

Core Immunology Laboratory
Keating
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I. INTRODUCTION

The Core Immunology laboratory has been an important resource for accomplishing high throughput immunological analysis of specimens that are part of larger research objectives. We have the capacity to run antibody or antigen detection, high throughput T cell response screening (ELISPOT), multiplex analyte analysis (Luminex), and flow cytometry facilities (FACS Aria and LSR II). In the past the immunology core has had the opportunity to serve the national and international research community by performing the less-sensitive EIA HIV detection test (also known as the detuned assay) to identify individuals recently infected with HIV who have already seroconverted on highly-sensitive, standard HIV tests. Currently the core lab is working as a key collaborator in developing and validating new assays to detect recent HIV infection in conjunction with the CDC, Johns Hopkins, University of North Carolina and the Health Protection Agency (UK). We have been working with these groups and the Gates Foundation to develop a real and virtual repository of specimens that would aid in the validation of new detuned assays. In addition, the core lab has received a number of contracts to perform immunological analysis of cytokines in humans and non-human primates during acute viral infection. Results from these studies have provided novel information and will be published within the coming year.

Major research areas

- Recently acquired HIV infection and detection systems for incidence calculations
- Stem cell isolation, immune cells sorting and detection of activation and proliferation of lymphocytes, and other projects done by flow cytometry.
- HLA antigens by Luminex (REDS II – HLA)
- Immune responses to blood-borne pathogen inactivation products.
- Analysis of cytokine responses in non-human primates during acute SIV infection.
- Analysis of cytokine responses during transfusion and concomitant infection with hepatitis C virus.
- Analysis of cytokine responses in women infected with HIV.

Major BSRI collaborations

- Marcus Muench – cell sorting
- Philip Norris – Luminex cytokine and chemokine analysis, flow cytometry analysis.
- Ed Murphy – spontaneous proliferation of cells from individuals infected with HTLV.
- Leslie Tobler and VRLRC – Investigating cell recovery after frozen storage of peripheral blood mononuclear cells (PBMC)
- Rachael Jackman – Luminex cytokine analysis for transfusions and cellular analysis after blood pathogen inactivation.
- Marion Lanteri – Luminex cytokine analysis for viral infection and cellular analysis after West Nile virus infection.

Major non-BSRI collaborations

- Center for HIV/AIDS Vaccine Immunology (CHAVI)
- Women's Interagency Health Study (WIHS)
- Novartis
- CaridianBCT – with Philip Norris

- Detuned testing collaborators: UCSF,UCSD, NYU, UCLA (Pamina Gorbach), Aaron Diamond (Martin Markowitz), Northwestern (Ann Ragin), University of Colorado Health Sciences Center (Michelle Baron), University of Alabama (Michael Kilby and Sonja Heath), University of Washington (Joanne Steckler and Beth Morrigan)
- Ortho Clinical Diagnostics
- Collaboration on establishing a repository of specimens for use in validating future early HIV detection assays

Staff

- Dale Hirschhorn, Senior Research Associate
- Mila Lebedeva, Research Associate

II. PROGRAM SUMMARY/ PROGRESS REPORT/PLANS

Detuned HIV EIA testing

The Core Immunology laboratory continued to perform detuned HIV EIA testing in 2008/2009. We supported the Acute Infection and Early Disease Research Project (AIEDRP). We also collaborated with Dr. Chris Pilcher and Frederick Hecht at UCSF and Bernie Branson at the CDC on HIV staging projects. We also furthered developing collaborations with the South African and Brazilian National Blood Service, performing detuned HIV testing on specimens identified as HIV RNA+ in their operational testing. We are currently working with these samples to identify the detection window period for individuals infected with various clades of viruses for Ortho Vitros and other methods of incidence testing.

Collaboration with Ortho Diagnostics has provided us with reagents for detuned HIV testing utilizing their Vitros platform; we have optimized a new low sensitivity HIV detection method and calibrated this to the Vironostika assay results using over 700 previously characterized HIV-1 positive American Red Cross blood donors. In addition to this low sensitive method, the core lab has also brought on board an avidity assay modification to the system that will enable us to test, in parallel, two ways to detect early HIV infection using the same system. With these studies finished, a draft of a manuscript has been completed and will be submitted in the coming months. In addition, a new assay called Avioq, based on the previous “gold standard” for the detection of recent HIV infection, has recently been approved by the FDA. We will begin to use this assay, comparing it to specimens previously tested by Vitros and Vironostika, and testing banked specimens of various HIV clades to compare to other HIV incidence tests. This will be an important step in validating a new assay for international use for the detection of recent acquisition of HIV.

Currently, the core lab is running and has approvals to run low sensitivity assays for the following collaborators: UCSD, NYU, UCLA (Pamina Gorbach), Aaron Diamond (Martin Markowitz), Northwestern (Ann Ragin), University of Colorado Health Sciences Center (Michelle Baron), University of Alabama (Michael Kilby, Sonja Heath), and University of Washington (Joanne Steckler and Beth Morrigan). These tests will support a variety of studies identifying individuals with early infection. The core laboratory, with the help of Dr. Chris Pilcher and UCSF Options and Scope studies, is collecting samples from recently infected, long term infected elite controllers and HIV+ individuals on HAART therapy, to set up panels for validation of new methods of testing for recent infection. A similar proposal was submitted for funding from the Gates Foundation but the proposal was scaled down and the laboratory portion was excluded from the final application. The core lab is also interested in collaborating with companies

developing new HIV tests by setting up the necessary HIV+ panels of various clades to monitor analytical sensitivity and the dynamic range of these new tests.

Flow cytometry

The core lab is continuing to expand its research in infectious disease using the FACS Aria flow sorter (Becton Dickinson) allowing multi-parameter flow cytometry sorting, currently capable of detecting up to nine different cellular stains simultaneously and sorting four distinct populations. This is bringing research projects from infectious diseases and stem cell research from within BSRI and from outside collaborators. This facility enhances the ability to make new collaborations in these fields. An example of this type collaboration is with companies focused on pathogen inactivation systems for blood products. The Flow core facilities have been vital in clinching these collaborations.

This core facility, the LSR-II, FACSaria and the expertise of Dale Hirsch Korn, have been instrumental in facilitating the optimization of study panels and trouble shooting for a number of projects that are based in the immunology and stem cell research departments. This work includes internal and external collaborative studies in exploring the pathogenesis of HIV (with CHAVI under Dr. Philip Norris), HTLV (under Dr. Ed Murphy) and WNV infection (under Dr. Philip Norris). In addition, external collaborations have increased this year. Under Dr. Norris, the core lab flow facility, with Dr. Rachel Jackman, has optimized panels to study immune responses to blood pathogen inactivation products manufactured by Caridian BCT. The core lab will also look at T regulatory cells after repeated transfusion with treated cells. Both of these experiments will require the FACS core to phenotype these cells and to optimize proliferation protocols.

ELISPOT

The ELISPOT plate reader continues to function well and allow high-throughput processing of 96 well-plates. This equipment has been supported both HIV and WNV pathogenesis projects and will continue to support these upcoming projects.

Luminex

The Luminex system that arrived at the end of 2005 has also led to new capabilities and opportunities for collaboration. Up to 100 different beads can be labeled with unique antibodies, allowing simultaneous tracking of 100 different analytes from a single 25 µl sample. We successfully lobbied the NIH to pay for the majority of the purchase of the new system as it will be used for the REDS II project, leukocyte antibody prevalence study (LAPS). The availability of the new technology also allowed successful competition for funding from the Center for HIV/AIDS Vaccine Initiative (CHAVI) and Women's Interagency Health Study (WIHS) to study cytokines in plasma donors.

This machine has served well in both the REDS II leukocyte antibody prevalence project and the CHAVI. This study helped to show our expertise in this technique and we were given the opportunity to work again for CHAVI looking at cytokine responses in non-human primate models of SIV infection. The main objective in this study was to identify the cytokine responses during acute non-human primate infection and to see whether the cytokine response induced were similar to that of acute HIV infection in humans. The data generated with the CHAVI group has been recently presented at the annual CHAVI retreat and is currently being analyzed in preparation for publication. A large cohort of HIV infected individuals has also been analyzed as part of the study with the WIHS pathogenesis group. The first part of the study has been completed and the results presented to the WIHS investigators and NIH program officers. Our success in this study has led to another year of funding to continue our work with the WIHS

program and to a \$1.5 million administrative supplement through the American Reinvestment and Recovery Act (ARRA) to expand this testing. More recently, the Luminex has been used to look at cytokine and chemokine responses to transfusion after trauma under the direction of Dr. Rachel Jackman and Dr. Philip Norris. The manuscript describing this study has recently been accepted for publication. Another study looking at 42 cytokine and chemokine responses in acute HCV and its impact on disease resolution has been completed and the analysis is currently underway. This study will be a very important addition to the current literature because it provides a rare glimpse at identifying immune responses during the first days to weeks after infection.

III. GRANTS, CONTRACTS, AND AWARDS

Current

Detuned HIV testing billed per test.

Ortho Clinical Diagnostics 01/01/09 – 12/31/09
 Calibration and validation of HIV-1/2 low sensitive enhanced chemiluminescence.

U01-AI-034993-15 (Minkoff) 09/30/09 – 09/29/11
 NIH/NIAID
 Women's Interagency HIV Study (WIHS)
 Salary support for Keating (20%) and supplies. Determine if cytokine levels across a broad panel of cytokines differ amongst HIV infected women with good immunological control of viral replication, poor control, or in HIV uninfected women.

U01-AI067854 (Haynes) 09/01/09 – 06/30/10
 NIH/NIAID
 CHAVI: HIV Genetics
 Salary support for Keating (10%) and supplies. Detailed molecular and virological studies to characterize genetic diversity and drug resistance in recently transmitted viruses from around the world.

R01-HL-095140-01 (Kaplan) 09/25/08 – 06/30/13
 NIH/NHLBI
 Inflammatory and Immune Mechanisms of Atherosclerosis in HIV-Infected Women
 Salary support for Lebedeva (10%) and supplies.

R01-HL-076902 (Busch) 09/01/03 – 08/31/10
 NIH/NHLBI
 Natural History of Acute and Chronic HCV in Blood Donors
 Salary support for Lebedeva (30%). Epidemiologic, viral and immunologic studies to understand the host-virus interaction from seroconversion through resolved or persistent HCV infection

Immunology Core 01/01/09 – 12/31/09
 Blood Systems Inc.
 Determine the relationship between HIV-specific cytolytic CD4+ T cells and control of HIV replication. Expand T cell immunology studies to examine West Nile virus immunity and the immunology surrounding the development of microchimerism.

Pending

Gates Foundation

Development of a specimen repository for the development of assays for acute HIV infection and estimation of HIV incidence in populations. Letter of intent has been submitted. Full proposal to be submitted in early 2010.

Completed

REDS II (Busch)

NIH/NHLBI

Leukocyte Antibody Prevalence Study (LAPS)

Unsuccessful Applications

Cerus (Prime)

Department of Defense

Blood Safety Technology: Pathogen Inactivation Treatment of Red Blood Cell, Platelet, and Plasma Components for Support of Combat Trauma Patients Requiring Massive Transfusion

IV. OTHER SIGNIFICANT ACTIVITIES

V. ABSTRACTS, PUBLICATIONS, AND PRESENTATIONS

Abstracts that included work done in the core immunology laboratory

Keating SM, Lebedeva M, Norris PJ, Laeyendecker O, Contestable P, Edwards S, Busch MP; Optimization and Calibration of a Less Sensitive (LS) Protocol for the Ortho Vitros Enhanced Chemiluminescence (ECi) HIV-1/2 assay for Detection of Early HIV Infections and Incidence Estimation (poster). Conference on Retrovirology and Opportunistic Infections (CROI), Montreal, February 2009.

Busch MP, Stramer S, Vermeulen M, Goncales T, **Keating S**, Remis R; Derivation of HIV Incidence Assay "Window Periods" from Seroconverting Blood Donors in Countries with Diverse HIV Clades (poster). Conference on Retrovirology and Opportunistic Infections (CROI), Montreal, February 2009.

Fiscus S, Cachafeiro A, Kshatriya R, Napravnik S, Owen M, **Keating S, Busch M**, Little S, Hogan C. Comparison of 5 HIV Incidence Assays with the Less Sensitive Vironostika HIV Antibody Assay for Screening Subjects for A5217. subm V-122. [accepted as poster] 16th Conference on Retroviruses and Opportunistic Infections (CROI 2009). Montreal, QC, Canada, Feb 8-11, 2009.

Delaney K, Charurat M, Constantine N, Owen M, **Keating S**, Curtis K, Saidu A, Croxton T, Villalba-Diebold P, Aliu G, Vertefueille J, Blattner W, Nasidi A. Evaluation of 5 HIV Incidence Assays Using a Panel of Nigerian Specimens; Implications for Surveillance Programs in Nigeria. "Distinction of Merit" Poster MOPEC006. International AIDS Society, 5th IAS Conference on HIV Pathogenesis, Treatment, and Prevention (IAS 2009). Cape Town, So Africa, July 19-22, 2009.

Pilcher C, Louie B, **Keating S**, Pandori M, Fish F, Keren T, **Lebedeva M**, Liska S, **Busch M**, Hecht F. A clinical Study of Antigen/Antibody Rapid Testing for Acute HIV Infection. subm

V-166, poster 992. 16th Conference on Retroviruses and Opportunistic Infections (CROI 2009). Montreal, QC, Canada, Feb 8-11, 2009.

Keating S, Heitman J Stacey A, Zahn R, Liu J, Borrow P, Barouch D, Letvin N, Schmitz J **Norris PJ**. Early Immune Responses to SIV Infection. Acute HIV meeting, Harvard University, September 2009. (poster).

Keating S, Heitman J Stacey A, Zahn R, Liu J, Borrow P, Barouch D, Letvin N, Schmitz J **Norris PJ**. Pathogenic SIVmac251 Infection of Rhesus Macaques is Associated with a Striking Acute-Phase Systemic Cytokine Response Paralleling That Observed in Acute HIV-1 Infection. (Oral abstract) Center for HIV/AIDS Vaccine Immunology (CHAVI). Durham NC. Oct 4-7, 2009.

Norris PJ, Carrick DM, Kleinman SH, Pandey S, Lee J-H, Vorhaben R, Roback J, Chance S, **Lebedeva M, Busch MP**. Comparison of Assays to Detect HLA Antibodies. Poster # Sp-372. 62nd Annual Mtg, American Association of Blood Banks (AABB). New Orleans LA. Oct 24-28, 2009. Transfusion 49(Suppl 3):189a-190a, Sep 2009.

Jackman RP, Heitman JW, Marschner S, Goodrich, RP, **Norris PJ**. Understanding Loss of Donor White Blood Cell Immunogenicity Following Pathogen Reduction: Mechanisms of Action in UV Illumination and Riboflavin Treatment. Oral Abstr S93-040A. 62nd Annual Mtg, American Association of Blood Banks (AABB). New Orleans LA. Oct 24-28, 2009. Transfusion 49(Suppl 3):38a-39a, Sep 2009.

Lanteri MC, O'Brien KM, Cameron MJ, **Owen RE, Heitman JW, Custer B, Hirschhorn DF, Tobler LH**, Kiely N, Prince HE, Ndhlovu LC, Nixon DF, Kamel HT, Kelvin DJ, **Busch MP**, Diamond MS, **Norris PJ**. Low Levels of Regulatory T Cells Associate with the Development of Symptomatic West Nile Virus Infection. Poster _____. Keystone Symposium C5: Regulatory T Cells, Keystone CO, March 1-6, 2009.

Norris PJ, Glynn SA, Todd DS, Likos AM, **Heitman JW**, Collins CS, Linnen JM, **Busch MP**. Assessment for Influenza A Virus in Blood Donors. Oral Abstr S103-040C. 62nd Annual Mtg, American Association of Blood Banks (AABB). New Orleans LA. Oct 24-28, 2009. Transfusion 49(Suppl 3):43a, Sep 2009.

Norris PJ, Hirschhorn DF, Devita D, Lee T-H, Murphy EL. HTLV-I Induced Spontaneous Lymphocyte Proliferation is not Limited to CD8+ T Cells. Poster # 87. 14th International Conference on Human Retrovirology: HTLV and Related Retroviruses. Salvador, Brazil, July 1-4, 2009.

Law JP, Hirschhorn DF, Owen RE, Biswas HH, Norris PJ, Lanteri M. The Importance of Foxp3 Antibody and Fixation/Permeabilization Buffer Combinations in Identifying CD4+CD25+Foxp3+ Regulatory T Cells. UCSF/UCB Joint Immunology Retreat. Asilomar, Monterey CA, Oct 10/23-25/09

Publications that included work done in the core immunology laboratory

1. Stacey AR*, **Norris PJ***, Qin L, Haygreen EA, Taylor E, **Heitman J, Lebedeva M, DeCamp A, Li D, Grove D, Self SG, Borrow P**. Induction of a striking systemic cytokine cascade prior to peak viremia in acute HIV-1 infection, in contrast to more modest and delayed responses in acute hepatitis B and C virus infections. *J Virol* 83(8):3719-3733 (2009). *These authors contributed equally to this work.
2. Triulzi DJ, Kleinman S, Kakaiya RM, Busch MP, **Norris PJ**, Steele WR, Glynn SA, Hillyer CD, Carey P, Gottschall JL, Murphy EL, Rios J, Ness PM, Wright DJ, Carrick D, Schreiber GB. The Effect of Previous Pregnancy and Transfusion on HLA Alloimmunization in Blood Donors: Implications for a Transfusion Related Acute Lung Injury (TRALI) Risk Reduction Strategy. *Transfusion* 49(9):1825-35 (2009).
3. **Jackman RP, Heitman JW**, Marschner S, Goodrich RP, **Norris PJ**. Understanding Loss of Donor White Blood Cell Immunogenicity after Pathogen Reduction: Mechanisms of Action in Ultraviolet Illumination and Riboflavin Treatment. *Transfusion* 2009 (submitted 4/6/09, accepted 5/26/09. MS # TRANS-2009-0196)
4. **Lanteri MC**, O'Brien KM, Cameron MJ, Purtha WE, Lund JM, **Owen RE, Heitman JW, Custer B, Hirschhorn DF, Tobler LH**, Kiely N, Prince HE, Ndhlovu LC, Nixon DF, Kamel HT, Kelvin DJ, **Busch MP**, Rudensky AY, Diamond MS, **Norris PJ**. Regulatory T Cells Control the Development of Symptomatic West Nile Virus Infection. *J Clin Invest* 2009 (submitted 3/30/09, MS # 39387-RG-1)
5. **Norris PJ, Hirschhorn DF, DeVita DA, Lee T-H, Murphy EL**. Human T Cell Leukemia Virus Type 1 Infection Drives Spontaneous Proliferation of Natural Killer Cells. *Virulence*, 1(1) Jan-Feb 2010 (subm 8/18/09, accepted 8/20/09. MS # 2009VIRULENCE0024)
6. **Law JP, Hirschhorn DF, Owen RE, Biswas HH, Norris PJ, Lanteri MC**. The Importance of Foxp3 Antibody and Fixation/Permeabilization Buffer Combinations in Identifying CD4⁺CD25⁺Foxp3⁺ Regulatory T Cells. *Cytometry Part A*, 2009 (submitted 4/6/09, MS # 09-039)
7. **Norris PJ**, Lee J-H, Carrick D, Gottschall JL, **Lebedeva M**, de Castro BR, Kleinman SH, **Busch MP**, for the National Heart, Lung, and Blood Institute (NHLBI) Retrovirus Epidemiology Donor Study-II (REDS-II). Long-Term in vitro Reactivity for Human Leukocyte Antigen Antibodies and Comparison of Detection Using Serum versus Plasma. *Transfusion*, 49(2):243-251, Feb 2009 (accepted 8/18/08)
8. Christopher Pilcher CD, Louie B, Facente S, **Keating S**, Pandori MW, Fish F, Hackett J., Keren T, Vallari A, Hall C, Dowling T, **Busch MP**, Liska S, Klausner JD, Hecht FM, and Colfax G. Performance of Screening Tests for Targeted HIV Testing in San Francisco. (manuscript submitted)