

WHO Collaborating Centre

2002 Annual Report

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1. Title of the centre:

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

2. Annual Report: 2002

3. Address:

341, Pojung-Ri, Koosung-Eup, Yongin-Si, Kyunggi-Do,
Republic of Korea (449-913)

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 vaccine 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. (M. H. Park, Ph. D, H. J. Park, Ph. D, and Y. J. Ha, Ph. D)
Consulting Green Cross Life Science Co. for quality control of HIV diagnostic kit which was developed by the Centre in 1994 and commercialized in 1995 by Green Cross Life Science Co. (H. J. Park, Ph. D, and Y. J. Ha, Ph. D)
- (2) Training for development of vaccine and diagnostic reagents
Training of the research scientist of Green Cross Life Science Co. for the development of ELISA diagnostic kits . (H. J. Park, Ph. D, and Y. J. Ha, Ph. D)

(3) Development of vaccines and diagnostic reagents

Development of vaccine against *O. tsutsugamushi*. (PI: Y. Choi)

Scrub typhus is an acute febrile illness caused by *Orientia tsutsugamushi* (former name; *Rickettsia tsutsugamushi*), an obligate intracellular bacterium, which belongs to the Rickettsiaceae. This disease is endemic in Asia-Pacific regions and it can be assumed that not less than billions of people are exposed to the disease. Therefore, effective vaccine against *O. tsutsugamushi* is desirable for the control of scrub typhus. There have been efforts to elucidate immunological mechanism of prevention of the pathogen and to develop vaccine candidates for scrub typhus. But no vaccine for humans is available at present. Considering the complexity of the antigenicity among serotypes of *O. tsutsugamushi* and difficulty of identifying specific molecules with protective activity, we designed the inactivated whole cell vaccine formulation with mixture of different serotypes. Although animal cell culture method and embryonated chicken egg culture method have been widely used to obtain *O. tsutsugamushi* cells, both methods have some limitation in producing the sufficient amount of cells required for commercial vaccine development. To overcome this limitation, we improved the infectivity by series of subcultivation for several years of the original *O. tsutsugamushi* strain in the embryonated egg. We established a mass culture process using embryonated chicken egg culture of the adapted strain with high infectivity and strong immunogenicity. A manufacturing process including purification of bacteria from the yolk sac membrane, inactivation of the purified antigens was demonstrated. Efficacy of the vaccine was confirmed by protection study in which mice were immunized with the vaccine and then challenged with live *O. tsutsugamushi* strains. Furthermore, preclinical toxicity test and stability test of the vaccine were successfully completed. The safety and immunogenicity of the Tsutsugamushi vaccine candidate in human needs now to be evaluated in clinical trials.

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

We have developed the HCV diagnostic kits which are used to detect the antibodies against HCV antigens in the blood. However, the window period in hepatitis C virus (HCV) infection is still a major problem in ensuring blood safety. Recently, the availability of an anti-core antigen monoclonal antibody allowed development of an enzyme-linked immunosorbent assay (ELISA) detecting and quantifying total HCV core antigen in peripheral blood of HCV-infected patients and this assay technology contributed to reduce the risk of HCV infection by blood

transfusion or by the blood products. To develop the ELISA detecting HCV core antigen, we constructed the recombinant HCV core antigens having different length and which were used for producing anti-core monoclonal antibodies. Using the anti-core monoclonal antibody, we will develop the ELISA diagnostic kit capable of detecting HCV core antigen in blood.

Development of virus screening system for virus therapeutics. (PI: H. J. Park, Ph. D)

HCV (Hepatitis C virus) is a positive single-stranded RNA virus to cause chronic liver damage. We are studying on the high-affinity ligands for HCV core protein or small RNA molecules as cofactors consisted in viral nucleocapsid particle, which inhibit the primary viral assembly step in HCV life cycle. To discover these novel RNAs, we introduced the SELEX (Systematic Evolution of Ligands by EXponential enrichment) technology by designing random oligonucleotide library. By an artificial evolution process, the finally selected RNAs are expected to regulate the cellular metabolism caused by HCV core protein or to inhibit nucleocapsid particle assembly competitively. In the future, the application with these molecules will be extended to the diagnostic area of the early HCV infection or of the individual drug sensitivity with high affinity and sensitivity for therapeutic treatment. Furthermore, this diagnostic system will be used for the screening of HCV inhibitors.

Development of DNA/protein vaccine against Hepatitis C virus. (PI: M. H. Park, Ph. D)

In spite of the availability of reliable serological tests for HCV diagnosis, HCV infection is still common and causes significant morbidity and mortality worldwide. In addition, interferon treatment, which is the most common anti-viral therapy available at the moment, is effective in only 20-30% of patients. Thus, development of HCV vaccines is a high priority project. Since there is no tissue culture system available for HCV, we studied immune responses of Hepatitis C viral genes delivered as DNA or HCV fusion protein produced from yeasts. In animal study, we confirmed that both DNA and fusion protein could induce strong humoral and cellular immune response against HCV. Based on these observations, MBRI is preparing to develop DNA/protein vaccine against HCV.

Development of epitope-based therapeutics vaccine for hepatitis B and C. (PI: K. Y. Lee, Ph. D)

Our approach to therapeutic vaccine against chronic viral hepatitis B and C is to rationally improve the epitope-based therapeutics capable of eliciting a strong cellular immune response. The basic concept is to identify the specialized epitopes (named as supertype 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, respectively, were identified and their effectiveness is being examined in *ex vivo* system such as in patients PBMCs. The future plan is to develop the practical ways to make the supertype-based therapeutics for chronic viral hepatitis.

(4) Publication

Yu Kyeong Hwang, *et. al*, HLA-A2.1 restricted peptides from the HBx antigen induced specific CTL responses *in vitro* and *in vivo*, *VACCINE* 20:3770 - 3777, 2002

The HBx-derived, HLA-A2.1 restricted peptides, XEP-3, XEP-4, and XEP-6, induced activation of specific CTLs from patients with HBV *in vitro*. XEP-6 peptide induced the strongest response among the three peptides in CTLs from the blood samples of patients that were HBsAg positive. It was not clear whether the stage of disease (chronic infection, cirrhosis or hepatoma) was related to the responsiveness of the CTLs to each peptide. We vaccinated HLA-A2/K(b) transgenic mice with these peptides encapsulated in pH-sensitive liposomes at various concentrations and tested their ability to protect against challenge with rVV-HBx. Mice immunized with encapsulated peptides were protected against viral challenge whereas those immunized with empty liposomes were not. In general, 5 micro g of each peptide per head inoculation was sufficient to give protection after 2 weeks. After 3 weeks, this protective effect was increased. This effect of time was more important on the level of protection than the initial dose of the peptide. To explain the protective effect, IFN-gamma secreting CD8(+) cells isolated from mice 3 weeks after immunization were analyzed *ex vivo*. There was little dose dependency of peptide on IFN-gamma secretion except for XEP-3. The variations in the results may reflect the chemical properties of the peptides, such as solubility and binding affinity. In conclusion, epitope peptides derived from HBx can induce specific CTL activation and lead to cellular immunity *in vitro* and *in vivo* by inducing the peptide-specific CD8(+) CTLs. Thus, pH-sensitive liposomes increase the immune response following immunization with a peptide vaccine. This could be used for the treatment of HBV-related disease.

Ki-Young lee, *et. al*, Characterization of HLA-A2.1-restricted epitopes, conserved in both Hantaan virus-infected patients, *Journal of General Virology* 1131 - 1136, 2002

Nine different CTL epitopes, conserved in both Hantaan virus (HTNV) and Sin Nombre virus (SNV), were selected for study. The binding affinity of each peptide

with HLA-A2.1 molecules in vitro was determined and antigen-specific responses from seven donors who had a previous field infection with HTNV were examined. Although the strength or frequency of CTL activity showed different patterns in the seven patients, five of seven patients showed significant activity against at least one or more epitope peptides. In particular, the peptide ILQDMRNTI (HTNV, aa 334-342; SNV, aa 333-341), which elicited CTL activity in five patients, was shown to be specifically HLA-A2.1-restricted in partially cloned CD8+ T cells and also induced activated and effector CD8+ T cell-producing T cytotoxic (Tc) type 1 cytokines, such as IL-2 and IFN-gamma. The results suggest that this epitope would serve as a useful component for the intervention of both HTNV and SNV infection.

(5) Patents

Construction and application of a novel poliovirus vaccine vector with a cloning site between 2C and 3A coding region. Korea Patent Registered No. 332614 (2002. 4. 2)

Novel detoxified mutants of *Escherichia coli* heat-labile enterotoxin. US Patent Pending No. 10/088,202 (2002. 3. 15)

Novel detoxified mutants of *Escherichia coli* heat-labile enterotoxin. Europe Patent Pending No. 99 944 897.0 (2002. 4. 12)

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)

Development of therapeutic drug against osteoporosis using recombinant human parathyroid hormone. (PI: D. H. Park, Ph. D)

Development of chemokine: the inhibitor on the differentiation of hematopoietic stem cell. (PI: D. H. Park, 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 liposome-mediated gene therapy for liver cancer. (PI: J. S. Chang, Ph. D)

Development of immunotherapy for Hepatitis B. (PI: D. H. Park)

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

(2) Patents

Liposome comprising peptide antigens derived from X protein of hepatitis B virus.

US Patent Registered No. 6,380,359 (2002. 4. 30)

Liposome comprising peptide antigens derived from X protein of hepatitis B virus.

Russia Patent Registered No. 2189989 (2002. 9. 27)

Process for preparing Amino-terminal Methionine free recombinant Interferon- γ .

Korea Patent Registered No. 327971 (2002. 2. 26)

Screening method of antiviral agent by protein-primase activities of hepatitis B virus

DNA Pol. Korea Patent Registered No. 358248 (2002. 10. 11)

Method for preparation of recombinant Guamerin and pharmaceutical compositions containing the recombinant Guamerin for wound healing. Korea Patent Registered No. 365533 (2002. 12.

7)

HCV as template for efficient de novo RNA synthesis by HCV S5B polymerase.

Korea Patent Registered No. 2002-3154 (2002. 1. 19)

Mass production method of shLkn-1 protein using recombinant yeast. Korea

Patent Registered No. 2002-5189 (2002. 1. 29)

Large-scale purification method of shLkn-1 protein with high purity using recombinant yeast. Korea Patent Registered No. 2002-18045 (2002. 4. 2)

Derivatives of hydroxyphenyl. a method for preparing thereof and their pharmaceutical composition. Korea Patent Registered No. 2002-20481 (2002. 4.

15)

Serum-free media for cell culturing. Korea Patent Registered No. 2002-58982

(2002. 9. 27)

Method for preparation of recombinant Guamerin and pharmaceutical compositions containing the recombinant Guamerin for wound healing. US Patent Registered No. 10/275,672 (2002. 11. 7)

Angiogenesis inhibitor containing sulfonamide derivatives. Korea Patent Registered No. 2002-69779 (2002. 11. 11)

(3) Publication

Ki-Young Lee, *et. al*, Investigation of antigen delivery route *in Vivo* and immune-boosting effects mediated by pH-sensitive liposomes encapsulated with K^b-

restricted CTL epitope. *Biochemical and Biophysical Research Communications*, 292(3):682 - 688, 2002

Meehyein Kim, *et. al*, Template requirements for De Novo RNA synthesis by Hepatitis C Virus nonstructural protein 5B polymerase on the viral X RNA. *Journal of Virology* 76(14):6944 - 6956, 2002

Hyung-Kwon Lim, *et. al*, Sustained release of PEGylated G-CSF from PLGA Microsphere. *Korean Journal of Biotechnology and Bioengineering* 17(1):33 - 37, 2002

8. Evaluation by the centre

In the year of 2002, the Centre made continuous efforts on research and development of vaccines and diagnostic reagents not simply because we were 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 we are determined to contribute for the welfare of human beings by trying to eradicate the infectious diseases together with WHO as a "WHO collaborating centre".

On the basis of technologies that have been accumulated for developments of recombinant Hepatitis B vaccine and the other novel vaccine such as Hantaan virus vaccine, the centre made excellent progress in the field of vaccine research in 2002. First of all, the centre has successfully finished the preclinical studies of novel tsutsugamusi vaccine, which is being developed for the first time in the world, and the centre is waiting to get permission for human clinical trials of this vaccine from Korea Food and Drug Administration. The novel tsutsugamusi vaccine is believed to be beneficial not only to peoples living in endemic area but also to peoples traveling to endemic area. Secondly, 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 is now being investigated. Based on these preliminary results, the centre is cautiously planning to test the feasibility of these HCV vaccine candidates in primate model. Thirdly, the centre has found several novel supertype-epitopes of HBV and HCV, 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 *ex vivo* system using patient's PBMCs. 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 the field of developing diagnostic reagents, the centre made several practical progress in 2002. First, the centre succeeded in upgrading the sensitivity and specificity of

several diagnostic kits, which were developed by the centre, such as HBV, HCV, and HIV antigen/antibody detection kits. This achievement will certainly be used to enhance the accuracy of diagnostic tests ensuring the biosafety of blood supply and blood products. Secondly, the centre launched the new project developing HCV core antigen detection diagnostic reagents because it 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. Highly specific anti-core monoclonal antibodies are now in the process of screening and selection as a major component of HCV core antigen detection kit. Thirdly, the centre is gaining access to proteomics technology and protein chip technology in order to develop the next generation of diagnostic reagents that are more accurate in terms of sensitivity and are simpler to perform the tests.

In addition to these achievements that the centre has made in research and development of vaccine and diagnostic reagents in 2002, the centre was actively involved in providing consulting services to and training fellows of Green Cross Life Science Co., diagnostic product specialized company, in order to upgrade the quality of diagnostic kits. The centre also had several scientific meetings with Green Cross Vaccine Co. and Rhein Biotech in order to exchange information regarding development of new vaccines for newly emerging infectious diseases and for chronic infectious diseases.