Application for Faculty Development Program

FACULTY SUMMER FELLOWSHIP

Name: Joseph Mester
Department: Biological Sciences
Office: SC 244
Office Phone: 572-5277
e-mail address: mesterj1@nku.edu

Project Title:

Immunogenicity of Novel Virus-Vectored Vaccines for Hepatitis C

Amount requested: $6,000

Short Project Description:

This Fellowship will accelerate the NKU collaboration with the virology group at the University of Pittsburgh. The goal is to characterize a series of novel virus-vectored vaccines for hepatitis C. These vaccines will be screened for activity in human skin cells, neurons, and liver cells. The results of this testing will indicate which cells are best at supporting vaccine activity, and which vaccines are most likely to induce protective immune responses to hepatitis C. The Summer Fellowship will focus equally on laboratory-based research and manuscript preparation. The manuscript will document the creation, characterization, and immunogenicity of the vaccines.

Joseph Mester October 1, 2013

signature* date

* By typing your name or pasting your signature in the space provided you are allowing this application to be reviewed by the Faculty Benefits Committee for a possible award. The applicant is also aware that failure to comply with the instructions may result in this proposal not being reviewed.

GOALS AND CRITERIA

Hepatitis C virus (HCV) is one of the most prevalent and deadly blood-borne pathogens. No vaccine is currently available for preventing infection or treating those with life-long chronic infections. The creation of novel vaccines capable of generating protective immune responses to HCV would be a major advance in worldwide public health efforts.

This Summer Fellowship is designed to bring the HCV vaccine project that will be initiated during sabbatical leave in January 2014 (at the University of Pittsburgh) to full fruition, and plan for its estimated completion in early 2015. It is anticipated that the sabbatical project will result in the generation of several novel herpes simplex virus (HSV)-based hepatitis C vaccine stocks. A related Faculty Project Grant submission seeks to determine the ability of these novel HSV-based vaccines to express HCV genes in human skin cells, neurons, and liver cells. Lastly, the immunogenicity of the hepatitis C vaccines will be assessed in animal models during Bio 430L, Immunology Lab.

The targeted outcomes of this Fellowship are to:

- Design and execute experiments to determine the ability of the novel HSV-based vaccines to express HCV genes in human skin cells, neurons, and liver cells, and to
- Compile a scientific manuscript summarizing the full extent of project activities over 2014 and early 2015, including the creation, characterization, and immunogenicity of the novel vaccines.

Measures of success will include achievement of the above goals and also a more in-depth collaboration between the molecular virology group at the University of Pittsburgh and NKU, leading to co-authored publications in the future and potentially joint federal grant submissions to obtain research funding.

DETAILED PROJECT DESCRIPTION

Vaccination has proven to be effective for disease prevention as well as the reduction of disease duration and severity. Traditional vaccines include killed or attenuated whole organisms, inactivated bacterial toxins, or isolated viral proteins, each of which has been shown to generate protective immune responses in humans. Recently, genetically-engineered viruses containing genes from other infectious organisms are being pursued as potentially more effective vaccines due to several inherent strengths, including cell and tissue specificity, safety, and enhanced immunogenicity. Several types of viral vaccine vectors are under investigation, including those based on adenoviruses, poxviruses, and herpes simplex viruses (HSV).

Hepatitis C Virus (HCV) is a major health concern worldwide. An estimated 170 million people are currently infected with HCV, which is about 3% of the world population (1). Nearly 3.2 million people are chronically infected with HCV in the United States, and each year 8,000 to 10,000 infected individuals die due to HCV-related ailments (2). No vaccines are currently

available for preventing HCV infection and transmission. Several vaccines for HCV are currently in clinical trials, and additional preclinical vaccine strategies are also being pursued, including the use of pox and adenovirus-vectored vaccines, virus like particles, and synthetic peptide vaccines (3-5).

The use of HSV-based vectors represents a novel approach to HCV vaccination. There are several advantages of using HSV as a vaccine vector. It is easy to create and characterize in the lab. It elicits a strong and durable immune response in the host (6). The viral genome remains independent of host chromosomes after colonization of the cell, so the possibilities of mutagenicity and tumor formation are minimized. The vector is capable of entering a broad range of cell types and can be administrated through a variety of routes, including intranasally (7). Finally, the large genome of HSV allows for several vaccine targets to be inserted and expressed simultaneously (8).

Previous experiments at NKU focused on non-replicating HSV vectors that were able to gain access to the cell and express the vaccine component (a generic reporter protein), but were unable to grow in the cell or spread to new cells. These are considered to be very safe vectors that do not produce symptoms of HSV infection. Two different "activators" (gene promoters) were used to produce the vaccine target. One expressed a large amount of the reporter protein, but only during the first week after injection (strong but transient expression). The second expressed a lower level of the reporter, but over a longer time frame after injection (low but long-term expression) (9). The ability of the two different activators to generate an immune response was determined by measuring the antibody and T cell response of vaccinated mice to the reporter at various times before and after vaccination. The mouse model is widely accepted as the standard model for the initial evaluation of experimental vaccines. Protective antibody and T cell responses are the goal of vaccines, and the techniques and reagents for measuring these responses are well-developed in the mouse model.

Following vaccination, the antibody response to the reporter in the strong/transient HSV vector peaked at one month post-vaccination, and then declined. The antibody response to the reporter in the low/long-term HSV vector showed similar dynamics. T cell responses were detected to both vaccine vectors, but the highest T cell activity 1-2 months post-vaccination was generated by the strong/transient HSV vaccine vector. These results suggest a slight advantage for the strong/transient HSV vector, although additional experiments are needed to reach a final conclusion.

Early in 2014, during my sabbatical project, similar HSV vaccine vectors will be constructed to express the structural proteins of HCV. Structural components of viruses are the major targets of protective antibody and T cells. Genes encoding HCV envelope proteins E1 and E2 and the core protein C will be inserted into the HSV vectors using standard recombinant DNA techniques.

The applicant is seeking salary support with this Summer Fellowship application to cover the time involved in the planning and execution of experiments, the interpretation and formatting of experimental results for presentation, and full manuscript preparation. A related Faculty Project Grant application requests funding to perform laboratory-based screening of the vaccines created during the sabbatical project. They will verify the ability of the newly created HSV-based vaccines to express the hepatitis C virus E1, E2, and C genes in cells from

human skin, nervous system, and liver. The HSV vector naturally infects cells from the skin and nervous system, so these tissues are natural targets for initial vaccine evaluation. Since HCV resides in the liver, and the HSV vectors are able to infect liver cells, these vaccines may also be useful for treating subjects already infected with HCV. Once the vaccines vectors are characterized at the cellular level, the next step would be to analyze the most promising vaccine constructs in small animal models.

It is anticipated that the Fellowship will begin in May 2014, and continue through mid-August 2014, with all activities being performed at NKU. The activities in May and June will center on manuscript research and writing, and the detailed planning of laboratory experiments. With funding of the related Faculty Project Grant in July, laboratory experiments will be conducted and refined so that most of the remaining experimental work will be able to be performed by undergraduate students supervised by the applicant in the Fall 2014 semester. Bio 430, Immunology lab, will be offered in Spring 2015, allowing 24 students to participate in small animal trials of the lead vaccine candidates identified in this project. Successful completion of these trials will provide sufficient data for manuscript submission for publication.

PROJECT DESCRIPTION REFERENCES

1. Knipe DM, Howley PM, Griffin DE, Lamb RA, Martin MA, Straus SE. Hepatitis C Virus *Fields Virology 5th edition*. Lippincott Williams & Wilkins, 2007.
2. Holmberg S. 2010. Hepatitis C Virus. http://wwwnc.cdc.gov/travel/yellobook/2012.
3. Eisenstein M. 2011. Vaccines a moving target. *Nature* 474: S16-17.
4. Torresi J, Johnson D, Wedemeyer H. 2011. Progress in the development of preventive and therapeutic vaccines for hepatitis C virus. *Journal of Hepatology* 54: 1273–85.
5. Meunier JC, Gottwein JM, Houghton M, Russell RS, Emerson SU, Bukh J, Purcell RH. 2011. Vaccine-induced cross-genotype reactive neutralizing antibodies against hepatitis C virus. *J Infect Dis*. 204(8): 1186-90.
6. Hocknell PK, Wiley RD, Wang X, Evans TG, Bowers WJ, Hanke T, Federoff HJ, Dewhurst S. 2002. Expression of human immunodeficiency virus type 1 gp120 from herpes simplex virus type 1-derived amplicons results in potent, specific, and durable cellular and humoral immune responses. *J Virol*. 76(11): 5565-80.
7. Brockman MA, Knipe DM. 2002. Herpes simplex virus vectors elicit durable immune responses in the presence of preexisting host immunity. *J Virol.* 76(8): 3678-87.
8. Watanabe D, Brockman MA, Ndung'u T, Mathews L, Lucas WT, Murphy CG, Felber BK, Pavlakis GN, Deluca NA, Knipe DM. 2007. Properties of a herpes simplex virus multiple immediate-early gene-deleted recombinant as a vaccine vector. *Virology*. 357(2): 186-98.
9. Goins WF, Sternberg LR, Croen KD, Krause PR, Hendricks RL, Fink DJ, Straus SE, Levine M, Glorioso JC. 1994. A novel latency-active promoter is contained within the herpes simplex virus type 1 UL flanking repeats. *J Virol.* 68(4): 2239-52.

VALUE OF THE PROJECT

To the applicant's professional growth and status:

Promising results have been obtained using HSV-based proto-type vaccines with a generic reporter target that is not related to a specific infectious disease. These test vaccines served as investigational models for determining antibody and T cell responses to a foreign protein expressed in the HSV vector system. Experimental results were derived over two years in an undergraduate laboratory class at NKU (Bio 430L, Immunology Lab) in Spring 2009 and Spring 2011 (the class is held every other Spring semester). The results, while promising, were preliminary and need further development.

A detailed characterization of the newly created HSV-based vaccine vectors for hepatitis C in human cells will increase the impact factor of the approach. The vaccine screening process described in this Fellowship is the next step in characterizing the hepatitis C vaccine vectors generated during the sabbatical period. The data generated will be an important addition to a manuscript, slated for submission/publication once animal testing of the vaccines has been completed. Publication of the results will increase the scientific visibility of the applicant and increase his chances of obtaining research funds in the future to further refine his work.

To the scholarly community:

Vaccines are one of the most cost-effective methods for preventing infection and disease. The ability of HSV to act as a vaccine vector has not been extensively tested. The applicant has expertise in vaccine construction and analysis, and so will be able to determine the potential of HSV to act as a vaccine vector. Related advances will be communicated to the scholarly community in local, regional, and national scientific conferences and in publications in the scientific literature.

To the applicant's teaching and students:

Viral vectors and vaccines are a common topic throughout the undergraduate Cell and Molecular Biology degree tract. The results of this fellowship will provide new "first-hand" material for teaching students enrolled in Bio 430 Immunology, Bio 475 Virology, Bio 470 Medical Microbiology, Bio 302 General Microbiology, and Bio 202 Microbiology for Health Professionals. The fellowship will also benefit students interested in performing independent research projects in the applicant's lab.

To the University:

The results of this fellowship will expose NKU undergraduates to current and novel techniques in the lab and current biomedical concepts in the classroom. Presentation of project-related results at local, regional, and national scientific conferences will enhance the visibility and status of the University's science programs. Funding of project-related research grant proposals will bring additional research money to NKU.

To the non-academic community:

Hepatitis C virus is highly prevalent worldwide, and is one of the most prevalent blood-borne pathogens in the United States. Anyone exposed to human blood is at risk for infection, especially those working in hospitals and IV drug users. No vaccine is currently available for preventing infection or treating those with life-long chronic infections. The identification of novel vaccine methods for generating protective immune responses to HCV would benefit public health efforts worldwide.

TIMETABLE OF THE PROJECT

It is anticipated that the Fellowship will begin in May 2014, and continue through mid-August 2014, with all activities being performed at NKU. The activities in May and June will center on manuscript research and writing, and the detailed planning of laboratory experiments. With funding of the related Faculty Project Grant in July, laboratory experiments will be conducted and refined so that most of the remaining experimental work will be able to be performed by undergraduate students supervised by the applicant in the Fall 2014 semester.

BACKGROUND OF APPLICANT RELEVANT TO THIS PROJECT

I have extensive experience in the creation, characterization, and testing of viral vectors expressing vaccine targets. As a post-doc at the University of Pittsburgh, I created proto-type herpes simplex virus (HSV) vectors expressing vaccine proteins that were the early foundation of this proposal. At Cincinnati Children's Hospital, I created vaccinia (pox) virus recombinants expressing genital herpes (HSV-2) proteins and evaluated their protective effect in animal models. At GeneMedicine, I developed immunogenicity assays to measure the effectiveness of novel DNA plasmid-based (non-viral) vaccine vectors. At Aventis and Wyeth, I developed testing methods to detect antibody and T cell responses to vaccines in phase III clinical trials. At Wyeth, I generated and characterized lentiviral (human immunodeficiency virus-based) vectors for vaccine delivery to specialized immune cells capable of generating strong immune responses. At NKU, I analyzed the proto-type HSV vectors created at the University of Pittsburgh in Bio 430L, Immunology lab, during the Spring 2009 and Spring 2011 semesters. During Spring 2014, my sabbatical project will be focused on creating several novel HSV-based vaccines for HCV with Dr. William Goins at the University of Pittsburgh. My laboratory at NKU has the basic equipment needed for human cell culture and virology research.

OTHER SUPPORT AND COMMITMENTS

No supply funds are currently available or pending to support this fellowship. The applicant has no other commitments during the time period of the fellowship.

JOSEPH C. MESTER, Ph.D.

PROFESSIONAL EXPERIENCE

NORTHERN KENTUCKY UNIVERSITY, Highland Heights, KY 2006 - current
Department of Biological Sciences
Associate Professor
Developed and coordinated lecture and laboratory courses such as Microbiology for Health Professionals. Developed contemporary upper level Virology and Immunology Lab courses. Sponsored and obtained grant funding for diverse undergraduate laboratory research projects. Established and chaired the University's Institutional BioSafety Committee. Acted as site BioSafety Officer.

MOUNT SAINT MARY COLLEGE, Newburgh, NY 2004 - 2006
Department of Natural Sciences
Adjunct Professor of Biology
Responsible for all lecture and laboratory aspects of Accelerated Microbiology.

WYETH VACCINES, Pearl River, NY 2001 - 2006
Principal Research Scientist
Performed and managed the development of human immunoassays to support clinical research and the Phase I, II & III evaluation of innovative vaccines. Designed clinical research protocols and associated business plans. Initiated and managed clinical research collaborations. Trained, supervised, and motivated technical personnel. Provided expert opinion in the areas of immunology, virology, molecular biology, and the *in vivo* and *in vitro* analyses of vaccines.

AVENTIS PASTEUR INC., Swiftwater, PA 1999 - 2001
Manager, Clinical Serology
Managed the Clinical Serology Laboratory within the Clinical Development Department of a global pharmaceutical company. Actively liaised with Clinical Directors, CRAs, Data Management, Biometry and Quality Assurance personnel to advance vaccine INDs and License Submissions. Managed Serology personnel, budget, and facilities/testing in cGLP/cGMP compliance.

GENEMEDICINE, INC., The Woodlands, TX 1997 - 1999
Senior Research Scientist
Led the In Vivo Antigen Presenting Cell Team effort in a small, fast-paced gene therapy company developing novel vaccine technologies and cancer therapies. Consistently contributed key advice for improved experimental design and played a pivotal role in focusing the group's research effort. Generated quarterly research reports that provided the operating framework for future endeavors.

CHILDREN'S HOSPITAL / J.N. GAMBLE INSTITUTE, Cincinnati, OH 1993 - 1997
Research Associate / Associate Research Scientist
Investigator in the fast-paced, clinically oriented Division of Infectious Diseases. Managed personnel assisting in several research projects and maintained a dynamic interpersonal work environment.

UNIVERSITY OF PITTSBURGH SCHOOL OF MEDICINE, Pittsburgh, PA 1990 - 1993
Research Associate
Investigator in an emerging gene therapy program within the Department of Molecular Genetics and Biochemistry. Trained graduate students and lab staff in molecular genetics, tissue culture, and animal handling techniques.

UNIVERSITY OF TENNESSEE, Knoxville, TN 1984 - 1989
Department of Microbiology and College of Veterinary Medicine
Graduate Teaching Assistant
Responsible for lecture/laboratory sessions in undergraduate Microbiology and graduate level Veterinary Bacteriology and Immunology. Led lecture and laboratory sessions in team-taught General Biology.

EDUCATION

University of Michigan, Ann Arbor, MI	*Post-doctoral, Human Genetics*	1989 - 1990
University of Tennessee, Knoxville, TN	*Ph.D., Microbiology*	1984 - 1989
University of Rochester, Rochester, NY	*B.A., Biology*	1979 - 1983

PUBLICATIONS

Mester, J.C. (2009) Integrated design of a virology course develops lifelong learners. *New Directions for Teaching and Learning* 119: 71-79.

Pommerville, J.C., Mester, J.C. and Revie, J. (2009) *Student Study Guide to Accompany Alcamo's Fundamentals of Microbiology, Body Systems Edition*. Jones and Bartlett Publishers, Boston.

Cooper, D.*, Mester, J.C.*, Guo, M., Nasar, F., Souza, V., Dispoto, S., Sidhu, M., Hagen, M., Eldridge, J.H., Natuk, R.J., and Pride, M.W. (2006) Epitope mapping of full-length glycoprotein D from HSV-2 reveals a novel $CD4^+$ CTL epitope located at the transmembrane-cytoplasmic junction. *Cellular Immunology* 239: 113-120. (*equal authorship)

Cooper, D., Pride, M.W., Guo, M., Cutler, M., Mester, J.C., Nasar, F., She, J., Souza, V., York, L., Mishkin, E., Eldridge, J. and Natuk, R.J. (2004) Interleukin-12 redirects murine immune responses to soluble or aluminum phosphate adsorbed HSV-2 glycoprotein D towards Th1 and $CD4^+$ CTL responses. *Vaccine* 23: 236-246.

Mester, J.C., Twomey, T.A., Tepe, E.T. and Bernstein, D.I. (2000) Immunity induced by DNA immunization with herpes simplex virus type 2 glycoproteins B and C. *Vaccine* 18: 875-883.

Bernstein, D.I., Tepe, E.T., Mester, J.C., Arnold, R.L., Stanberry, L.R., and Higgins, T. (1999) Effects of DNA immunization formulated with bupivacaine in murine and guinea pig models of genital herpes simplex virus infection. *Vaccine* 17: 1964-1969.

Coleman, M., Muller, S., Quezada, A., Mendiratta, S.K., Wang, J., Thull, N.K., Bishop, J., Matar, M., Mester, J., and Pericle, F. (1998) Nonviral interferon α gene therapy inhibits growth of established tumors by eliciting a systemic immune response. *Human Gene Therapy* 9: 2223-2230.

Mester, J.C., Milligan, G.N. and Bernstein, D.I. (1996) The immunobiology of herpes simplex virus. In Stanberry, L.R. (ed.): *Genital and Neonatal Herpes*. John Wiley and Sons, NY, pp. 49-91.

Mester, J.C., Pitha, P.M. and Glorioso, J.C. (1995) Anti-viral activity of herpes simplex virus vectors expressing murine alpha interferon. *Gene Therapy* 2: 187-196.

Mester, J.C. and Rouse, B.T. (1991) The mouse model and understanding immunity to HSV. *Rev. Inf. Dis.* 13: S935-S945.

Mester, J.C., Glorioso, J.C. and Rouse, B.T. (1991) Protection against the zosteriform spread of herpes simplex virus by monoclonal antibodies. *J. Inf. Dis.* 163: 263-269.

Mester, J.C., Highlander, S.L., Osmand, A.P., Glorioso, J.C. and Rouse, B.T. (1990) HSV-1-specific immunity induced by peptides corresponding to an antigenic site of glycoprotein B. *J. Virol.* 64: 5277-5283.

EXTERNAL RESEARCH SUPPORT

Ongoing:

BEI Resources Level 2 Registration, Biodefense and Emerging Infections Research Resources Repository, NIAID, NIH
Project Period: 09/01/2010 to current (no expiration date)
Role: Registrant
Amount: ~$200,000 of viral research reagents have been received to date
Goals/Aims: Obtain reagents necessary to characterize the effect of components of influenza A virus, human respiratory syncytial virus, and hepatitis C virus on immune cells of the monocytic, natural killer, T, and B cell lineages.

Completed within the Last Five Years:

KY-INBRE, 2P20 RR016481, NIH
Immunomodulation by Influenza A and Respiratory Syncytial Virus Virions
Project Period: 05/01/2009 through 04/30/2012
Role: PI/New Investigator
Amount: $300,000
Goals/Aims: Characterize the immunomodulatory effect of influenza A virus and HRSV virions and subcomponents on human immune effector cells of the monocytic, natural killer, T, and B cell lineages.

KBRIN AREA, NIH
Immunomodulation by Influenza A and Respiratory Syncytial Virus Virions
Project Period: 05/01/2008 through 04/30/2009
Role: PI/Fellowship
Amount: $12,500
Goals/Aims: Characterize the immunomodulatory effect of influenza A virus and HRSV virions on human immune effector cells of the monocytic, natural killer, T, and B cell lineages.

KY EPSCoR Research Start-up Award RSF-026-05 (Pearce, PI)
Project Period: 06/01/2006 through 07/31/2008
Role: Co-PI
Amount: $75,000
Goals/Aims: Establish research lab focusing on cellular immunology, virology, and molecular biology. Develop and direct undergraduate research projects.

PREVIOUS FBC AWARDS

None (Sabbatical Grant for Spring 2014)