, we are of the opinion that developing anti-idiotype vaccines against this type of infections and cultivating sufficient amounts of Ab2 antibodies are of great benefit to public health.

INTRODUCTION

Vaccine studies have been on the agenda ever since the first case of Covid-19 was detected in China. Covid-19 is a pandemic that caused the death of tens of thousands of people and today (2019-2020), continue to have a severe impact worldwide. In parallel with every pandemic that scared the world, studies on the vaccines of the pandemic have promptly begun and some vaccines were found and offered for use while others were analyzed for years. (1-9)

Vaccines are biological materials that stimulate immune system cells in humans and animals to protect them against infections. Animals and humans are immunologically protected in two ways: active immunization and passive immunization. The innate natural resistance is the first agent in body's defense. The history of active and passive immunization goes back as far as microbes. The vaccines that were used early on were inactive vaccines. Due to failure in achieving desired levels of immunization, active vaccines have come to the forefront. Inactive vaccines are prepared by killing microorganisms with various methods. Active vaccines are prepared by eliminating the virulence of infectious agents such as virus or bacteria, in other words, by attenuating microbes. Although immunization with attenuated vaccines is much stronger than that with inactivated vaccines, it has a one-in-a-million risk of infection. Studies have been carried out to eliminate their risk of infection. Today, these studies are rapidly and intensely carried out. Biotechnological vaccines that are prepared away from the infective genes of microorganisms only contain the gene sequence coding the protein that stimulate the immune system. They are safer than conventional vaccines, but their cost is much higher. Recently examined vaccines that are prepared using advanced technologies and genetic engineering are completely free from the risk of infection. Synthetic peptides, anti-idiotype antibodies, subunits, recombinant mutants are among vaccine types that are produced using genetic engineering and advanced technologies. These vaccines are prepared using state-of-the-art technologies and the mutants and recombinant mutants that were created by manipulating the genomes of agent microbes and their products are used as vaccines.

In anti-idiotype vaccines, the antibodies (Ab1) produced in a laboratory animal against a microorganism (antigen) are re-injected into a different laboratory animal to produce second antibodies (Ab2) against the first antibodies produced in the first animal. The antibodies produced in the second animal are referred to as anti-idiotype antibodies. They are used as antigens to develop immunization. Anti-idiotype antibodies are similar to the epitope of the first injected microorganism and stimulate the production of protective antibodies. The risk of infection in anti-idiotype vaccines is zero. Thus, they can be used safely for immunization against pathogens for which a vaccine was not developed. (10-14)

The virulence of the agent of Covid-19, which maintains its impact and causes numerous deaths worldwide, can be low or quite high. The production of the agent and directly preparing a vaccine from the agent pose risks. Thus, vaccine production studies that use its products are common. As of today (21/05/2020) when our study is carried out, there hasn't been a report of a Covid-19 vaccine that is available for use. As individuals who are among those trying to produce vaccines around the world, we planned a modified anti-idiotype antibody immunization study against Covid-19 by changing the anti-idiotype antibody vaccine preparation method with no infection risk.

MATERIALS AND METHOD

The blood samples were collected 22 days after treatment from patients who were treated for Covid-19. They were also collected from patients who recovered from the disease without hospitalization and developed antibodies. Then, the samples were centrifuged to separate their plasmas and serums. The currently available rapid test kit, Coronavirus disease (Covid-19) IgM/IgG Antibody Rapid Test (Colloidal Gold) (Beijing Hotgen Biotech Co. Ltd.,

China), was used to investigate the presence of antibodies. The samples that were positive for antibodies without dilution were manually diluted with sterile distilled water and re-analyzed. The serums and plasmas that tested positive at the highest titer (1/300) were identified.

Antibody purification:

The samples with high antibody levels were precipitated with ammonium sulfate and majority of undesired proteins was removed. (15) The plasma and serum samples were collected using the PPD injector from the bottom to the top of the tubes and about 1-2 cm from the top and intramuscularly administered to the animals.

Animal experiments:

We included five of each animal group comprising rabbits, rats and mice. In immunization using anti-idiotype antibody method, the infection agent is given to the laboratory animal as antigen to obtain antibodies called Ab1. The Ab1s are then given to a different animal species to produce the second antibodies, namely Ab2s, against Ab1s. The Ab2s are used as antigens to produce vaccines. (16,17)

In this study, the antibodies (Ab1) that are produced due to the agent of Covid-19, SARS-CoV-2, were directly obtained from humans. Three different animal species comprising rabbits, Wistar rats and BALB mice were used. The antibodies collected from humans were separately given at 10-day intervals in the amounts of 0.7 ml, 0.3 ml and 0.05 ml to rabbits, rats and mice, respectively. Twenty-eight days after the first injection, the presence of antibodies against the Ab1s was examined using lam agglutination with the positive human plasmas we had. The antibodies were purified from the Ab2-positive animal bloods using the same method. At 10-day intervals, the Ab2s obtained from the rabbits were transversely injected twice into rats and mice (new animals); the Ab2s obtained from the mice were transversely injected twice into the rabbits and rats; the Ab2s obtained from the rats were transversely injected twice into the rabbits and mice. The presence of antibodies in the blood samples collected 28 days after the first injection (against SARS-CoV-2 and Ab2 similar to its epitope) was investigated using Ab2-positive plasma and the Coronavirus disease (Covid-19) IgM/IgG Antibody Rapid Test (Colloidal Gold) (Beijing Hotgen Biotech Co. Ltd., China) kit.

RESULTS

We detected antibodies in the blood samples that were collected from patients who were treated for the infection or had an inapparent infection using the ‘Coronavirus disease (Covid-19) IgM/IgG Antibody Rapid Test (Colloidal Gold) (Beijing Hotgen Biotech Co. Ltd., China) kit. We produced antibodies in rabbits, rats and mice (Ab2) against the antibodies collected from human plasmas (Ab1). We discovered that the second animal group developed immunization against the Ab2s developed in the laboratory animals.

DISCUSSION

The blood samples that were collected from the treated patients and those who had mild symptoms and recovered without treatment were analyzed in the Atatürk University Medicinal Microbiology Laboratory. The antibodies produced against Covid-19 were detected. These antibodies are the equivalents of the Ab1s that were obtained in the anti-idiotype vaccine method. In other words, they are similar to the Ab1s, which were produced against the agent in the laboratory animals, and were produced in humans after infection. Ab1s were injected to phylogenetically different rabbits, rats and mice to produce Ab2s. The

Ab2s are antibodies of Ab1s and they are anti-idiotype antibodies that are similar to the epitope of SARS-CoV-2, agent of Covid-19 (18-20).

Each stage of our four-stage study was carried out with utmost care using available technical means. We will be able to produce denser and purer Ab1 antibodies, which were obtained from humans, and Ab2 antibodies, which are similar to the epitope of SARS-CoV-2, in following days as more sensitive test kits are made available. The development of antibodies in humans was observed to be weak at the beginning of the pandemic. In following days in Erzurum, antibody development especially in those who did not receive any treatment and recovered with mild clinical manifestations was pleasing and indicated the presence of either a natural or acquired resistance in the local community. Looking at the stages of our study, the simple injection of pure antibodies, which were purified within the bounds of available means, and Ab2 without the addition of supportive adjuvants (21-25) into animals yielded considerably promising results in terms of *in vivo* immunization. With the hope and courage this study has given us, we are sure that using more technologically advanced and sensitive tests and supportive adjuvants will yield better results.

In conclusion, considering the risk of directly preparing a vaccine from the Covid-19 agent with a very high rate of infection and spread, we are of the opinion that developing anti-idiotype vaccines against this type of infections and cultivating sufficient amounts of Ab2 antibodies are of great benefit to public health.

We will carry out vaccine production at an industrial scale in 4.6-L bioreactors in further studies by combining monoclonal antibody types that will be identified in cell culture medium with myeloma cells using hybridization.

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