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Institution Name MOGAM Biotechnology Research Institute

Name of the relevant department, unit, section or area of the institution

Vaccine Unit

City Yonginsi

Country REPUBLIC OF KOREA

Reference KOR-28

Title WHO Collaborating Centre for the Research and Development of Vaccines and Diagnostic Reagents

Number

Report Year 11/2009 to 11/2010

1. Implementation of the work plan. For each main activity briefly explain how the activity was implemented, the outcome and impact and, if available, the results of the evaluation (e.g. evaluation of a course by the participants). Also explain difficulties (if any). Do not provide technical results in this form (technical results, if applicable, are to be sent directly to the WHO Department you work with).

Activity 1 Development of a pre-pandemic influenza virus vaccine

Explanation

(PI: Dr. Gyu-jin Woo, Research period: 2005-2013)

To develop an efficient vaccine against pandemic influenza virus (H5N1), we are developing an egg-based inactivated whole virion vaccine with apathogenic H5N1 virus (NIBRG-14).

The protective efficacy of pre-pandemic influenza viruses (H5N1) was confirmed in ferrets by challenging them with wild-type H5N1 viruses (A/Vietnam/1203/04 or A/Indonesia/5/05 strains). In addition, their safety was confirmed in both rats and dogs by using non-clinical toxicity tests. On the basis of these results, we obtained approval for a clinical trial of an IND (Investigational New Drug) from the Korea Food and Drug Administration in November 2009.

This year, we conducted the phase I trial to evaluate the safety and efficacy of the H5N1 vaccine. This trial was performed with 40 healthy volunteers in a randomized, double-blind, placebo-controlled dose escalation study with 4 cohorts. In the first cohort, 8 individuals were administered a 3.75 µg dose and 2 individuals were given a placebo. After reviewing the data for 1 week, the second cohort was started and another 8 individuals were administered a 7.5 µg dose and 2 individuals were given a placebo. The third and fourth cohorts were performed in the same manner with 15 µg and 30 µg doses, respectively. The vaccine was administered intramuscularly and a second dose was given 3 weeks later. On days 0, 21, and 42, serum samples were collected to assess the antibody responses to the vaccine.

The safety data showed that all vaccine formulations were well-tolerated and there were no serious adverse events. Antibody responses against H5N1 were observed in the 15 µg and 30 µg dose cohorts, as shown by hemagglutinin inhibition assays.

As a result, we plan to conduct the phase II trial, and if it is successful, to stockpile the vaccine.

This work has been funded by the Korea National Institute of Health.

Activity 2 Development of a CTL-based therapeutic vaccine for chronic hepatitis C

Explanation

(PI: Dr. Yukyeong Hwang, Research period: 2004-2013)

Non-A non-B hepatitis, which is the leading cause of chronic liver infections, is due to hepatitis C virus (HCV) infection. Chronic HCV infection is associated with an increased risk of liver cirrhosis and hepatocellular carcinoma. It is estimated that there are more than 170 million HCV carriers worldwide. Despite much research, there is neither a prophylactic vaccine nor an effective treatment. The development of a HCV vaccine has been hindered by the lack of a small animal model.

Our approach to develop a HCV vaccine was to rationally improve CTL-based immunotherapy, which is capable of eliciting strong cellular immune responses. Briefly, we identified novel HCV epitopes that bound major histocompatibility complex (MHC) molecules with a broad specificity and induced HCV-specific CTL responses in vivo. Our goal was to increase the application of epitope-based immunotherapy, regardless of MHC restriction. We examined the effectiveness of HCV epitopes ex vivo and in vitro by using peripheral blood mononuclear cells (PBMCs) from HCV patients. Previously, we screened and selected several novel epitopes from more than 100 patients, and then incorporated them into an expression vector for a stimulatory cytokine gene. This cytokine augmented cellular immunity in a chimpanzee model of chronic HCV infection. The DNA vaccine was tested in the chimpanzee model to determine whether HCV could be controlled by increasing cellular immunity. In this study, the epitope-based DNA vaccine induced strong cellular immunity against pre-existing epitopes that were suppressed by HCV. Interestingly, 2 of 3 chimpanzees showed increased secretion of interferon gamma after DNA vaccination with human leukocyte antigen (HLA)-restricted epitopes. However, despite their increased cellular immunity, there was no reduction in their viral titers. Due to the limitations of this study, we designed a study with monkeys and used the TriGrid™ delivery system (Ichor Medical Systems, San Diego, CA), an in vivo electroporation system, to administer DNA and improve the expression of epitopes and cytokines. This study was performed in collaboration with MBRI and Ichor Medical Systems.

The monkey study was performed at the National Primate Research Centre, Korea Research Institute of Bioscience and Biotechnology. The TriGrid™ system was used for intramuscular delivery and enhanced gene expression in immune cells, which is likely to improve the cellular immune responses between vaccinations. There were no adverse events or tissue pathology. As a result, we concluded that administration of the epitope-based DNA vaccine with in vivo electroporation is safe and effective in primates.

The goal of our research is to develop effective HCV vaccines that can be produced economically in Korea as well as many other countries. In addition, our collaborators at MBRI believe that these studies will aid economically disadvantaged patients, which is an objective of WHO research programs.

Unfortunately, the development of a DNA vaccine for HCV was suspended in 2010 due to a lack of human resources and low priority. We hope to restart this project soon.

This work was financially supported by the Department of the Ministry of Knowledge and Economy, South Korea, which was formerly the Korea Evaluation Institute of Industrial Technology.

Activity 3 Development of a recombinant protective antigen vaccine against Anthrax

Explanation

(PI: Dr. Dongho Ahn, Research period: 2006-2013)

Anthrax is a highly lethal and infectious disease that is caused by the spore-forming bacteria *Bacillus anthracis*. Naturally occurring cases of inhalational anthrax infections in humans are rare. In the 20th century, only 18 cases of inhalational anthrax were reported in the United States, and the most recent case was in 1976. Biothrax is the only vaccine that is approved by the U.S. Food and Drug Administration (FDA) for the prevention of anthrax infection. It is manufactured from the filtrate of a culture of a non-virulent strain of *B. anthracis*.

The Korea Centers for Disease Control and Prevention (KCDC) has developed a second generation anthrax vaccine from recombinant protective antigen (rPA), which has been tested in non-clinical and clinical trials for several years. Single and repeated dose toxicity tests in rabbits and monkeys have been conducted at the Korea Institute of Toxicology and the Huntingdon PRC in the United States, respectively. A single intramuscular injection of the anthrax rPA vaccine was well-tolerated and there were no local or systemic reactions at any of the doses that were tested. Three lots of this vaccine that was stored for up to 24 months continued to be stable; however, its potency tended to decrease as the duration of the storage period increased.

In the phase I clinical trial of the rPA vaccine, 16 healthy adult men who were 18–45 years old received an injection of either 50 or 100 µg of purified rPA with 250 µg of aluminum hydrogel as an adjuvant. These subjects were followed up for safety and immunogenicity studies. The rPA vaccine was well-tolerated with a low rate of local reactions and no systemic reactions, which demonstrated the safety of this vaccine. The immunoglobulin G (IgG) response against the rPA was not detectable in either group. The formulation study to enhance the product stability and the adsorption capacity of the aluminum gel was completed in 2010. These newly formulated vaccines showed improved stability in the stability test. Currently, we are preparing for the phase II clinical trial, which will begin next year.

This work has been funded by the Korea Centers for Disease Control and Prevention.

Activity 4 Development of a Prophylactic vaccine against Herpes Zoster

Explanation

(PI: Dr. Songyong Park, Research period: 2008-2013)

Herpes zoster (shingles) is caused by reactivation of the varicella zoster virus. The initial infection with this virus causes chickenpox, which usually occurs in children. Since the virus is not eliminated from the body after this illness, it can reappear later in life and cause shingles, which is characterized by painful rashes. Most commonly, shingles occurs in elderly and immunocompromised people. In Korea, shingles is an increasingly common dermatologic disease. According to the National Health Insurance Corporation, the incidence of shingles in Korea increased 45% from 2000 to 2004. As a result, it is expected that the demand for shingles vaccines will increase. Since our institute already has the technology for producing varicella vaccines, we could easily adapt it to develop a herpes zoster vaccine.

In 2010, we selected a cell line with a high growth rate and susceptibility to the varicella zoster virus to increase the production yield of vaccines with considering quick and easy approval and global market. Then, we produced an in-house research cell bank (RCB) to manufacture the master cell bank (MCB) and working cell bank (WCB), which were characterized by a clinical research organization (CRO) (BioReliance, UK) that has current good manufacturing practices (cGMP) facilities.

To generate high yields of viruses from the master virus bank (MVB) and working virus bank (WVB), we determined the factors that affect infectivity, such as media condition, harvest time, or multiplicity of infection (MOI). Then, using our results, we manufactured the MVB and WVB under cGMP conditions and outsourced them to the same CRO for characterization studies, which confirmed the suitability of the MVB and WVB.

To estimate the degree of attenuation of the original virus strain in the vaccine and confirm the safety of the MVB, we performed a neurovirulence test in rhesus monkeys at a CRO that specializes in animal studies (WIL Research Lab, USA).

After scaling up vaccine production, we will conduct pre-clinical tests of the vaccine. Specifically, we will determine its immunogenicity in a small animal model, such as guinea pigs. In addition, we will assess the safety of the vaccine by performing single or repeated dose toxicity tests in rodent and non-rodent species, local tolerance tests, and pharmacological studies.

If the results of the in vivo immunogenicity test, short-term stability test, and pre-clinical toxicity test are favorable, we will file an Investigational New Drug (IND) application with the Korea Food and Drug Administration (KFDA) to obtain approval for a human clinical trial.

This work is funded by Green Cross Corporation and the Ministry of Health and Welfare, South Korea.

Activity 5 Development of a prophylactic vaccine against human papillomavirus infection

Explanation

(PI: Dr. Yeup Yoon, Research period: 2009-2013)

Human papillomavirus (HPV) is the causative agent of cervical cancer, which is the second most common cause of death in women with cancer worldwide. To develop a HPV vaccine, our strategy is to use an insect cell expression system to produce L1 proteins, which self-assemble into virus-like particles (VLP), from Korean cervical cancer patients who were infected with HPV subtypes 16, 18, and 58. Last year, we determined the efficacy of vaccines against HPV 16 and 58 L1 VLP in mice.

This year, we determined the efficacy of a vaccine against HPV 18 L1 VLP in mice. The purified HPV 18 L1 VLP was formulated with an aluminum adjuvant and administered to mice by 2 intramuscular injections. The antibody titer against the VLP was monitored for 12 weeks by using an enzyme-linked immunosorbent assay (ELISA). We also used a secreted alkaline phosphatase (SEAP)-based pseudovirus neutralization assay (provided by Dr. John T. Schiller at the National Cancer Institute) to confirm that the purified HPV 18 L1 VLP was antigenically effective for producing neutralizing antibodies.

On the basis of these findings, we compared our HPV vaccine with the Gardasil® HPV vaccine (Merck &

Co.). Two groups of BALB/c mice were immunized by injecting them with 2 intramuscular injections (3 weeks apart) of 1/2.5 human dose of Gardasil® or the corresponding amount of our HPV vaccine, and then their immune responses were assessed for 16 weeks. The serum IgG antibody titers to HPV 16 and 18 L1 VLP were comparable in both groups of mice. In addition, a pseudovirion-based assay showed comparable levels of neutralizing antibodies in both groups. These results suggested that our vaccine may be a potentially effective prophylactic HPV vaccine.

Unfortunately, this project was suspended in the second half of 2010 due to a lack of production facilities and concerns about clinical trials.

This work was funded by the Green Cross Corporation, South Korea.

Activity 6: Establishment of a cell culture platform for influenza vaccine production (We added one more research activity as below)
(PI: Dr. Dongho Ahn, Research period: 2010-2015)

The development of cell culture systems for virus propagation has led to major advances in the development of virus vaccines. For example, continuous cell lines have been used to produce polio and rabies vaccines.

For human influenza vaccines, significant progress has been made since the mid-1990s to develop alternatives to the traditional egg-based manufacturing process. Many cell lines have been used, including baby hamster kidney (BHK-21), human embryonic retinal (PER.C6), African green monkey kidney (Vero), and Madin-Darby canine kidney (MDCK) cells. Among these cell lines, MDCK cells are the easiest to handle, the most sensitive, and the most reliable; therefore, they are the gold standard for influenza virus propagation. However, their anchorage dependence limits large-scale virus production. Although the use of microcarrier beads to increase the surface area for propagation has been used industrially, this strategy is less convenient than suspension cell cultures.

At the beginning of this year, we began researching ways to make highly productive suspension cell cultures of the MDCK cell line. In 1 strategy, we adapted an adherent MDCK cell line to suspension growth in serum-free medium. The development of this cell line served as a pilot experiment to determine its potential as host cells for producing influenza vaccines in lab-scale bioreactors.

In another strategy, MDCK cells were stably transfected with 2 genes from the human sialyltransferase gene family to increase their susceptibility to influenza virus infection, since the concentration of α -2,6-linked sialic acid receptors on their cell surface is relatively low compared with that of cells in the human respiratory system. We confirmed that the transformed MDCK cells overexpressed the α -2,6-linked sialic acid receptor by using a species-specific lectin-binding assay. Currently, we are verifying the productivity of various human influenza virus strains in these candidate cell lines. We expect that these cell lines will increase the number of interactions between human influenza virions and the cell surface, and consequently, improve virus propagation and hemagglutinin (HA) production.

This work has been funded by the Green Cross Corporation, South Korea.

2. Other information related to the Collaboration between the centre and WHO. Briefly describe visits by WHO staff to the centre, visits by the centre staff to WHO (HQ and/or Regional Office), use of the centre staff by WHO, support provided by centre staff for courses cosponsored or organized by WHO (HQ and/or Regional Office), WHO financial support to the centre through contractual or Technical Services Agreement or other type of support provided by WHO, any other collaborative activities. Please mention any difficulties encountered in the collaboration and suggestions for increased and improved collaboration with WHO.

- We attended WHO and DCVMN (Developing Country Vaccine Manufacturer's Network) in Japan. (J.W. Kim, Patent Attorney, Nov. 15~17, 2009)

- We attended WHO IFPMA IVS in Switzerland and presented "Successful case study of H1N1 vaccine development and preparedness in Korea." (Dr. B.G. Rhee, Feb. 18, 2010)

- We attended a WHO meeting in Nha Trang, Vietnam, the "3rd Meeting with International Partners on Prospects for Influenza Vaccine Technology Transfer to Developing Country Vaccine Manufacturers," which was organized by the Initiative for Vaccine Research (IVR). Dr. Jin Won Youn gave a presentation entitled "GCC's Influenza Vaccine Development" (May 5~6, 2010).

3. Collaboration with other WHO Collaborating Centres: Briefly describe the nature and outcome of the collaboration and the name(s) of the other WHO collaborating centre(s) with which the centre has collaborated. If applicable, please mention the name of the network of WHO CCs to which the centre belongs. Also include suggestions for increased and improved collaboration with other WHO CC

No activities in this period.