

WHO Collaborating Centre

2003 Annual Report

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**1. Title of the centre:**

WHO Collaborating Centre for Research and Development of Vaccines and Diagnostic Reagents, MOGAM Biotechnology Research Institute

**2. Annual Report:** 2003

**3. Address:**

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**4. Head of the centre:** Young-Sup Huh

**5. Terms of Reference for the centre**

- (1) To participate in collaborative studies with WHO on the research and development of new vaccines and diagnostic reagents,
- (2) To provide consulting services on the development and quality control of vaccine and diagnostic reagent,
- (3) To provide training for fellow who are developing vaccines and diagnostic reagents,
- (4) To provide reference for vaccines and diagnostic reagents,
- (5) To collect and disseminate information on the development of vaccines and diagnostic reagents ;

**6. Work performed in relation to the terms of reference**

- (1) Consulting service on the development and quality control of vaccines and diagnostic reagents  
Consulting Green Cross Life Science Co. for upgrading of HCV diagnostic kit that was developed by the Centre in 1994 and commercialized by Green Cross Life Science Co. in 1995. (H. J. Park, Ph. D, and Y. J. Ha, Ph. D)  
Consulting Green Cross Life Science Co. for the development of Severe Acute Respiratory Syndrome(SARS) diagnostic Kit (H.J.Park, PH.D, Y.J.Ha, Ph.D, and M.H.Kim, PH.D)
- (2) Training for development of vaccines and diagnostic reagents

Training of the research fellows of Green Cross Life Science Co. for the development of SARS diagnostic kits . (H.J.Park, PH.D, Y.J.Ha, Ph.D, and M.H.Kim, PH.D)

Training of the research fellows of Yonsei University Medical school(K.H.Han, MD) for the development of therapeutic vaccine against chronic Hepatitis C. (M.H. Park, Ph.D)

(3) Development of vaccines and diagnostic reagents

Development of Severe Acute Respiratory Syndrome(SARS) diagnostic kit using real-time RT-PCR (PI: H.J.Park, Ph.D.)

A global outbreak of a new emerging illness which is called "severe acute respiratory syndrome(SARS)", was found to be associated with a novel coronavirus(CoV), SARS CoV. SARS causes significant morbidity and mortality and by the end of 2003, more than 1,500 peoples were diagnosed with SARS in the world. Therefore, rapid laboratory confirmation of SARS CoV infection was very important for managing patient care and for prevention of worldwide epidemic. The identification of the etiological agent and its partial gene sequence data made it possible for us to develop molecular diagnostic methods for SARS CoV using real-time reverse transcription(RT)-PCR, which was suitable for diagnosis of infection in the early phase of SARS using patient's blood. This work has been done in collaboration with CDC of Republic of China(Dr. Kan) since there has been no confirmed patient in Korea

Development of Hepatitis C virus core antigen detecting diagnostic kits (PI: H. J. Park, Ph. D)

Even though the Centre has developed very sensitive HCV diagnostic kit which is intended to detect the antibodies against hepatitis C virus(HCV) in the blood, this kit cannot be used for detection of HCV infection in the early window period when viruses exist , but no antibodies appear yet. Missing this window period of HCV infection causes a major problem in ensuring the safety of blood supply and blood products. In order to solve this problem, the Centre has initiated the project for development of an enzyme-linked immunosorbent assay (ELISA) diagnostic kit that can be used to detect and to quantify total HCV core antigen in peripheral blood of HCV-infected patients. To develop the ELISA detecting HCV core antigen, we have expressed the recombinant HCV core antigens with different length ,and these core antigens were used for producing anti-core monoclonal antibodies. By screening and selection of several anti-core monoclonal antibodies, we are in the process of testing the sensitivity and the

specificity of ELISA diagnostic kit capable of detecting HCV core antigen in blood.

Development of prophylactic vaccine against Hepatitis C virus.

(PI: M. H. Park, Ph. D)

The hepatitis C virus is the etiological agent of non-A non-B hepatitis, the leading cause of chronic liver infections. Chronic HCV infection is correlated with the risk of developing liver cirrhosis and hepatocellular carcinoma. It is estimated that there are more than 170 million chronic carriers worldwide. To date, there is neither a prophylactic vaccine nor a satisfactory therapeutic treatment. The development of an HCV vaccine has been problematic due to there is no tissue culture system available for HCV and furthermore due to the lack of small animal model system except chimpanzees. In order to develop the prophylactic vaccine against HCV in these circumstance, the Centre decided to use modified HCV glycoprotein gene as DNA vaccine in order to induce strong cellular immune response in addition to use HCV glycoprotein fusion proteins produced in yeast in order to induce humoral immune responses. In animal study, we confirmed that both DNA and fusion protein could induce the humoral and cellular immune response against HCV, and we are now in the process of testing the protective immunogenicity of these candidate vaccine using the surrogate challenge systems.

Development of CTL-based therapeutic vaccine for chronic hepatitis B and C.

(PI: M.H.Park, Ph. D)

Our approach to therapeutic vaccine against chronic viral hepatitis B and C is to rationally improve the CTL-based immunotherapy capable of eliciting a strong cellular immune response. The basic concept is to identify the specialized novel epitopes capable of both binding to broad MHC molecules and inducing their specific CTL response *in vivo*. The goal of this approach is to expand the applicability of the epitope-based immunotherapy independent of MHC restriction. Practically, the potent epitopes derived from HCV and HBV were identified and their effectiveness is being examined in *ex vivo* using the patients PBMCs. We already have screened and selected the several novel epitopes with more than 100 patient's PBMCs, and we are validating the selected epitopes using animal model systems.

## 7. Other research activities

### (1) Research Projects

Development of novel immunomodulator (PI: J. H. Won, Ph. D)

Development of therapeutic drug against cancer through inhibition of new blood vessel. (PI: K. H. Jung, Ph. D)

Adeno-associated virus mediated gene therapy against metastatic tumor and hemophilia. (PI: E. C. Jo, Ph. D)

Development of G-CSF delivery system for cancer therapy using PLG and PEG. (PI: K. H. Jung)

Development of Haemophilia therapeutic drug using recombinant blood coagulation factor (PI: Y. Yoon, Ph. D)

## (2) Patents

Method for mass production of angiogenesis inhibitor LK8 Protein.

Korea Patent Registered No.2003-6391 (2003. 1. 30)

Anticancer agent comprising LK8 protein as an active ingredient.

Korea Patent Registered No.2003-10797 (2003. 2. 20)

Expression vector using for animal cell.

US Patent Registered No.10/343,303(2003.1.27)

Expression vector using for animal cell.

CH Patent Registered No.01813557.9(2003.1.29)

Expression vector using for animal cell.

JP Patent Registered No.2002-561951(2003.1.29)

Expression vector using for animal cell.

EP Patent Registered No.01957013.4(2003.2.13)

Crystalizing method of kringle proteins

Korea Patent Registered No.2003-21982(2003.4.8)

Derivatives of Hydroxyphenyl a method for preparing thereof and their pharmaceutical composition,

PCT Registered No.2002-21982(2003.4.8)

Derivatives of Hydroxyphenyl a method for preparing thereof and their pharmaceutical composition,

US Registered No.10/411.772(2003.4.11)

## (3) Publication

Mi-Aei Kang, *et. al*, Rosemarinic acid inhibits Ca<sup>2+</sup>-dependent pathways of T-cell, antigen receptor mediated signaling by inhibiting the PLC-gamma 1 and Itk activity. *Blood* ;101(9):3534-3542, 2003 May.

Jong-Wha Won, *et. al*, Rosemarinic acid inhibits TCR-induced T Cell activation

and proliferation in an LCK dependent manner. Eur.J Immunol.;33(4):870-9, 2003 Apr.

Eun-Young Jun, *et. al*, Tumor eradication by Hepatitis B Virus X antigen specific CD8+ T Cells in xenografted nude mice. J. of Immunology ;170(3):1183-90, 2003 Feb.

Su-Young Yun, *et. al*, Synergistic immunosuppressive effects of Rosmarinic acid and Rapamycin in vitro and in vivo. Transplantation, 27(75):10):1758-60, 2003 May.

Hyung-Kwon Lim, *et. al*, Dissolved-oxygen-stat controlling two variables for methanol induction of rGuamerin in *Pichia pastoris* and its application to repeated fed-batch. Applied Microbiology and Biotechnology ;62(4):342-8, 2003 Sep.

Jang-Seong Kim, *et. al*, Inhibition of angiogenesis and angiogenesis-dependent tumor growth by the cryptic kringle fragments of human apolipoprotein(a). J. of Biological Chemistry ;278(31):29000-8, 2003 Aug.

Kyu-Hyun Lee, *et. al*, Shuttle PCR-based cloning of the infectious adeno-associated virus type 5 genome, J.of Virological Method;111(2):75-84, 2003 Aug.

Hyung-Kwon Lim, *et. al*, Induction of the T7 promoter using lactose for production of recombinant plasminogen kringle 1-3 in *Escherichia coli*. J.of Microbiology and Biotechnology, Inprint

Jong-Mook Kim, *et. al*, Improved recombinant gene expression in CHO cells using matrix attachment regions, J.of Biotechnology, Inprint

Hyung-kwon Lim, *et. al*, Enhanced production of rhLK68, the cryptic apolipoprotein(a) kringle domains as a novel anti-angiogenic protein, by continuous lactose induction strategy in *Escherichia coli*. J.of Biotechnology, Inprint

Hyung-Kwon Lim, *et. al*, Statistical selection of amino acids fortifying minimal defined medium for a high-level production of the cryptic apolipoprotein(a) Kringle domains, rhLK68, in *Escherichia coli*. J.of Microbiology and Biotechnology, Inprint

Yun-Kyung Huh, *et. al*, Rosmarinic acid induces p53-dependent apoptosis through the activation of caspase-8 and -3 and the disruption of the mitochondrial membrane permeability, J.of Immunology, Inprint

## 8. Evaluation by the centre

In the year of 2003, the Centre made continuous efforts on research and development of vaccines and diagnostic reagents not simply because the Centre was founded on the firm belief that accurate diagnosis and mass vaccination are the best ways to fight against the infectious diseases of mankind but because the Centre is determined to contribute to the welfare of human beings by developing the innovative methods to fight against the emerging infectious diseases together with WHO as a "WHO collaborating centre".

In the field of developing diagnostic reagents, the centre made remarkable progresses in 2003. First, the Centre has succeeded in developing SARS real-time RT-PCR diagnostic kit that can be used for diagnosis of infection in the early phase of SARS using patient's blood, and the centre is waiting to get registration for use in clinical laboratory from Korea Food and Drug Administration. This SARS diagnostic kit is believed to be beneficial not only to peoples living in endemic area but also to peoples traveling to endemic area for quick and accurate test. Since we have established the real-time RT-PCR diagnostic technology for SARS, we will be able to develop the similar kits for detection of early phase of HIV and HCV infection. We believe that these kits play very important role to monitor the safety of blood supply. Secondly, the centre made the practical progress in developing HCV core antigen detection diagnostic kit which is critical to monitor the early phase of HCV infection before antibody starts to be detected in blood for the biosafety of blood supply and for treatment of disease. Several highly specific anti-core monoclonal antibodies are selected as a major component of HCV core antigen detection kit, and the specificity and the sensitivity of the kit is being evaluated using these selected monoclonal antibodies.

On the basis of technologies that have been accumulated for developments of recombinant Hepatitis B vaccine and the other novel vaccines such as Hantaan virus vaccine, the centre made excellent progress in the field of vaccine research in 2003. First of all, the centre has tested immune responses of HCV DNA/protein vaccine candidates in small animal model. The humoral immune response elicited by HCV vaccine candidates was very strong and persistent for 6 months after vaccination and the cellular immune response induced by HCV vaccine candidates was easily detected by ELISPOT assay after the second booster injection. Based on these preliminary results, the centre is now examining the protective immunogenicity of these HCV vaccine candidates using the surrogate challenge systems such as recombinant vaccinia viruses expressing HCV genes or pseudotype retroviruses containing HCV glycoproteins. Once we get these results, we will be able to determine whether we can go for the primate

challenge test using chimpanzees. Secondly, the centre has screened and selected several novel epitopes of HBV and HCV by ex vivo ELISPOT assay using more than 100 patients PBMCs, which could be used for the CTL-based therapeutic vaccine for chronic viral hepatitis B and C. The CTL-induction capacities of these epitopes are being evaluated by use of small animal models such as HLA transgenic mice. The centre has great expectations for the CTL-based therapeutic vaccine since it can be, in principle, applied to treat cancers as well as chronic viral diseases.

In addition to these achievements that the centre has made in research and development of vaccine and diagnostic reagents in 2003, the centre was actively involved in providing consulting services to and training fellows of Green Cross Life Science Co. in order to upgrade the quality of several diagnostic kits. The centre was actively engaged in collaboration with CDC of Republic of China (Dr. Kan) in order to develop the SARS diagnostic kits, and also in collaboration with Yonsei University Medical School (K.H.Han, MD) to develop the therapeutic vaccine for chronic hepatitis C. Moreover, we had several scientific meetings with Berna vaccine Co. in order to exchange information regarding the development of new vaccines for newly emerging infectious diseases and for chronic infectious diseases.