AD_____

Award Number: W81XWH-17-2-0031

TITLE: AMRMC Resident Research Associateship Program

PRINCIPAL INVESTIGATOR: Dr. Ray Gamble

CONTRACTING ORGANIZATION: The National Academy of Sciences, Engineering and Medicine

Washington, DC 20001

REPORT DATE: Sept 2018

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
Sept 2018	Annual	1 Sep 2017 - 31 Aug 2018

4. TITLE AND SUBTITLE

AMRMC Resident Research Associateship Program

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-17-2-0031

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Dr. Ray Gamble

E-Mail: rgamble@nas.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

The National Academy of Sciences, Engineering and Medicine
500 5th Street NW, Washington, DC 20001

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

The NRC Research Associateship Program provides postdoctoral and senior research fellowship awards to outstanding applicants who perform research supporting the mission of AMRMC laboratories. These awards provide opportunities that enable AMRMC/NRC Research Associates to increase their proficiency in conducting research, advance the research programs of AMRMC, and make AMRMC laboratory facilities, often including unique equipment, available to the scientific community. The program further provides a pool of talented researchers who may be retained as contractors or civilian employees, thereby contributing to scientific workforce development.

15. SUBJECT TERMS

Postdoctoral fellowships research, bio-medical research, training, education

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U Unclassified	U Unclassified	U Unclassified	UU Unclassified	14	19b. TELEPHONE NUMBER *(include area code)*

The National Academies of
SCIENCES • ENGINEERING • MEDICINE

RESEARCH ASSOCIATESHIP PROGRAM

with

AMRMC - U.S. Army Medical Research Institute of Infectious Diseases

Annual Contract Technical Report

Contract No. W81XWH-17-2-0031
Contract Period: 09/01/2017-08/31/2022
Report Period: 09/01/2017-08/31/2018

During the reporting period, the National Academies of Sciences, Engineering, and Medicine (the Academies) NRC conducted the following activities in support of the subject contract:

Outreach and Promotion

The promotional schedule to advertise the NRC Research Associateship Programs included the following: 1) attendance at meetings of major scientific and engineering professional societies; 2) advertising in programs and career centers for these and other professional society meetings; 3) direct mailing and emailing of announcements and program materials to presidents, graduate deans, and heads of appropriate science and engineering departments and minority-affairs offices of all academic degree-granting institutions in the United States; 4) posting announcements on internet job sites, electronic newsletters and professional society websites; 5) print advertising in high profile publications (e.g., Science magazine, the Chronicle of Higher Education); and, 6) maintaining a presence on social media sites such as Facebook.

The Academies attended a number of minority focused events in which we maintained exhibit booths, participated in workshops and advertised in meeting literature, newsletters and websites or submitted materials for distribution. In addition, ads were placed in a variety of minority publications (e.g., Affirmative Action, Black Collegian).

In advertising the Research Opportunities available to prospective applicants, the Academies maintained an up-to-date listing of all active Research Advisers, current Adviser contact information and details of each Research Opportunity.

Processing and Review of Applications

Applications to the Research Associateship Programs were submitted via a web-based application system. Each application cycle opened two months prior to the application deadline. Academies staff provided support to prospective applicants including providing application instructions, technical support and additional information as requested.

A summary of applications for the reporting period is shown in Table 1.

For each applicant, the Academies received and processed an application form, a research proposal, transcripts, a statement of previous and current research, and confidential reference reports. An application file check was made prior to the review and each applicant was notified if required documents were missing.

The Academies convened panels in five broad discipline areas for the competitive review of applications in the NRC Research Associateship Programs. Results of the review were made available to Laboratory Program Representatives immediately following the conclusion of the each review.

A summary of the outcome of the review of applications for the reporting period is shown in Table 1.

Administration of Awards

The Academies made awards to applicants based on sponsor authorization. A summary of awards authorized and the acceptance or declination by the applicant during the current reporting period is shown in Table 1.

For NRC Research Associates beginning or continuing tenure, the Academies provided the administrative functions described in the contract Statement of Work. These functions included stipend payments,

management of a major medical benefits insurance program, and reimbursement for relocation and travel to professional meetings.

A summary of NRC Research Associates on tenure during the reporting period is shown in Table 2.

Outcomes Reporting

All NRC Research Associates who completed tenure were requested to submit a final report that described the outcome of their Research Associateship award. A summary of the activities of NRC Research Associates who submitted final reports during this reporting period, including publications, presentations and patents, as well as an assessment of their experience in the program, are summarized in Table 3. Specific accomplishments of NRC Research Associates completing tenure during the reporting period are summarized in individual Final Reports (attached to annual technical reports).

Table 1. Summary of applications and awards

Table 2. NRC Research Associates on tenure during the reporting period

Table 3. Activities of NRC Research Associates who completed tenure during the reporting period

Attachments: NRC Research Associates Final Reports, including Research Accomplishments and Scholarly Productivity

AMRMC - U.S. Army Medical Research Institute of Infectious Diseases

Table 1: Summary of applications and awards

	Applications			Lab Decision/Outcome		
	Submitted	**Reviewed**	**Recommended**	**Awards Offered**	**Awards Accepted**	**No Offer**
Nov 2017	0	0	0	0	0	0
Feb 2018	0	0	0	0	0	0
May 2018	1	1	1	1	1	0
Aug 2018	3	1	1	0	0	0
Total (All Reviews)	4	2	2	1	1	0

Table 2: NRC Research Associates on tenure during the reporting period

Associate	Adviser	Tenure Dates	Country of Citizenship	Final Report
U.S. Army Medical Research Institute of Infectious Diseases				
Arnold, Catherine Elizabeth	Palacios, Gustavo F	9/22/2017-9/21/2019	United States	
Bachert, Beth Alexandra	Bozue, Joel A	1/3/2017-1/2/2018	United States	Received
Coate, Eric Allan	Bozue, Joel A	12/30/2015-2/8/2018	United States	Received
DeLaine-Elias, BreOnna C.	Palacios, Gustavo F	3/1/2017-4/13/2018	United States	Received
Di Paola, Nicholas	Palacios, Gustavo F	7/2/2018-7/1/2019	United States	
Duy, Janice	Minogue, Timothy Devins	8/1/2013-2/28/2018	United States	Received
Espy, Nicole Joy	Palacios, Gustavo F	10/2/2017-7/31/2018	United States	Received
Gardner, Christina Lynn	Glass, Pamela J	10/10/2017-10/9/2018	United States	
Hollidge, Bradley Sherman	Schmaljohn, Connie	5/2/2016-9/30/2017	United States	Received
Kohler, Lara Juliette	Cote, Christopher Kevin	1/17/2017-10/6/2017	United States	Received
Krishnamurthy, Malathy	Panchal, Rekha G.	10/5/2015-10/4/2018	India	
Maxson, Tucker	Minogue, Timothy Devins	2/1/2017-1/31/2019	United States	
Mielech, Anna Maria	Ulrich, Robert Glenn	2/2/2016-9/15/2018	Poland	
Ricks, Keersten Michelle	Schoepp, Randal J.	12/7/2015-12/6/2017	United States	Received
Smith, Jessica L	Ulrich, Robert Glenn	6/24/2013-6/23/2018	United States	Received
Stefan, Christopher Patrick	Minogue, Timothy Devins	1/2/2014-1/1/2018	United States	Received
Stojadinovic, Marija	Panchal, Rekha G.	12/1/2014-11/30/2018	Serbia	
Suschak, John Joseph	Schmaljohn, Connie	5/1/2017-6/5/2018	United States	Received
Tursiella, Melissa Lynne	Schmaljohn, Connie	4/1/2014-5/2/2018	United States	Received

Table 3: Activities of NRC Research Associates who completed tenure during the reporting period

12 Associates ended tenure during the report period
27 months was the average tenure length
60 months was the longest
9 months was the shortest
12 submitted final reports

In the final reports, Associates indicated the following scholarly activity while on tenure.

22 Articles published in refereed journals
2 Articles other (Proceedings, Book Chapters, other)
14 Domestic presentations
3 International presentations
1 Patent applications
4 Awards

After ending their tenure, Associates indicated their future plans as follows:

0 Permanent position at the NRC host agency
5 Contract or temporary position at the NRC host agency
1 Research/administrative position with another U.S. government agency
0 Research/administrative position with foreign government agency
0 Research/teaching at US college/university
0 Research/teaching position at a foreign college or university
0 Research/administrative position in private industry in the U.S.
0 Research/administrative position in private industry outside of the U.S.
1 Research/administrative position with a non-profit
0 Self-employed/consulting
2 Postdoctoral Research
3 Other
0 No information provided

In their final reports, Associates were asked to evaluate certain aspects of their experiences on a scale of 1 (low) to 10 (high). The average rating for each item follows:

8.7 Short-term value (lab)-Development of knowledge, skills, and research productivity at lab
9.1 Long-term value (career)-How your Research Associateship affected your career to date
9.0 Laboratory Support-Equipment, funding, orientation, safety & health training, etc.
9.0 Adviser Mentoring-Quality of mentoring from the Research Adviser
8.5 LPR Support-Quality of administrative support from the LPR
8.8 NRC Support-Quality of administrative support from the NRC

Attachments: NRC Research Associates Final Reports, including Research Accomplishments and Scholarly Productivity, follow.

NRC Research Associateship Program Associate Final Report

Associate: Bachert, Beth Alexandra
Program: AMRMC - U.S. Army Medical Research & Materiel Command
U.S. Army Medical Research Institute of Infectious Diseases
US Army Medical Research Insti Infec Diseases
Fort Detrick, MD 21702-5011
Opportunity: B8095/Molecular Pathogenesis of Yersinia pestis, Francisella tularensis, and Burkholderia
Adviser: Bozue, Joel A

Research Proposal: Characterization and Virulence Assessment of Naturally Derived Streptomycin-resistant Francisella Tularensis Variants
Tenure Dates: 01/03/2017-01/02/2018

Research Accomplishments

Previous work in our lab showed that antibiotic resistant variants of Francisella tularensis, derived from passaging on increasing concentrations of antibiotic, accumulate mutations directly linked to antibiotic resistance, as well as mutations in potential virulence factors. The goal of this project is to investigate the impact of these mutations on virulence in order to identify new therapeutic targets, as well as to assess the risk of antibiotic resistant F. tularensis using the live vaccine strain (LVS). Preliminary work showed that ciprofloxacin-resistant (CipR) variants of LVS had acquired mutations mainly in outer membrane proteins, including LPS-assembly proteins and potential virulence factors. Major accomplishments from these studies are outlined as follows: i) A series of five CipR strains, each harboring a unique combination of mutations, was assessed for virulence in murine macrophages. We show that CipR mutants passaged on high concentrations of ciprofloxacin had diminished replication in macrophages compared to WT, indicating attenuated virulence. ii) Two of the CipR mutants, one with the most mutations (Cip128-3) and one with the least mutations (Cip80-1), were tested in a BALB/c mouse model of intranasal infection. The Cip80-1 mutant was slightly but significantly attenuated while the Cip128-3 mutant displayed complete attenuation even at the high dose of 64,000 CFU compared to wild-type LVS, revealing a role for these mutated genes in virulence. Interestingly, the Cip128-3 strain acquired mutations in wbtC and lptE genes, encoding proteins presumed to be involved in LPS assembly and transport, respectively. iii) A lptE mutant was generated via group II intron insertion, and tested in the intranasal infection model. We observed significant attenuation in the lptE mutant which had an LD50 measurement of 3,388 CFU compared to 174 CFU for WT LVS ($P<0.0001$), revealing an important link between LPS transport and virulence. iv) Additional plasmids have been constructed for mutagenesis of the wbtC gene, using both in-frame allelic replacement and group II intron insertion methods. These plasmids are currently being utilized to generate a wbtC mutant of LVS. v) Finally, a serial sacrifice experiment was performed using wild-type LVS for intranasal infection in mice over a period of 7 days. This study allowed us to determine the dissemination characteristics of LVS to the lungs, spleen, and blood and therefore determine critical time points for later studies comparing WT and mutant LVS. Overall, our studies show that mutations acquired during ciprofloxacin resistance acquisition have a significant impact on virulence. Importantly, we highlight the LptE protein as a key virulence factor in LVS. Current work aims to complete characterization and virulence assessment of the CipR strains, as well as determine the impact of individual genes on virulence by constructing mutants for genes of interest.

Scholarly Productivity

Articles - Peer Reviewed

Articles - Other (Proceedings, Book Chapters, other)

Presentations - Domestic

Presentations - International

Patents

AWARDS

NRC Research Associateship Program Associate Final Report

Associate: Coate, Eric Allan
Program: AMRMC - U.S. Army Medical Research & Materiel Command
U.S. Army Medical Research Institute of Infectious Diseases
US Army Medical Research Insti Infec Diseases
Fort Detrick, MD 21702-5011
Opportunity: B8095/Molecular Pathogenesis of Yersinia pestis, Francisella tularensis, and Burkholderia
Adviser: Bozue, Joel A

Research Proposal: Identification of Novel Bacterial Targets for Medical Countermeasures to Combat Antibiotic Resistant Bacterial Threats
Tenure Dates: 12/30/2015-02/08/2018

Research Accomplishments

During my tenure I was able to develop and characterize a new animal model to study antibiotic resistance in Yersinia pestis. This model allowed us to test and determine resistance profiles along with look at potential targets for resistance. These resistance profiles that were determined have homology in other pathogens making this work very interesting. While the development of my initial constructs took longer than expected, I was able to learn more molecular techniques that will help me in the future. Lastly, I was able to present my work at several national meetings (American Society of Microbiology). Overall, my experience in a federal laboratory and teachings under Dr. Joel Bozue guided me onto a career path as a commissioned Army Medical Service Officer.

Scholarly Productivity

Articles - Peer Reviewed

Articles - Other (Proceedings, Book Chapters, other)

Presentations - Domestic

Presentations - International

Patents

Awards

01/01/2017, ASM Post-Doctoral Fellowship Travel Award, ASM

NRC Research Associateship Program Associate Final Report

Associate: DeLaine-Elias, BreOnna C.
Program: AMRMC - U.S. Army Medical Research & Materiel Command
U.S. Army Medical Research Institute of Infectious Diseases
US Army Medical Research Insti Infec Diseases
Fort Detrick, MD 21702-5011
Opportunity: B7865/Dissecting the Immunological Response against Extremely Dangerous Pathogens
Adviser: Palacios, Gustavo F

Research Proposal: Longitudinal Dissection of the Immune Response to Vaccine Using Novel Ngs Techniques
Tenure Dates: 03/01/2017-04/13/2018

Research Accomplishments

During my tenure as an NRC fellow at USAMRIID, I worked on the optimization B cell receptor (Ig-Seq) sequencing protocols to characterize IgG and IgM repertoire responses in humans or nonhuman primates (NHPs). Ig-Seq analysis will be used to directly compare humoral responses against vaccination or viral infection in both species. IgM sequences (mostly naïve B cells) would be mainly used to infer the germ line genes at the Ig locus of the animal/human subject. IgG sequences to detect and characterize the B cell clones responding to the vaccination or infection.

I focused in three different and complementary strategies to perform the Ig-Seq: 1) infer the Ig transcripts directly from the transcriptome, 2) sequence using short reads (Illumina), and 3) sequence using long reads (Pacific Biosciences).

Ig transcriptome analysis: After isolating RNA from peripheral blood mononuclear cells (PBMCs) of vaccinated or naïve animals, we performed standard RNA-sequencing and tested two available bioinformatic pipelines, ImReP and MiXCR, in their ability to reconstruct B cell repertoires from RNA-sequencing data. Initial analysis showed that the pipeline can detect the most abundant B cell clonotypes but fail to detect the clonotypes with low frequency.

Targeted Ig-Seq: Ig primers directed toward the constant region of the Ig were used to specifically target Ig transcripts. The sequences, containing the VDJ rearrangement of the Ig, were sequenced by MiSeq Illumina (2 x 250 base pairs) or PacBio sequencing. The raw data was annotated with IMGT and analyzed using bioinformatic tools to compare the B cell repertoires (BCR) described with both technologies. Using this combined approach, we developed a standard procedure to dissect changes in the BCR following infection or immunization. The dual approach, also allowed me to deeply characterize the VDJ repertoire (short reads) and link the paratope of the Ig with the Ig isotype (long reads). This strategy permits the association of the Ig specificity (paratope) with it biological function (Fc region).

Additionally, my work included researching and testing various single-cell sequencing platforms. The goal of using single-cell sequencing was pairing the variable domains of the Ig heavy and light chain sequences within a single B cell to reconstitute the antibody and determine antigen specificity. We compared the ICell8 system single cell imaging system to microfluidic droplet techniques and have found the ICell8 system to produce better quality results.

Scholarly Productivity

Articles - Peer Reviewed

Articles - Other (Proceedings, Book Chapters, other)

Presentations - Domestic

DeLaine-Elias, B.; Bartlett, M.; Koroleva, G.; Hill, B.; Garcia, K.; Nagle, E. ; Arnold, C.; Sanchez-Lockhart, M.; and Palacios, G., 10/03/2017, Comparative Immunoglobulin Sequencing for Immune Repertoire Analysis, Immunogenomics Conference by HudsonAlpha, Huntsville, Alabama

Presentations - International

PATENTS

AWARDS

08/22/2017, 2017 ABRCMS Judge Travel Award, Annual Biomedical Research Conference for Minority Students

NRC RESEARCH ASSOCIATESHIP PROGRAM ASSOCIATE FINAL REPORT

Associate: Duy, Janice
Program: AMRMC - U.S. Army Medical Research & Materiel Command
U.S. Army Medical Research Institute of Infectious Diseases
US Army Medical Research Insti Infec Diseases
Fort Detrick, MD 21702-5011
Opportunity: B7377/Development of Novel Diagnostic Assays, Platforms, and Capabilities for Detection of Biothreat Agents
Adviser: Minogue, Timothy Devins

Research Proposal: Presymptomatic Biomarker Discovery for Ebola Virus and Other Biothreat Agents
Tenure Dates: 08/01/2013-02/28/2018

RESEARCH ACCOMPLISHMENTS

- Circulating viral miRNAs may be presymptomatic markers of Ebola virus disease
- Ebola virus miRNAs are conserved in 4 infection models (mouse, cynomolgus macaque, rhesus macaque, human)
- Circulating host miRNAs can indicate Ebola virus infection
- Nonhuman primate blood components (whole blood, PBMCs, plasma, and serum) contain overlapping and unique miRNA profiles
- Human dermal interstitial fluid contains detectable mRNAs and miRNAs that can be used as health markers

SCHOLARLY PRODUCTIVITY

ARTICLES - PEER REVIEWED

Smith, Rosemary L.; Collins, Scott D.; Duy, Janice; Minogue, Timothy D., 2018, Silicon microneedle array for minimally invasive human health monitoring, SPIE Photonics West
Duy, Janice; Koehler, Jeffrey W.; Honko, Anna N.; Schoepp, Randal J.; Wauquier, Nadia; Gonzalez, Jean-Paul; Pitt, M. Louise; Mucker, Eric M.; Johnson, Joshua C.; O'Hearn, Aileen; Bangura, James; Coomber, Moinya; Minogue, Timothy D., 2016, Circulating microRNA profiles of Ebola virus infection, Scientific Reports, 6//24496
Duy, Janice; Koehler, Jeffrey W.; Honko, Anna N.; Minogue, Timothy D., 2015, Optimized microRNA purification from TRIzol-treated plasma, BMC Genomics, 16/95/1

ARTICLES - OTHER (PROCEEDINGS, BOOK CHAPTERS, OTHER)

PRESENTATIONS - DOMESTIC

Duy, Janice; Koehler, Jeffrey W.; Minogue, Timothy D., 11/28/2017, miRNA markers of early exposure to the bacterial biothreat pathogen Burkholderia mallei in nonhuman primates, DTRA Chemical and Biological Science Defense and Technology Conference, Long Beach, CA
Duy, Janice; Koehler, Jeffrey W.; Honko, Anna N.; Schoepp, Randal J.; Wauquier, Nadia; Gonzalez, Jean-Paul; Pitt, M. Louise; Mucker, Eric M.; Johnson, Joshua C.; O'Hearn, Aileen; Bangura, James; Coomber, Moinya; Minogue, Timothy D., 04/12/2017, miRNAs in Ebola virus infection, Invited presentation - University of Nebraska Medical College, Omaha, NB/USA
Duy, Janice; Koehler, Jeffrey W.; Honko, Anna N.; Schoepp, Randal J.; Wauquier, Nadia; Gonzalez, Jean-Paul; Pitt, M. Louise; Mucker, Eric M.; Johnson, Joshua C.; O'Hearn, Aileen; Bangura, James; Coomber, Moinya; Minogue, Timothy D., 03/28/2017, miRNAs in biothreat infections, UMaine/USAMRIID DTRA dermal interstitial fluid grant kickoff meeting
Duy, Janice; Koehler, Jeffrey W.; Honko, Anna N.; Schoepp, Randal J.; Wauquier, Nadia; Gonzalez, Jean-Paul; Pitt, M. Louise; Mucker, Eric M.; Johnson, Joshua C.; O'Hearn, Aileen; Bangura, James; Coomber, Moinya; Minogue, Timothy D., 06/27/2016, Circulating miRNA profiles of Ebola virus infection, Biodefense World Summit, Baltimore, MD/USA
Duy, Janice; Koehler, Jeffrey W.; Honko, Anna N.; Minogue, Timothy D., 05/12/2015, Longitudinal plasma microRNA profiling of rhesus macaques infected with aerosolized Ebola virus, ASTMH Annual Meeting, New Orleans, LA/USA
Duy, Janice; Koehler, Jeffrey W.; Honko, Anna N.; Minogue, Timothy D., 05/04/2015, Longitudinal plasma microRNA profiling of rhesus macaques infected with aerosolized Ebola virus, NICBR Spring Research Festival, Frederick, MD/USA

Duy, Janice; Koehler, Jeffrey W.; Honko, Anna N.; Minogue, Timothy D., 02/09/2015, Longitudinal plasma microRNA profiling of rhesus macaques infected with aerosolized Ebola virus, ASM Biodefense Meeting, Washington, DC/USA
Duy, Janice; Koehler, Jeffrey W.; Honko, Anna N.; Minogue, Timothy D., 11/17/2014, Longitudinal plasma microRNA profiling of rhesus macaques infected with aerosolized Ebola virus, DTRA Chemical and Biological Defense Science and Technology Conference, St. Louis, MO/USA

PRESENTATIONS - INTERNATIONAL

Duy, Janice; Koehler, Jeffrey W.; Honko, Anna N.; Schoepp, Randal J.; Wauquier, Nadia; Gonzalez, Jean-Paul; Pitt, M. Louise; Mucker, Eric M.; Johnson, Joshua C.; O'Hearn, Aileen; Bangura, James; Coomber, Moinya; Minogue, Timothy D., 01/26/2016, Circulating microRNA profiles of Ebola virus infection, MDPI AG (Viruses 2016 meeting), Basel/Switzerland

PATENTS

AWARDS

03/02/2017, Funded grant in collaboration with the University of Maine: Microneedle extraction of dermal interstitial fluid for minimally invasive disease diagnostics, Defense Threat Reduction Agency

NRC RESEARCH ASSOCIATESHIP PROGRAM ASSOCIATE FINAL REPORT

Associate: Espy, Nicole Joy
Program: AMRMC - U.S. Army Medical Research & Materiel Command
U.S. Army Medical Research Institute of Infectious Diseases
US Army Medical Research Insti Infec Diseases
Fort Detrick, MD 21702-5011
Opportunity: B8339/Viral Evolution of Emerging Pathogen Threats under Positive Selection Center for Genomic Sciences
Adviser: Palacios, Gustavo F

Research Proposal: In Vivo Characterization of Ebov Infection in the Presence of Antiviral Nucleoside Inhibitors
Tenure Dates: 10/02/2017-07/31/2018

RESEARCH ACCOMPLISHMENTS

1. Learned the methods of next generation sequencing (NGS).
2. Learned computational methods to analyze NGS data. Able to publish a scientific article in a peer reviewed journal using these methods and present this work in a poster at an international conference.
3. Gained scientific knowledge about a number of viruses.
4. Learned about drug discovery pipelines and guidelines.

SCHOLARLY PRODUCTIVITY

ARTICLES - PEER REVIEWED

Espy, Nicole; Perez-Sautu, Unai; Ramirez de Arellano, Eva; Negredo, Anabel; Wiley, Michael Rl Bavari, Sina; Diaz Menendez, Marta; Paz Sanchez-Seco, Maria; Palacios, Gustavo, 2018, Ribavirin Had Demonstrable Effects on the Crimean-Congo Hemorrhagic Fever Virus (CCHFV) Population and Load in a Patient With CCHF Infection, The Journal of Infectious Diseases, Volume 217, Issue 12, 25 May 2018, Pages 1952–1956
Espy, Nicole; Nagle Elyse; Pfeffer, Brad; Bixler, Sandra; Warren, Travis; Bavari, Sina; Sanchez-Lockhart, Mariano; Palacios, Gustavo, 2018, Favipiravir induces lethal mutagenesis in Ebola and Marburg populations in macaques, Manuscript in preparation

ARTICLES - OTHER (PROCEEDINGS, BOOK CHAPTERS, OTHER)

PRESENTATIONS - DOMESTIC

PRESENTATIONS - INTERNATIONAL

PATENTS

AWARDS

NRC RESEARCH ASSOCIATESHIP PROGRAM ASSOCIATE FINAL REPORT

Associate: Hollidge, Bradley Sherman
Program: AMRMC - U.S. Army Medical Research & Materiel Command
U.S. Army Medical Research Institute of Infectious Diseases
US Army Medical Research Insti Infec Diseases
Fort Detrick, MD 21702-5011
Opportunity: B3460/Molecular Virology and Vaccine Development
Adviser: Schmaljohn, Connie

Research Proposal: Sounding the Alarmin: HMGB1 promotes Venezuelan Equine Encephalitis Virus Neuroinvasion
Tenure Dates: 05/02/2016-09/30/2017

RESEARCH ACCOMPLISHMENTS

We developed a highly susceptible mouse model of ZIKV infection using immune-intact C57Bl/6 mice. In this model, immunocompetent mice have impaired interferon (IFN) signaling by treatment with a non-cell depleting monoclonal antibody (mAb-5A3) that efficiently targets the type I IFN receptor subunit of the mouse IFN-α/β receptor. Using a Senegal strain of ZIKV, we found mAb-5A3-treated mice infected with ZIKV by subcutaneous or intraperitoneal injection resulted in 40% and 100% mortality, respectively. The brains from these mice showed significant neuropathology indicating severe neuroinflammation including astrogliosis and microgliosis. In addition, we have shown the Senegal strain in the mAb-5A3 model and IFN receptor knockout mice results in higher mortality than several other ZIKV strains tested. Moreover, we have tested our mAb-5A3 mouse model in pregnant mice and found mildly decreased size from embryos of dams infected with ZIKV.

Using positron emission tomography-computed tomography (PET/CT), we have assessed neuroinflammation in the mAb-5A3 mouse model at days 3, 6, and 10 postinfection (PI). Two PET tracers were used, 18-fluorodeoxyglucose ([18F]-FDG) and [18F]-DPA-714. [18F]-FDG is a glucose analog used to measure changes in tissue metabolism and inflammation. [18F]-DPA-714 is a PET radiotracer specific for the 18kDa translocator protein, which is a biochemical marker of neuroinflammation that is upregulated in activated microglia, macrophages, and reactive astrocytes. [18F]DPA-714 PET imaging demonstrated significant neuroinflammation at day 3 PI compared to uninfected, mAb-5A3-treated mice and this neuroinflammation continued to increase at days 6 and 10 PI. In contrast, [18F]FDG uptake in the brains of the mAb-5A3-treated, infected mice peaked at day 6 PI returning to normal levels again by day 10 PI. The [18F]DPA-714 PET imaging was similar to the Iba1, a marker for activated microglia, staining from the brains of these mice confirming the specificity of the PET tracer.

My studies of neuroinflammation in VEEV have shown high-mobility group box 1 (HMGB1), which is normally a nuclear protein, is present in the cytoplasm of cells within the CNS from VEEV-infected mice. Using VEEV-TC-83, I have found increased HMGB1 release in the supernatants of primary mouse neurons.

SCHOLARLY PRODUCTIVITY

ARTICLES - PEER REVIEWED

Smith, Darci; Hollidge, Bradley; Daye, Sharon; Zeng, Xiankun; Blancett, Candace; Kuszpit, Kyle; Bocan, Thomas; Koehler, Jeff; Coyne, Susan; Minogue, Tim; Kenny, Tara; Chi, Xiaoli; Yim, Soojin; Miller, Lynn; Schmaljohn, Connie; Bavari, Sina; Golden, Joseph, 2017, Neuropathogenesis of Zika Virus in a Highly Susceptible Immunocompetent Mouse Model after Antibody Blockade of Type I Interferon, PloS Neglected Tropical Diseases, 11(1): e0005296
Kuszpit, Kyle*; Hollidge, Bradley*; Zeng, Xiankun; Stafford, Robert; Daye, Sharon; Zhang, Xiang; Bhattacharyya, Falguni; Swenson, Rolf; Smith, Darci; Bocan, Thomas, 2017, Imaging Neuroinflammation in an Immunocompetent Mouse Model of Zika Virus Infection, Mol. Imaging Biol. *Authors contributed equally

ARTICLES - OTHER (PROCEEDINGS, BOOK CHAPTERS, OTHER)

PRESENTATIONS - DOMESTIC

PRESENTATIONS - INTERNATIONAL

PATENTS

AWARDS

NRC RESEARCH ASSOCIATESHIP PROGRAM ASSOCIATE FINAL REPORT

Associate: Kohler, Lara Juliette
Program: AMRMC - U.S. Army Medical Research & Materiel Command
U.S. Army Medical Research Institute of Infectious Diseases
US Army Medical Research Insti Infec Diseases
Fort Detrick, MD 21702-5011
Opportunity: B8336/Bacillus anthracis Spore Biology in Context of Certain Practical Applications
Adviser: Cote, Christopher Kevin

Research Proposal: Inducing Germination in Spores of Bacillus Anthracis to Improve Wide Area Decontamination and Patient Therapy
Tenure Dates: 01/17/2017-10/06/2017

RESEARCH ACCOMPLISHMENTS

Validated novel organic compounds from a high-throughput screen that impact spore germination. These compounds may potentially be used to enhance wide-area decontamination after an accidental or intentional release of Bacillus anthracis by enhancing germination and thus the impact of a secondary disinfectant because germinated spores are less resistant to common disinfectants than ungerminated spores. One compound in particular has a very strong effect in a fluorescenct kinetic assay. I have also investigated the effects of this compound on spore germination using the optical density and heat assays. I also found that the magnitude of the effect of the identified compounds on germination depends on the concentration of germinant added. Outside of my main research project I tested aptamers that could be potentially used for detection or therapeutics against the anthrax toxin. These aptamers were generated by collaboraters in academia. Additionally, I have performed an analysis of a large-scale cytokine data-set from an animal infection experiment. Lastly, I have written a review article on spore decontamination using germination-induction that is currently under review.

SCHOLARLY PRODUCTIVITY

ARTICLES - PEER REVIEWED

Kohler, Lara; Quirk, Avery; Welkos, Susan; Cote, Christopher, 2017, Incorporating germination-induction into decontamination strategies for bacterial spores, Kohler, Lara; Quirk, Avery; Welkos, Susan; Cote, Christopher. Incorporating germination-induction into decontamination strategies for bacterial spores. Under review.

ARTICLES - OTHER (PROCEEDINGS, BOOK CHAPTERS, OTHER)

PRESENTATIONS - DOMESTIC

PRESENTATIONS - INTERNATIONAL

PATENTS

AWARDS

NRC RESEARCH ASSOCIATESHIP PROGRAM ASSOCIATE FINAL REPORT

Associate: Ricks, Keersten Michelle
Program: AMRMC - U.S. Army Medical Research & Materiel Command
U.S. Army Medical Research Institute of Infectious Diseases
US Army Medical Research Insti Infec Diseases
Fort Detrick, MD 21702-5011
Opportunity: B5422/Molecular Diagnostics and Pathogenesis
Adviser: Schoepp, Randal J.

Research Proposal: Teaching an Old Dog New Tricks: DNA Vaccination for Target-specific Immunodiagnostic Assay Development
Tenure Dates: 12/07/2015-12/06/2017

RESEARCH ACCOMPLISHMENTS

During the first year of tenure, I worked on two major projects. The first, on which I wrote my initial proposal, was a collaborative proof of concept project with the Schmaljohn and Hooper labs in the Virology division at USAMRIID. This project aimed to demonstrate the use of DNA vaccines as a safe, low-cost route toward target-directed monoclonal antibody production for future use in diagnostic immunoassays. We successfully developed IgM monoclonals against the Hantavirus Dobrava. We also vaccinated mice with a DNA construct to make Sudan NP monoclonals. I started an immunoassay development project, funded by DTRA in collaboration with Beckton Dickinson, to distinguish a bacterial or viral infection using host biomarkers. The goal of this project is to develop multiplex MAGPIX assays for procalcitonin, human neutrophil lypocalin (HNL), TNF-related apoptosis-inducing ligand (TRAIL), and interferon gamma-induced protein 10 (IP-10), using commercially-available reagents, and to optimize assay performance in a laboratory setting in preparation for future validation in a clinical setting. Clinically relevant limits of detection were achieved for each biomarker in both the singleplex and multiplex formats.
As a continuation of my research in my first year of tenure as an NRC, I worked toward assay development to gear up for the deployment of an emerging infectious disease immunoassay panel we deem the West African Panel. This panel will identify antigen in sera for Ebola, Lassa, Marburg, CCHF, Rift, Flaviviruses, and Alphaviruses. In collaboration with the Joint West African Research Group (JWARG), we conducted training in both Ghana and Nigeria, to teach the lab technicians how to use the Magpix platform, on which the West African Panel is based.
In addition to this, I collaborated with Dr. Charles Shoemaker, a former NRC postdoc in the Virology Division at USAMRIID, to address pathways toward sustainable immunodiagnostic reagents. This included creation and application of alternative capture reagents, such as virus like particles and recombinant proteins, as well as alternate routes toward monoclonal antibody production. We have begun to incorporate these reagents into the Mapgix platform. We filed for a provisional patent anticipate to have a manuscript out by the end of the year.

Significant accomplishments include: 1) Filing a provisional patent application for an immunodiagnostic reagent we developed, 2) conducting training protocols in West Africa for serosurveillance using MAGPIX, 3) fostering strong collaboration with the Schmaljohn lab in the Virology Division at USARMIID, 4) fully developing both an antigen and antibody multiplex MAGPIX assay for West African viruses, and 5)mentoring a local high school student wishing to pursue a career in science.

SCHOLARLY PRODUCTIVITY

ARTICLES - PEER REVIEWED

Ricks, Keersten; Shoemaker, Charles; Dupuy, Les; Flusin, Olivier; Voorhees, Matthew; Six, Carolyn; Badger, Catherine; Schmaljohn, Connie; Schoepp, Randal, 2017, Virus-Like Particles and Magnetic Microspheres Provide a Flexible and Sustainable Multiplex Immunodiagnostic Platform ,

ARTICLES - OTHER (PROCEEDINGS, BOOK CHAPTERS, OTHER)

PRESENTATIONS - DOMESTIC

Ricks, Keersten, 08/23/2017, Sustainability Isn't Just for Energy: Development of Sustainable Immunoassays for Point-of-Care Detection of West African Infectious Diseases, ACS National Meeting, Washington, DC, USA

PRESENTATIONS - INTERNATIONAL

PATENTS

Ricks, Keersten
Shoemaker, Charles
Schmaljohn, Connie
Schoepp, Randal, 11/03/2017, Virus-like Particle Bound Magnetic Particles as Reagents for Immunodiagnostics, 62/581,023

AWARDS

NRC RESEARCH ASSOCIATESHIP PROGRAM ASSOCIATE FINAL REPORT

Associate: Smith, Jessica L
Program: AMRMC - U.S. Army Medical Research & Materiel Command
U.S. Army Medical Research Institute of Infectious Diseases
US Army Medical Research Insti Infec Diseases
Fort Detrick, MD 21702-5011
Opportunity: B3470/Proteomics of Host-Pathogen Interactions
Adviser: Ulrich, Robert Glenn

Research Proposal: Development of Protein Microarrays for the Detection of Biomarkers of Arboviral Infection
Tenure Dates: 06/24/2013-06/23/2018

RESEARCH ACCOMPLISHMENTS

Cloned, expressed, and purified 48 structural proteins from 16 arboviruses.
Determined antibody-antigen recognition patterns for natural human alphaviral infections, presented the findings at in international conference, and published the findings in a peer-reviewed journal.
Cloned, expressed and purified 19 Dengue envelope proteins for vaccine immune-response studies and am in the process of publishing the findings in a peer-reviewed journal.
Wrote a proposal and got funding for an international collaborative research effort to study antibodies to filoviruses, alphaviruses, bunyaviruses, and flaviviruses in Africa.

SCHOLARLY PRODUCTIVITY

ARTICLES - PEER REVIEWED

Smith, JL; Pugh CL; Cisney, ED; Keasey, SL; Guevara, C; Ampuero, JS; Hontz, RD; Ulrich, RG., 2018, Human Infections caused by Mayaro, Venezuelan Equine Encephalitis and Chikungunya Viruses Detected by Specific Antibody Recognition of Microarrayed Structural Proteins, mSphere, Mar 2018, 3 (2)e00003-18; DOI: 10.1128/mSphere.00003-18
Smith, JL; Ulrich, RG. et al., 2018, Comparative Analysis of Antibody Responses to Chikungunya Vaccine Versus Natural Chikungunya Infection,
*Keasey, S.; *Smith, JL; Fernanedez, S.; Durbin, A.; Zhao, B.; Ulrich, RG, 2018, "The impact of dengue virus serotype 2 strain diversity on serological immune responses to dengue". *These authors contributed equally to this work,
Keasey SL, Pugh CL, Jensen SM, Smith JL, Hontz RD, Durbin AP, Dudley DM, O'Connor DH, Ulrich RG., 2017, Antibody Responses to Zika Virus Infections in Environments of Flavivirus Endemicity, Clin Vaccine Immunol. 2017 Apr 5;24(4). pii: e00036-17.

ARTICLES - OTHER (PROCEEDINGS, BOOK CHAPTERS, OTHER)

PRESENTATIONS - DOMESTIC

PRESENTATIONS - INTERNATIONAL

Smith, Jessica; Cisney, Emily; Pugh, Christine; Keasey, Sarah; Guevara, Carolina; Ampuero, Julia; Comach, Guillermo; Gomez, Doris; Ochoa, Margarita; Hontz, Robert; Ulrich, Robert, 11/16/2016, "Protein Specificity of Antibody Responses to South American Alphavirus Infections Using a Novel Multiplexed Assay", American Society of Tropical Medicine & Hygiene, Atlanta, GA

PATENTS

AWARDS

10/31/2016, Fiscal year 2017 Funded project "Seroprevalence of infections caused by four major groups of zoonotic and vector-borne viruses endemic to Africa", Global Emerging Infections Surveillance and Response System (GEIS)

NRC Research Associateship Program Associate Final Report

Associate: Stefan, Christopher Patrick
Program: AMRMC - U.S. Army Medical Research & Materiel Command
U.S. Army Medical Research Institute of Infectious Diseases
US Army Medical Research Insti Infec Diseases
Fort Detrick, MD 21702-5011
Opportunity: B7377/Development of Novel Diagnostic Assays, Platforms, and Capabilities for Detection of Biothreat Agents
Adviser: Minogue, Timothy Devins

Research Proposal: Antibiotic Resistance Gene Detection by Next-generation Sequencing
Tenure Dates: 01/02/2014-01/01/2018

Research Accomplishments

- Optimized Molecular Inversion Probe (MIP)protocol to create amplicons for NGS in under 5 hours
- Created a MIP panel for 16S gene detection and submitted a paper currently in review
- Created a MIP panel for detecting SNPs which lead to ciprofloxacin resistance in biothreat agents and published a paper
- Created a 90 probe MIP panel for the detection of organisms endemic to west africa
- Created a 80 probe MIP panel for the detection of antimicrobial resistance elements
- Optimized a protocol and several assays to measure the expression of SOS response genes in response to antibiotic treatment to determine strain susceptibility

Scholarly Productivity

Articles - Peer Reviewed

Stefan, Christopher; Chase, Kitty; Coyne, Susan; Kulesh David; Minogue Timothy; Koehler Jeff, 2016, Development of real-time reverse transcriptase qPCR assays for the detection of Punta Toro virus and Pichinde virus., Virology Journal, 13:54. doi: 10.1186/s12985-016-0509-3.
Stefan, Christopher; Koehler, Jeff; Minogue, Timothy, 2016, Targeted next-generation sequencing for the detection of ciprofloxacin resistance markers using molecular inversion probes., Scientific Reports, 13;6:25904. doi: 10.1038/srep25904.

Articles - Other (Proceedings, Book Chapters, other)

Presentations - Domestic

Presentations - International

Patents

Awards

NRC RESEARCH ASSOCIATESHIP PROGRAM ASSOCIATE FINAL REPORT

Associate: Suschak, John Joseph
Program: AMRMC - U.S. Army Medical Research & Materiel Command
U.S. Army Medical Research Institute of Infectious Diseases
US Army Medical Research Insti Infec Diseases
Fort Detrick, MD 21702-5011
Opportunity: B3460/Molecular Virology and Vaccine Development
Adviser: Schmaljohn, Connie

Research Proposal: Assessment of Immune-Mediated Protection by a DNA-Based Filovirus Vaccine Formulated with a Molecular Adjuvant
Tenure Dates: 05/01/2017-06/05/2018

RESEARCH ACCOMPLISHMENTS

The goal of this research was to investigate the ability of genetic adjuvants to increase the immunogenicity of DNA vaccines delivered by intramuscular (IM) injection. In previous studies we showed that DNA vaccines expressing the codon-optimized glycoprotein (GP) genes of Ebola (EBOV) or Marburg (MARV) viruses protect both mice and nonhuman primates from viral challenge when delivered by intramuscular electroporation (EP). To determine if we could achieve equivalent immunogenicity and protective efficacy in the absence of EP by using improved DNA vaccines, we tested co-expression of the EBOV DNA vaccine and genetic adjuvants designed to potentiate immune responses. The genetic adjuvant genes evaluated included those for the Th1-inducing cytokine IL-12 and the granulocyte growth factor GM-CSF, both of which have demonstrated significant adjuvant effect when included in clinical DNA vaccine formulations. Additionally, we tested enhancement of IFN-α/β production by a plasmid encoding the cytosolic RNA innate immune sensor retinoic acid-inducible gene 1 (RIG-I), which has been shown to be required for clearance of filovirus infections.

Initially we endeavored to determine if co-transfection of the genetic adjuvant and DNA vaccine plasmids impact expression of the EBOV GP gene in vitro. Importantly, no decrease in cytokine production or EBOV GP expression was observed when the genetic adjuvant and EBOV plasmids were transfected separately or in combination. We then tested the ability of the genetic adjuvants to improve the immunogenicity of our EBOV DNA vaccine when delivered by IM injection in an in vivo mouse model. Our preliminary data suggest that IM vaccination of mice with plasmid DNA encoding the genetic adjuvants allows for improved antigen-specific IgG and neutralizing antibody responses compared to vaccination with EBOV DNA vaccine alone. Genetic adjuvants also allowed for skewing of the anti-EBOV GP IgG antibody isotype, yielding a shift towards Th1 or Th2 humoral responses. Inclusion of genetic adjuvants also resulted in significantly increased populations of antigen-specific IFN-γ+ and IL-2+ T cells. Finally, IM vaccination with either plasmid EBOV and IL-12 provided complete protection against viral challenge. We also tested the immunological benefit of including the genetic adjuvants in our Venezuelan equine encephalitis virus (VEEV) DNA vaccine. This study yielded similar results to our EBOV studies, in that inclusion of the genetic adjuvants significantly improved the cellular immune responses. Additionally, IM vaccination with VEEV + IL-12 provided complete protection from aerosol VEEV challenge in mice. Overall, our data suggest that co-delivery of genetic adjuvants with filovirus or alphavirus DNA vaccines using IM delivery can provide comparable efficacy to the same DNA vaccines when delivered using IM-EP devices. We have submitted a manuscript detailing our EBOV results as well as a second manuscript describing the VEEV studies.

SCHOLARLY PRODUCTIVITY

ARTICLES - PEER REVIEWED

Suschak, John; Bagley, Kenneth; Six, Carolyn; Kwilas, Steven; Schmaljohn, Connie, 2018, The genetic adjuvants IL-12 and GM-CSF enhance the immunogenicity of an Ebola virus DNA vaccine in mice,
Suschak, John; Bagley, Kenneth; Six, Carolyn; Shoemaker, Charles; Kwilas, Steven; Dupuy, Lesley; Schmaljohn, Connie, 2018, The genetic adjuvant IL-12 enhances the protective efficacy of a DNA vaccine for Venezuelan equine encephalitis virus in mice,
Suschak, John; Dupuy, Lesley; Williams, James A; Six, Carolyn; Shoemaker, Charles; Kwilas, Steven; Schmaljohn, Connie,, 2018, Evaluation of Next-Generation Nanoplasmid Vectors for Alphavirus and Filovirus DNA Vaccines,
Suschak, John; Williams, James A; Schmaljohn, Connie, 2017, Advancements in DNA vaccine vectors, non-mechanical delivery methods, and molecular adjuvants to increase immunogenicity., Hum Vaccin Immunother. 2017 Dec 2;13(12):2837-2848

Garrison AR1, Shoemaker CJ1, Golden JW1, Fitzpatrick CJ1, Suschak JJ1, Richards MJ1, Badger CV1, Six CM1, Martin JD1, Hannaman D2, Zivcec M3, Bergeron E3, Koehler JW4, Schmaljohn CS5., 2017, A DNA vaccine for Crimean-Congo hemorrhagic fever protects against disease and death in two lethal mouse models., PLoS Negl Trop Dis. 2017 Sep 18;11(9)
Bounds CE, Terry FE, Moise L, Hannaman D, Martin WD, De Groot AS, Suschak JJ, Dupuy LC, Schmaljohn CS., 2017, An immunoinformatics-derived DNA vaccine encoding human class II T cell epitopes of Ebola virus, Sudan virus, and Venezuelan equine encephalitis virus is immunogenic in HLA transgenic mice., Hum Vaccin Immunother. 2017 Dec 2;13(12):2824-2836

ARTICLES - OTHER (PROCEEDINGS, BOOK CHAPTERS, OTHER)

Suschak, John; Schmaljohn, Connie, 2018, Future Approaches to DNA Vaccination Against Hemorrhagic Fever Viruses., Methods Mol Biol. 2018;1604:339-348

PRESENTATIONS - DOMESTIC

Presentation-International: Suschak, John; Bagley, Kenneth; Shoemaker, Charles; Dupuy, Lesley; Schmaljohn, Connie,, 05/10/2018, Enhancement of DNA Vaccines for Filoviruses and Alphaviruses with Genetic Adjuvants , Fort Detrick Spring Research Festival, Frederick, MD/USA
Suschak, John; Bagley, Kenneth; Dupuy, Lesley; Schmaljohn, Connie, , 11/27/2017, Enhancement of DNA Vaccines for Filoviruses and Alphaviruses with Genetic Adjuvants , CBD S&T Conference, Long Beach, CA/USA
Suschak, John; Shoemaker, Charles; Williams, James; Dupuy, Lesley; Schmaljohn, Connie, 11/27/2017, Evaluation of Next-Generation Nanoplasmid™ Vectors for Alphavirus and Filovirus DNA Vaccines, CBD S&T Conference, Long Beach, CA/USA
Suschak, John; Williams, James; Dupuy, Lesley; Schmaljohn, Connie, , 10/01/2016, Evaluation of Next-Generation Nanoplasmid Vectors for Alphavirus and Filovirus DNA Vaccines, ISV 2017, Boston, MA/USA

PRESENTATIONS - INTERNATIONAL

Suschak, John; Shoemaker, Charles; Williams, James; Bagley, Kenneth; Dupuy, Lesley; Schmaljohn, Connie, 10/05/2017, Enhancement of DNA Vaccines for Ebola virus with Genetic Adjuvants and Improved Plasmid Designs, ISV 2017, Paris, France

PATENTS

AWARDS

NRC RESEARCH ASSOCIATESHIP PROGRAM ASSOCIATE FINAL REPORT

Associate: Tursiella, Melissa Lynne
Program: AMRMC - U.S. Army Medical Research & Materiel Command
U.S. Army Medical Research Institute of Infectious Diseases
US Army Medical Research Insti Infec Diseases
Fort Detrick, MD 21702-5011
Opportunity: B3460/Molecular Virology and Vaccine Development
Adviser: Schmaljohn, Connie

Research Proposal: Defining the Mechanism of Coagulopathy and Fibrinolysis in Viral Hemorrhagic Fever
Tenure Dates: 04/01/2014-05/02/2018

RESEARCH ACCOMPLISHMENTS

The goal of this project was to assess the effects of pathogenic hantavirus infection on the extrinsic coagulation cascade (initiated by tissue factor (TF)). We first assessed the infectivity of primary human umbilical vein endothelial cells (HUVECs) with Andes (ANDV) and Hantaan (HTNV) viruses and determined through flow cytometry and high content imaging (HCI) that 10-39% and 2-29% of cells were infected, respectively. Through RT-PCR experiments, we discovered that both ANDV and HTNV resulted in a significant upregulation of TF mRNA at 5 days post infection.

To assess TF protein expression, we pursued a variety of approaches, including immunoblot, ELISA, on-cell ELISA and flow cytometry and ultimately found one antibody that sufficiently captured TF expression. Through these HCI studies, we found that consistent with our RT-PCR data, that total TF protein was also upregulated in both ANDV and HTNV infected HUVEC cultures. However, cell surface-associated TF was only significantly upregulated in ANDV-infected cultures.

These TF data led us to investigate the functional outcome of TF upregulation. We optimized an assay from various protocols in the literature to assess TF-mediated activation of coagulation Factor X (FXa) since the commercially available kits are unreliable. Through these assays, we consistently found that both ANDV and HTNV delayed FXa activity when compared to mock-infected samples, as determined by cleavage of an FXa-specific chromogenic substrate. These results were surprising given that we hypothesized that the increase in TF expression would result in an increase in FXa activity. However, these results suggested that perhaps an inhibitor of the TF-FVIIa-FXa complex was simultaneously upregulated thus reducing the activity of the extrinsic cascade and likely contributing to hemorrhage.

The TF-FVIIa-FXa complex is regulated by tissue factor pathway inhibitor (TFPI), which binds to and functionally inactivates this complex. There are two isoforms of TFPI, alpha (a) and beta (b), which can both functionally inhibit FXa activity. In endothelial cells, TFPI-a is reported to be primarily secreted, while TFPI-b is largely cell surface-associated. Therefore, we first collected supernatants from ANDV and HTNV-infected endothelial cells and analyzed TFPI-a, since it is believed to be the most predominant form in endothelial cells. By performing ELISAs for TFPI, we determined that secreted TFPI, likely TFPIa was reduced in ANDV and HTNV-infected cell populations. We next used HCI to assess the expression levels of TFPI at the surface of ANDV and HTNV infected endothelial cell populations and determined that ANDV, but not HTNV, had statistically significantly upregulated TFPI at the cell surface. In summary, we conclude that ANDV and HTNV inhibit the activity of the extrinsic coagulation cascade, thus yielding valuable insight into the molecular mechanisms of how these hemorrhagic viruses result in coagulopathy.

SCHOLARLY PRODUCTIVITY

ARTICLES - PEER REVIEWED

Tursiella, ML, Smith JM, Lindquist ME, Taylor SL and Schmaljohn CS, 2018, The Effect of Pathogenic Hantavirus Infection on Components of the Extrinsic Coagulation Cascade , In preparation for submission to JVirol

ARTICLES - OTHER (PROCEEDINGS, BOOK CHAPTERS, OTHER)

Tursiella, ML, Taylor SL and Schmaljohn CS, 2018, Protocols to Assess Coagulation Following In Vitro Infection with Hemorrhagic Fever Viruses., Salvato M. (eds) Hemorrhagic Fever Viruses. Methods in Molecular Biology, vol 1604. Humana Press, New York, NY

PRESENTATIONS - DOMESTIC

PRESENTATIONS - INTERNATIONAL

PATENTS

AWARDS