

**Busch Research Program
October 2009**

I. INTRODUCTION

Major research areas

My research interests are diverse and multifaceted, and hence pursued through extensive collaborations with scientists at BSRI and UCSF, and through multi-center collaborative epidemiologic, clinical, and laboratory-based studies with colleagues at blood centers and universities, in the government, and at commercial organizations throughout the world. Areas of primary focus include:

- 1) The pathogenesis and clinical laboratory evaluation of transfusion-associated infections, including epidemiology and natural history of human retroviral (HIV-1/2; HTLV-I/II) and other (HBV, HCV, HHV-8, CMV, EBV, T cruzi) infections in blood donor and recipient populations, with special emphasis on new and emerging infectious agents (West Nile Virus, Dengue Virus, influenza viruses, HERV-K, XMRV); and mechanisms of viral latency, disease, and viral reactivation following transfusion or other immunological stimulus;
- 2) The development and implementation of improved or novel laboratory assays and blood donor screening and processing protocols, including clinical evaluation and management of infected donors, new methods for prevention of blood-borne infections such as pathogen reduction; and development of new techniques (e.g. nucleic acid amplification testing, NAT; 4th generation Ag/Ab serological assays' sensitive/less-sensitive enzyme immunoassay (S/LS) or "detuned EIAs" for incidence estimation; HIV SelecTest, for discrimination of HIV vaccine response from infection) to reduce the infectious window periods, and track incidence rates in donor and other populations;
- 3) Immunological and other consequences of transfusion, including: alloimmunization, graft-vs-host disease (GvHD), transfusion-related acute lung injury, TRALI), and the mechanisms of allogeneic donor leukocyte clearance post-transfusion versus persistent microchimerism with donor cell engraftment and tolerance.

Major BSRI collaborations

1. D. Capon
2. B. Custer
3. E. Delwart
4. T. Lee
5. M. Muench
6. E. Murphy
7. P. Norris
8. G. Simmons
9. L. Tobler

Major BSI collaborations

1. Medical Affairs – H. Kamel, P. Tomasulo
2. Blood Systems Laboratories – S. Cagliotti, G. Robertson, S. Cyrus, J. Dunn-Williams

Major non-BSRI collaborations

1. REDS-II Domestic – Investigators at all clinical centers and the coordinating center (Westat)
2. REDS-II International – E. Sabino, A. Barbara-Proetti, T. Gonzales and other REDS-II International investigators Brazil
3. NIH – S Glynn, G Nemo, H Alter
4. FDA – H Golding, I Hewlett, M Rios
5. American Red Cross – S Stramer, R Dodd, D Leiby
6. CDC – L Petersen, R Lanciotti
7. UCSF – K Page, M. McCune, S. Deeks, C Pilcher, R. Hecht

Staff

1. T-H Lee, PhD
2. L. Tobler, DrPH
3. E. Bloch, MD

II. PROGRAM SUMMARY/ PROGRESS REPORT/PLANS

At BSRI, I am responsible for a growing staff of researchers and technologists who engage in cutting edge epidemiological and laboratory research in a highly collaborative manner. I take pride in our record of recruiting new scientists who are establishing their own research programs and mentoring students from universities and blood banking institutions around the world to pursue their own studies to advance blood safety, understand relevant disease pathogenesis, and promote evidence-based health policy development.

Program activities that are pursued in collaboration with other BSRI Investigators and Core Scientists and summarized in their annual progress reports will not be reviewed below. Instead I will focus on several key programs on which I am PI that are not covered in other reports.

REDS-II: Central Laboratory

During the 2008-2009 contract year the REDS-II Central Laboratory (CL) activities focused on conducting extensive testing in support of five major REDS-II programs: 1) the Parvo B19 Transmission and Persistence and Compartmentalization Studies; 2) the HIV/HV/HCV Molecular Surveillance Study; 3) the Leukocyte Antibody Prevalence Study (LAPS-I), including completion of HLA and HNA specificity testing; 4) the Influenza Viremia Study and 5) the REDS-II Donor Iron Status Evaluation (RISE) Study, including completing genetic polymorphism studies at BSRI and coordination of work at and compilation of results for sTfR and ferritin from ARUP. We also initiated and have completed several supplemental studies such as the HLA antibody assay comparison study, and a Steering Committee-approved study that involves the recall of selected BCP female LAPS-I donors for investigation of associations between pregnancy, alloimmunization, microchimerism and autoimmune disease (a collaboration with investigators at UCB/UCSF and more recently ITxM). For each of these projects the BSRI CL staff have worked closely with Westat, the NHLBI program officers, and REDS-II investigators to assure development and execution of laboratory protocols and testing algorithms both within the central laboratory and for laboratory work at REDS-II blood centers. We have worked effectively with Westat to utilize systems and procedures for sample accessioning, repository management, testing, and reporting of results generated by the CL and subcontract laboratories (BCW and ARUP) on donor-derived and quality control specimens.

BSRI performed effectively in each of the above areas of work. The PI (Dr. Busch) and key laboratory scientific staff at BSRI (Drs. Norris, Delwart, Tobler and Lee) worked closely with Steering Committee members and Westat staff to develop and revise study protocols for the projects with laboratory components. For each of the approved projects (Parvo B19, LAP, Molecular Surveillance, RISE, and Influenza) testing algorithms and protocols were developed and assays established and validated at BSRI to support the necessary analyses. During the 2008-2009 contract year extensive laboratory testing was completed on REDS-II clinical specimens for B19, LAPS, Molecular Surveillance, Influenza and RISE studies. Several novel assays developed and validated by BSRI scientists were employed to support unique study needs (e.g., B19 TC-RT-PCR and RISE SNP PCR assays for iron-related polymorphisms), while a series of other commercial assays have been implemented at BSRI to enable high through-put and cost-effective testing for HLA antibodies and other markers (e.g., Luminex HLA Ab detection and B19 serological assays). BSRI CL scientists developed several REDS manuscripts which have already been published (Phase I and II B19 Prevalence and Transmission papers, LAPS Serum vs Plasma paper, LAPS primary outcomes papers, Molecular Surveillance and Influenza Viremia papers). CL staff have actively participated in development of several additional draft papers and a large number of abstracts and presentations at the 2009 AABB Annual meeting. All of these abstracts are now moving into manuscript development phases.

REDS-II International Brazil

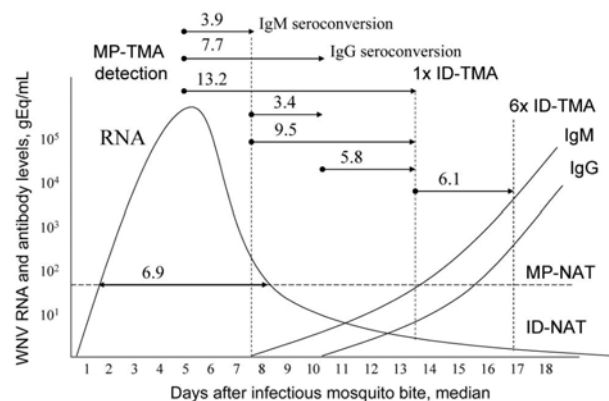
During the past year the REDS-II program in Brazil has matured into an extremely productive study group. Major studies that have been launched include extensive analyses of donor demographics and infectious disease markers in accepted and deferred donors, a case-control study of HIV risk factors, and the *T. cruzi*/Chagas disease cohort study. Several of these studies are summarized in Dr. Custer's progress report. The breadth of productivity of the Brazil investigators is evidenced by the scope of presentations which they developed for the recent AABB meeting in New Orleans: 1) Brazilian Death Index: Mortality Sensitivity and Specificity, 2) Demographic profile of blood donors in Brazil: results from the International REDS II Study, 2007-8, 3) Correlates of return among first time blood donors in Brazil, 4) Temporal analysis of blood donation in three blood centers from Brazil, 5) Analysis of donor deferral at three blood centers in Brazil, 6) The impact of simple donor education on donor behavioral deferral and infectious disease rates in São Paulo, Brazil, 7) HIV Testing and Confidential Unit Exclusion users in São Paulo, Brazil, 8) Demographic Characteristics And Prevalence Of Serologic Markers Among Blood Donors Who Use Confidential Unit Exclusion (CUE) In São Paulo, Brazil, 9) Enhanced classification of Chagas serological results at 3 large blood centers in Brazil, 10) Low rate of seroconversion to *T. cruzi* (Chagas disease) antibodies in Brazilian blood donors indicates low risk for travelers to this region, 11) Performance of US-licensed Ortho *T. cruzi* antibody assay on 2 large Latin American donor panels and comparison with kits used in Latin America. Each of these studies are being developed into manuscripts. We have also received significant (~\$500,000) in supplemental funding from NIH to add genetic and proteomic biomarker studies to the REDS Chagas project. We are confident that we will be successful in competing for a REDS-III International Site contract, which will begin in 2011 and run seven years. This next phase of the REDS Brazil program will extend our studies to recipient populations and in particular to sickle cell disease and HIV infected recipients. We are also developing a second REDS-II International application with our colleagues in the South Africa National Blood Services, which will focus on HIV in donor and recipient populations and transfusions of women in the peri-partum period.

Viral/immune parameters of WNV and Dengue in blood donors

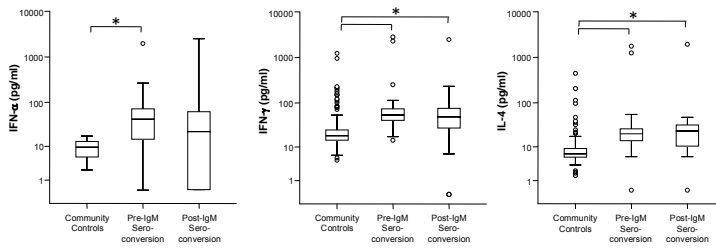
Mosquito-borne arboviruses, including West Nile (WNV) and Dengue viruses (DENVs), are now widely recognized as important transfusion-transmitted pathogens in the United States and worldwide. Although screening for WNV by RNA amplification assays (NAT) is now routine in the U.S., breakthrough infections continue to occur resulting in consideration of further enhancement to screening methods. BSRI has contributed extensively to our understanding of acute WNV infection both with respect to development of enhanced screening methods and disease pathogenesis. Systematic screening of blood donors for WNV RNA provides a key setting to investigate the natural history and clinical outcomes of acute WNV infection. Donors are healthy at the time of donation, thus allowing a relatively unbiased sampling of persons with incident infection to follow in order to determine rates and severity of subsequent symptomatic disease.

Our follow-up studies of over 300 acutely infected donors at BSRI enabled detailed characterization the time-course and kinetics of viremia and immune responses in infected donors and allowed examination of inter-relationships between viral load dynamics and the timing and magnitude of IgM, IgA, IgG and neutralizing antibody (PRNT) responses. The period of acute pre-seroconversion viremia detectable by MP-NAT averages 6.9 days, followed by seroconversion to WNV-specific IgM, IgA, and IgG. Although RNA levels became undetectable by ID-NAT at 13.2 days after index donations, WNV RNA detection by replicate (6x) TMA persists for an additional week and occasionally for >40 days post index. These data have guided development of ID-NAT triggering strategies now used in donor WNV screening throughout the US.

As important, our studies defined the basic immune mechanisms controlling viremia and development of symptomatic WNV disease. Viral load and chemokine and cytokine assays performed on serial samples from donors whose index and first follow-up samples tested negative for IgM



demonstrated that 84% of early viremic donors with decreasing viral loads had innate immune mechanisms primarily responsible for initial control of viremia. Levels of IFN- α were significantly increased before IgM seroconversion, relative to those in control specimens. CXCL10 and CCL2 levels were also significantly elevated in donor plasma obtained before vs. after IgM seroconversion, suggesting that IFN-mediated innate immunity plays a key role in initial control of WNV replication.



In a project led by Dr. Custer BSRI conducted case-control studies to characterize the rates and patterns of symptoms in WNV-reactive donors who were later confirmed as infected (based on WNV seroconversion) or were false positive (controls). Collecting symptom data from over 1000 enrolled WNV NAT-reactive blood donors, we found that the number rather

than type of symptoms was associated with confirmed WNV infection. Using this cut-off number of symptoms we were able to classify WNV+ donors as symptomatic or asymptomatic and then compare humoral and T cell responses in subjects with symptomatic and asymptomatic WNV infection to better understand host factors influencing evolution of WNV disease. Detailed assessment of T cell responses against WNV were conducted by Dr. Norris' team, and immunoreactive regions of the virus were defined. Frozen PBMCs from 35 WNV-infected blood donors were screened for virus-specific T cell responses by an interferon-gamma (IFN- γ) ELISPOT assay using 452 overlapping peptides spanning all WNV proteins. A subset of 8 frequently recognized peptides from the regions of the genome encoding membrane, envelope, and nonstructural 3 and 4b proteins were identified. Phenotypic study revealed that most responses were mediated by cytotoxic CD8+ cells. These findings were the first to define the human T cell response to WNV. Then levels of regulatory CD4 T cells (Treg) were compared in a subset of asymptomatic (AS), symptomatic (S) and persistently symptomatic (PS) donors, pursuing the hypothesis that Treg might be differentially regulated in subjects with symptomatic vs. asymptomatic WNV infection. Dr. Norris group showed that in 32 blood donors with acute WNV infection, Treg expanded significantly in the 3 months after index RNA⁺ donations in all subjects. A direct correlation between lower levels of Treg and progression toward a symptomatic WNV infection was observed. In parallel, mice with an acquired deficiency of Treg developed lethal WNV infection at a significantly higher rate than controls. Together, these results indicated that levels of peripheral Treg that develop after infection determine the clinical phenotype of WNV in immunocompetent animals and humans (this paper was recently accepted by Journal of Clinical Investigation).

In collaboration with ARC and Gen-Probe, our group has led the way in establishing the prevalence of viremia for DENV in donor populations in Latin America and the Caribbean. Screening of donors is under consideration, but basic information regarding viral dynamics and infectivity during serial stages of primary and secondary infections is insufficient to establish screening policies. We recently received a \$ 2 million Grand Opportunities grant from NIH, under the American Recovery and Reinvestment Act, that will enable us expand our continue our studies of WNV and extend them to donors in the acute stages of DENV infections. We will enroll and follow approximately 100 WNV and DENV donors in the acute viremic phase of infection. These donors will be rigorously followed to generate systematic data on the dynamics of viral replication, innate and adaptive immune responses, and infectivity of acute and convalescent viremia. The data generated will guide U.S. and international donor screening policies for WNV and DENV, contribute unique insights into viral and host determinants of disease, and establish repositories of plasma and cells for pathogenesis research.

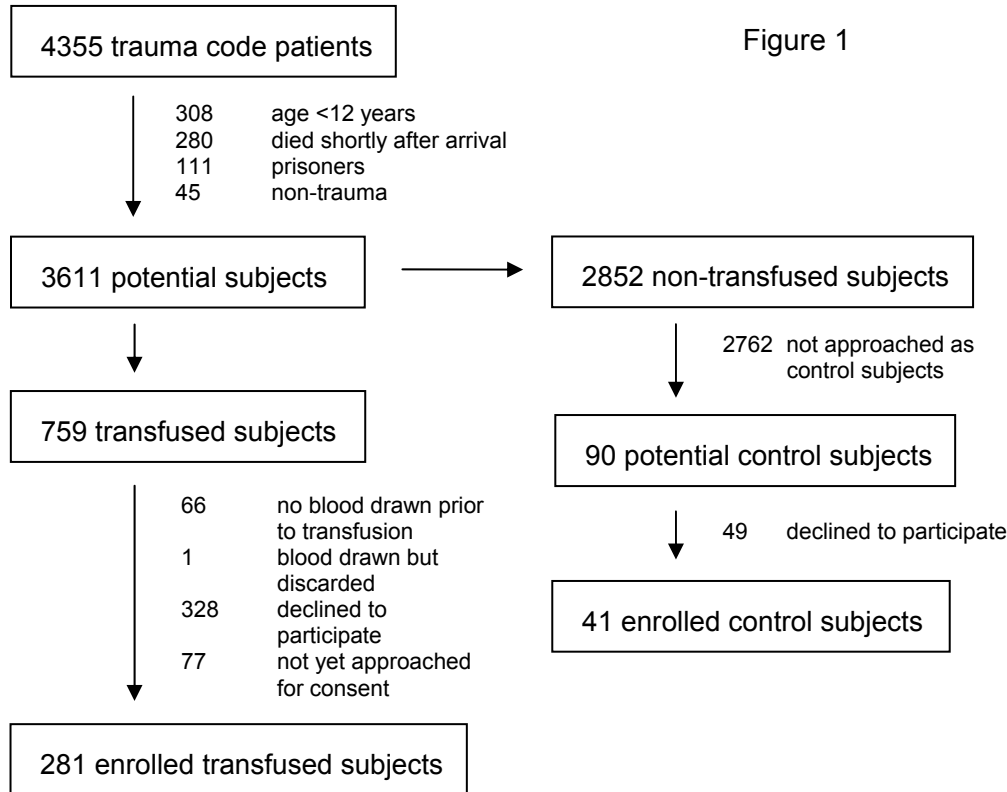
Microchimerism

This project continues to represent a major new research effort concerning transfusion-associated microchimerism (TA-MC) in the clinical setting of traumatic injury, with broader implications for tolerance and alloimmunization. The research consists of a set of closely related studies designed to investigate the clinical epidemiology, immunology, and hematopoiesis of TA-MC. With the recent approval of our request for a restored fifth year of funding, we have been able to make several key strategic decisions on how best to complete the research goals we consider most critically important and we are pleased to report

that we now believe we have resources needed for long-term follow-up of those crucial subjects identified with durable long-term TA-MC.

Enrollment for the main study (Aim 1) has continued to grow over the past year. With the addition of the fifth year of funding, we have been able to re-double our efforts to reach the originally stated prospective enrollment goal of 360 subjects while allocating additional staff time specifically for the follow-up of those subjects with long-term high-level TA-MC. It is this combination of prospective enrollment and follow-up of those with durable TA-MC that provides crucial subjects for the more detailed studies of and hematopoiesis that constitute Specific Aims 2 and 3.

The following flow diagram (Figure 1) illustrates overall study enrollment to date with enrollment and follow-up:



The success of this research program depends not only upon enrollment, but also upon our ability to follow these subjects forward in time with minimal attrition. As noted, both enrollment and follow-up present difficult challenges related to the nature of severe traumatic injury and to the poor, disenfranchised, and mobile nature of the trauma population. Our current overall consent rate is 46% and only 11% of potential subjects have been excluded due to lack of a pre-transfusion sample. Table 1 depicts the status of these critically important related problems of enrollment and follow-up along with the limited TA-MC batch testing that has been completed to date (TA-MC results by batch testing lag enrollment data as samples are accrued to save testing cost):

Follow-up Period (days)	#Subjects	#TA-MC Positive*	
		Short-term	Long-term
Pre-transfusion	281 of 281	NA	NA
7	121 of 281	NA	NA
14	139 of 277	2	NA
28	156 of 273	8	NA
90 (3 months)	87 of 245	3	NA
180 (6 months)	74 of 217	4	2
365 (12 months)	82 of 162	3	7
570 (18 months)	2 of 7	0	0
730 (24 months)	0 of 3	0	0
910 (30 months)	0 of 0	0	0
Total†	250	20	9

*Positive signal detected on at least one time point; †Total excludes pre-transfusion samples

At the current rate, we anticipate completing enrollment during the second half of 2009. With restoration of year-five funding, we are simultaneously deploying key resources and personnel time to assure forward follow-up and serial bleed sampling to identify those relatively few subjects who develop long-term high-level TA-MC. This follow-up will necessarily last well into year five. Regarding the retrospective study of enrollment of trauma, burn and orthopedic surgery patients transfused > 5 years ago to assess long-term consequences of TA-MC, we have been able to deploy resources with restoration of funding for the fifth year to fully launch this work. We have collected samples on over 100 subjects for the retrospective study.

Analyzing samples from prospectively enrolled subjects from Aim 1, we have completed the collection of cytokine data on a subset of subjects to assess the effect of trauma and transfusion on immunity. We analyzed the levels of 41 soluble immune mediators (see table) in the blood over the first year following trauma in 56 subjects, both transfused (n=39) and non-transfused (n=17). Healthy blood donor samples were also included in the analysis as controls (n=50). A number of the proteins appeared to fluctuate in response to trauma, particularly in the first four weeks following injury. These included proteins involved in immune modulation, inflammation, immune suppression, lymphocyte homeostasis, lymphocyte homing, and wound healing. In addition to the overall response to trauma, some differences were observed between the patients who received transfusions and those who did not. Preliminary data suggest that failure to upregulate IL-10 immediately following trauma is associated with the development of microchimerism. Analysis of cytokines in additional subjects who go on to develop microchimerism is planned to verify this preliminary finding. Additional validation work was completed to address the impact of sample age at processing on cytokine measurements. We are now working to incorporate clinical data on type/severity of injury and transfusion volume into our analysis of the cytokine response to trauma and transfusion.

EGF	IL-1Ra	IL-6	IL-12 (p70)	MCP-3/CCL7	MPO	sIL-2Ra
Eotaxin	IL-1 α	IL-7	IL-13	MDC/CCL22	PAI-1	sVCAM-1
FGF-2	IL-1 β	IL-8	IL-15	MIF	sE-selectin	TNF α
Fractalkine	IL-2	IL-9	IL-17	MIP-1 α	sFas	TNF β
GM-CSF	IL-4	IL-10	IP-10	MIP-1 β	sFasL	VEGF
IFN γ	IL-5	IL-12 (p40)	MCP-1	MMP-9	sICAM-1	

RNA expression analysis of the early immune response to trauma is planned to both confirm observations at the protein level and broaden our study to a wider range of immune mediators. We have selected 384 genes involved in the immune response for analysis using the Illumina system. We have collected whole blood samples in tempus tubes (which stabilize the RNA for later analysis) from approximately 70 trauma subjects at baseline and are currently collecting follow-up samples from these

subjects at their 6 month or 1 year visits for use as matched controls. At present, we have follow-up tempus samples from 27 subjects.

Aim 3 of this project involves analysis of hematopoietic engraftment and T cell receptor diversity in 5-10 subjects with long-term high-level TA-MC ($\geq 3\%$ of circulating leukocytes present consistently ≥ 6 months post-transfusion). These subjects are to be derived from long-term follow-up of the prospective enrollees in Aim 1. The data we presented in our original application showed that, in approximately 10% of subjects, TA-MC increased over 2-3 years time, eventually reaching 1-3% of all circulating WBCs in some recipients. We plan to approach these questions from the perspectives of both clinical research and cell biology, as described below. Human subjects protocols have been completed and approval is now in place for these studies.

Two additional clinical research projects have been developed that also address the questions of hematopoietic engraftment and the occurrence of TA-MC in pediatric and trauma populations from a different perspective - study of human transfusion recipients who receive irradiated blood products. First, as we have reported on at length, TA-MC is well documented to occur in the US where non-irradiated relatively fresh blood products are transfused in the setting of severe traumatic injury. Interestingly, in Japan, due to much less HLA diversity in the population, there is much greater risk for TA-GVHD with the transfusion of randomly selected allogeneic blood products compared with the US. To address this, Japan has implemented a policy of universal irradiation of all cellular blood products including those used in trauma. Although TA-MC is known to occur in trauma, the question of whether it occurs in trauma when units are irradiated has not been addressed. In collaboration with Dr. Mashahiro Satake of the Tokyo West Blood Center of Japan's Central Blood Institute, we have hypothesized that TA-MC will not occur in the Japanese trauma population receiving irradiated blood because the irradiation should inactivate both lymphocytes and HSCs. However, it is possible that HSCs, being relatively quiescent, are more radio-resistant than lymphocytes. Initial studies of blood irradiation were designed to demonstrate prevention of TA-GVHD through the inactivation of lymphocytes and it remains conceivable that HSCs may not be fully inactivated by standard-dose blood irradiation. In addition, we continue working with Dr. Harvey Alter of the NIH Department of Transfusion Medicine and Dr. Naomi Luban of National Children's Hospital Medical Center in Washington, DC, on the NIH-supported Transfusion Related Infections Prospective Study (TRIPS). The TRIPS study is unique in that they have pre-transfusion, serial post-transfusion specimens and all donor specimens from select patient groups. To date, we have tested 435 post-transfusion samples from female recipients, of which 53 (12.2%) were positive for Y chromosome sequences representing a lower limit on the prevalence of TA-MC. These preliminary data are especially interesting as they represent some of the first findings of TA-MC in pediatric and adult populations with varied clinical diagnoses but not traumatic injury, many of whom received gamma-irradiated blood. Rosa Sanchez at BSRI is currently writing up these findings.

Finally we have developed productive collaborations with colleagues at UCSF and in South Africa to extend our microchimerism assays to studies of fetal-maternal tolerance and disease. Our studies with Mike McCune's group, published last December in Science, established that trafficking of maternal cells into fetal lymphoid tissues induces maternal specific Tregs that are responsible for tolerance of the fetus to the mother. These studies are now extending to projects looking at the effects of pre-term labor and fetal surgery on MC and tolerance, and to studies of the role of MF- and TA-MC on transmission of HIV, SIV and HCV in both animal model and clinical studies (see Dr. Bloch's report).

III. GRANTS, CONTRACTS, AND AWARDS

Current

R01-HI-083388 (Busch) NIH/NHLBI	9/1/06 - 7/31/11	1.2 calendar
------------------------------------	------------------	--------------

Mechanisms and Clinical Effects of Microchimerism in Transfused Trauma Patients
Investigating the frequency of persistence of donor leukocytes in patients transfused following severe injury. The grant includes both retrospective (registry) and prospective studies of severely injured subjects with detailed laboratory and clinical studies to investigate the mechanisms and clinical consequences of persistent donor leukocyte microchimerism.

<p>R01-HL-076902 (Busch) NIH/NHLBI Natural History of Acute and Chronic HCV in Blood Donors Epidemiologic, viral and immunologic studies to understand the host-virus interaction from seroconversion through resolved or persistent HCV infection.</p>	<p>9/01/03-8/31/10</p>	<p>0.3 calendar</p>
<p>N01-HB-57181 (Busch) NIH/NHLBI Retrovirus Epidemiology Donor Study, Part II (REDS-II) - Central Laboratory BSRI established and maintains a central laboratory for all REDS specimen testing. Specific projects include testing for anti-HLA antibodies in blood donors and determining the presence of influenza viremia in donor populations.</p>	<p>3/15/05-8/31/10</p>	<p>3.0 calendar</p>
<p>HHSN268200417175C (Busch) NIH/NHLBI Retrovirus Epidemiology Donor Study, - Part II (REDS-II) - International Component – Brazil Builds on 3 decades of BSRI/UCSF blood safety & policy research, and 12+ years collaborative research and training with transfusion medicine (TM) colleagues in Brazil. Compilation of extensive blood donor/donation data and specimens as stipulated in the RFP, we will conduct 4 research projects on critical TM issues in Latin America.</p>	<p>2/14/06-8/31/11</p>	<p>1.8 calendar</p>
<p>R01-DA-021550 (Edlin) NIH/NIDA HCV Transmission Among Young Injection Drug Users in NYC To determine the risks of acquiring HCV infection associated with specific injection practices in a cohort of young, high-risk injection drug users (IDUs) on the Lower East Side of Manhattan, and the possible protective effect of pre-existing HCV-specific immune responses.</p>	<p>6/1/08-5/31/13</p>	<p>0.6 calendar</p>
<p>RC2-HL-101632 (Busch) NIH/NHLBI Viral/immune parameters of Dengue and WNV in donors: blood safety implications The goals of this grant are to establish the infectivity of low-level WNV viremic units in the early convalescent stage of infection not detected by current NAT screening, to implement sensitive NAT screening in Puerto Rico under an FDA IND, and to launch follow-up studies of DENV+ donors In addition, we will establish an NHLBI repository of extensively characterized, longitudinal specimens from Dengue and WNV infected donors to advance research into the pathogenesis of these important agents.</p>	<p>9/30/09-9/29/11</p>	<p>0.6 calendar</p>
<p>R01 AI-084109 (McCune) NIH/NIAID Interruption of maternal-fetal transmission of HIV The experiments of this proposal address a novel approach towards vaccination against HIV disease, namely: to create a vaccine that will prevent, rather than to elicit, a robust immune response against the virus.</p>	<p>7/1/09-6/30/13</p>	<p>0.3 calendar</p>
<p>U19-AI-067854 (Haynes) NIH/NIAID Study to characterize global HIV genetic diversity in recently infected donors As part of CHAVI, the project proposes to study viral isolate sequences in conjunction with viral load and Fiebig stage from blood donors from 3-5 different countries.</p>	<p>7/1/09-6/30/10</p>	<p>0.6 calendar</p>

Pending

R03-TW-008623 (Busch) NIH/Fogarty Transfusion-associated microchimerism in the peripartum population The goal of this ancillary study is to determine if transfusion in a South African cohort of women in the peripartum period will result in sustained, high-level TA-MC.	12/1/09-11/30/12	1.2 calendar
Pending U19 (McCune) NIH/NIAID Bay Area Hepatitis C Cooperative Research Center	4/1/12-3/31/15	No effort/salary
Pending R01 (McCune) NIH/NIAID Tolerance and protection of the newborn from lentiviral disease	7/1/10-6/30/12	0.3 calendar

Completed

R01 CI-000214 (Busch) NIH/NCID; CDC Natural History and Pathogenesis of WNV in Viremic Blood Donors Identify, enroll blood donors with early (pre-seroconversion), acute, and/or resolved WNV infection, characterize the dynamics and kinetics of acute viremia, and time course of immune response in primary WNV infection.	9/30/04 - 9/29/08	
---	-------------------	--

Unsuccessful Applications

Tolerance and protection of the newborn from lentiviral disease (McCune); NIH/NIAID
Fetal immune tolerance mechanisms during in utero infection (McCune); NIH/NIAID
Molecular basis of symptomatic WNV infection (Kelvin); CIHR
Rapid PCR diagnostic device for HIV at POC in resource-limited settings (Kelso); NIH/NIAID
Maximizing the sensitivity of nucleic acid testing for blood screening (Busch); NIH/NHLBI
Role of engineered immunoadhesins in transfusion-associated disease (Busch); NHLBI
Acute HCV in longitudinal studies of IDUs (Page); NIH/NIDA

IV. OTHER SIGNIFICANT ACTIVITIES

Academic Affiliations: Professor of Laboratory Medicine at UCSF; member of UCSF AIDS Research Institute Executive Committee

Advisory Committees: AABB Transfusion Transmitted Diseases Committee; ARC Medical Advisory Committee; Canadian Blood Services Advisory Committee; ISBT TTID Working party

Reviewer for professional publications: Associated Editor for Transfusion, Transfusion Medicine, and Chimerism; invited reviewer for numerous journals

Reviewer for grants: Reviewer of NIH Special Emphasis Panel and ARRA grants

V. ABSTRACTS, PUBLICATIONS, AND PRESENTATIONS

Peer-Reviewed Papers (Professional Journals):

Published in 2009

1. Hatano H, Delwart EL, Norris PJ, Lee T-H, Dunn-Williams J, Hunt PW, Hoh R, Stramer SL, Linnen JM, McCune JM, Martin JN, **Busch MP**, Deeks SG. Evidence for Persistent Low-Level Viremia in Individuals Who Control HIV in the Absence of Antiretroviral Therapy. *J Virol*, 83(1):329-35, Jan 2009. (submitted 8/20/08, MS # JVI-01763-08, accepted 10/13/08).
2. Zeremski M, Shu MA, Brown Q, Wu Y, Des Jarlais DC, **Busch MP**, Talal AH, Edlin BR. HCV-Specific T-Cell Immune Responses in Seronegative Injection Drug Users. *J Viral Hepatitis*, 16(1):10-20, Jan 2009 (
3. 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)
4. Custer B, Kamel H, Kiely NE, Murphy EL, **Busch MP**. Associations Between WNV Infection and Symptoms Reported by Blood Donors Identified Through Nucleic Acid Test Screening. *Transfusion*, 49(2):278-88, Feb 2009 (accepted 8/18/08)
5. Ma Z-M, Stone M, Piatak M Jr., Schweighardt B, Haigwood NL, Montefiori D, Lifson JD, **Busch MP**, Miller CJ. High Specific Infectivity of Plasma Virus from the Pre-Ramp Up and Ramp Up Stages of Acute Simian Immunodeficiency Virus Infection. *J Virology*, 83(7):3288-92, April 2009 (accepted
6. Anderson SA, Yang H, Gallagher LM, O'Callaghan S, Forshee RA, **Busch MP**, McKenna MT, Williams I, Williams A, Kuehnert MJ, Stramer S, Kleinman S, Epstein J, Dayton AI. Quantitative Estimate of the Risks and Benefits of Possible Alternative Blood Donor Deferral Strategies for Men Who Have Had Sex with Men. *Transfusion*, 49(6):1102-1114, June 2009 (accepted 12/9/08)
7. Nugent CT, Dockter J, Bernardin F, Hecht FM, Smith D, Delwart E, Pilcher CD, Richman D, **Busch M**, Giachetti C. Detection of HIV-1 in Alternative Specimen Types Using the APTIMA® HIV-1 RNA Qualitative Assay. *J Virolog Meth*, 159(1):10-14, July 2009 (accepted 2/12/09)
8. Kleinman SH, Dunn Williams J, Robertson GF, Caglioti S, Williams RC, Spizman R, Morgan L, Tomasulo P, **Busch MP**. West Nile Virus Testing Experience in 2007: Evaluation of Different Criteria for Triggering Individual-Donation Nucleic Acid Testing. *Transfusion*, 49(6):1160-1170, June 2009 (accepted 12/23/08)
9. Triulzi DJ, Kleinman S, Kakaiya RM, **Busch MP**, Norris PJ, Steele WR, Glynn SA, Hillyer CD, Carey P, Gottschall JL, Murphy EL, Rios JA, Ness PM, Wright DJ, Carrick D, Schreiber GB, for the Retrovirus Epidemiology Donor Study-II (REDS). The Effect of Previous Pregnancy and Transfusion on HLA Alloimmunization in Blood Donors: Implications for a Transfusion Related Acute Lung Injury Risk Reduction Strategy. (TRALI). *Transfusion*, 49(9):1825-1835, Sept 2009. (accepted 3/3/09)
10. Thomas DL, Thio CL, Martin MP, Qi Y, Ge D, O'hUigin C, Kidd J, Kidd K, Khakoo SI, Alexander G, Goedert JJ, Kirk GD, Donfield SM, Rosen HR, Tobler LH, **Busch MP**, McHutchison JG, Goldstein DB, Carrington M. Genetic Variation in IL28B and Spontaneous Clearance of Hepatitis C Virus. *Nature*, 461(7265):798-801, Oct 8, 2009, + on-line suppl mat'l. (accepted 8/28/09)
11. Page K, Hahn JA, Evans J, Shiboski S, Lum P, Delwart E, Tobler L, Andrews W, Avanesyan L, Cooper S, **Busch MP**. Acute Hepatitis C Virus Infection in Young Adult Injection Drug Users: A Prospective Study of Incident Infection, Resolution, and Reinfection. *J Infectious Disease*, 200(8):1216-26, 2009 Oct 15 (accepted 5/15/09)
12. Lee HY*, Giorgi EE*, Keele BF, Gaschen B, Athreya GS, Salazar-Gonzalez JF, Pham KT, Goepfert PA, Kilby JM, Saag MS, Delwart EL, **Busch MP**, Hahn BH, Shaw GM, Korber PT, Bhattacharya

T, Perelson AS. Modeling Sequence Evolution in Acute HIV-1 Infection. *J Theoret Biol* 261(2):341-360, Nov 21, 2009 (accepted 7/29/09)

13. Endres R, Carrick D, Randolph Steele W, Wright D, Norris P, Triulzi D, Kakaiya R, **Busch M**. Identification of Specificities of Antibodies against Human Leukocyte Antigens in Blood Donors. *Transfusion* 2009-2010 (submitted 9/4/09, MS TRANS-209-0511)

in press - will be published in 2009

1. Bekker V, Channock SJ, Yeager M, Hutchinson AA, von Hahn T, Chen S, Xiao N, Dotrang M, Brown M, **Busch MP**, Edlin BR, Rice CM, O'Brien TR. Genetic Variation in CLDN1 and Susceptibility to Hepatitis C Virus Infection. *J Viral Hepatitis*, 2009. Epub 2009 Aug 5.

2. Kleinman SH, Glynn SA, Lee T-H, Tobler LH, Schlumpf KS, Todd DS, Qiao H, Yu MW, **Busch MP**, for the NHLBI Retrovirus Epidemiology Donor Study-II (REDS-II). A Linked Donor-recipient Study to Evaluate Parvovirus B19 Transmission by Blood Component Transfusion. *Blood*, 2009 (submitted 6/2/09, accepted 7/29/09, MS #2009-255706)

3. Carneira-Proietti AB, Sabino EC, Sampaio D, Proietti FA, Goncalves TT, Oliveira CDL, Ferreira JE, Liu J, Custer B, Schreiber GB, Murphy EL, **Busch MP**. Demographic profile of blood donors in Brazil: Results from the International REDS II Study, 2007-2008. *Transfusion* 2009-2010 (submitted 9/4/09, accepted 10/11/09, MS # TRANS-2009-0505)

4. Khurana S, **Norris PJ**, **Busch MP**, Haynes BF, Park S, Sasono P, Mlisana K, Salim AK, Hecht FM, Mulenga J, Chomba E, Hunter E, Allen S, Nemo G, Rodriguez-Chavez IR, Women's Interagency HIV Study (WIHS), The Multicenter AIDS Cohort Study (MACS), Hana Golding. HIV-SelectTest EIA and Rapid Test: Ability to Detect Seroconversion Following HIV Infection. *J Clin Microbiology*, 2009-2010 (submitted 5/09, accepted 10/12/09, MS #JCM01573-09).

5. Guy R, Gold J Garcia Calleja JM, Kim A, Parekh B, **Busch M**, Rehle T, Hargrove J, Remis R, Kaldor J, on behalf of the WHO Working Group on HIV Incidence Assays. The Accuracy of Serological Assays for Detecting Recently Acquired HIV Infection and Estimating Population Incidence: A Systematic Review. *Lancet Infect Disease* 2009-2010 (submitted 11/10/08, accepted 10/13/09).

6. 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)

Submitted in 2008 - 2009, under review

1. Tobler LH, Bahrami SH, Kaidarova Z, Pitina L, Winkelmann VK, Vanderpool SK, Guiltinan AM, Cooper S, **Busch MP**, Murphy EL. A Case-Control Study of Factors Associated with Resolution of Hepatitis C Viremia. (REVELL #2) *Transfusion* 2009 (submitted 7/09).

2. Edgren G, Kamper-Jørgensen M, Eloranta S, Rostgaard K, Custer B, Ullum H, Murphy EL, **Busch MP**, Reilly M, Melbye M, Hjalgrim H, Nyrén O. Duration of Red Blood Cell Concentrate Storage and Survival of Transfused Patients. *Transfusion* 2009-2010 (submitted 10/15/09, MS # TRANS-2009-0585)

3. Kleinman SH, Glynn SA, Lee T-H, Tobler LH, Schlumpf KS, Todd DS, Qiao H, Yu MW, **Busch MP**, for the NHLBI Retrovirus Epidemiology Donor Study-II (REDS-II). A Linked Donor-Recipient Study to Evaluate Parvovirus B19 Transmission by Blood Component Transfusion. *Blood*, 2009 (submitted 6/2/09, MS #2009-255706)

4. Agapova M, **Busch MP**, Custer B. Cost-Effectiveness of Screening the US Blood Supply for *Trypanosoma cruzi*. *Emerg Infect Dis*, 2009-2010 (subm 7/31/09, MS #EID-09-1153)
5. Wong H-L, Chanock SJ, Pfeiffer RM, Chen S, Dotrang D, **Busch MP**, Edlin BR, O'Brien TR. IL18 5' Untranslated Region Polymorphism and Hepatitis C Virus Clearance among African American and European American Injection Drug Users. *J. Infect Dis*, 2009-2010 .
6. Mosbrugger TL, Duggal P, Goedert JJ, Kirk GD, Hoots WK, Tobler LH, **Busch MP**, Peters MG, Rosen HR, Thomas DL, Thio CL. Large-Scale Candidate Gene Analysis of Natural Hepatitis C Virus Clearance. *Hepatology*, 2009 - 2010 (subm 8/5/09, MS # HEP-09-1313).
7. Zeremski M, Hooker G, Shu MA, Winkelstein E, Brown Q, Des Jarlais DC, Tobler LH, Rehermann B, **Busch MP**, Edlin BR, Talal AH. Induction of CXCR3- and CCR5-Associated Chemokines during Acute Hepatitis C Virus Infection. *J Infect Dis*, 2009-2010 (submitted 8/24/09).
8. Kakaiya RM, Triulzi DJ, Wright D, Randolph Steele W, Kleinman SH, **Busch MP**, Norris PJ, Hillyer C, Gottschall J, Rios J, Carey P, Glynn S, for the National Heart, Lung and Blood Institute (NHLBI) Retrovirus Epidemiology Donor Study-II (REDS-II). Prevalence of HLA Antibodies in Remotely Transfused or Alloexposed Volunteer Blood Donors. *Transfusion*, 2009-2010 (subm 9/4/09. MS # TRANS-2009-0508).
9. Bebell LM, Pilcher CD, Dorsey G, Havlir D, Kanya M, **Busch MP**, Nugent C, Bentsen C, Rosenthal PJ, Charlebois ED. Acute HIV-1 Infection is Highly Prevalent in Ugandan Adults with Suspected Malaria. *AIDS* 2009-2010 (MS # AIDS-D-09-01074).

Book Chapters, Published Talks, and Invited Editorials / Review Articles (both Professional and General Interest Publications):

Published in 2009

1. Menitove JE, Fiebig EW, **Busch MP**. Transfusion-Transmitted Diseases. *Transfusion-Transmitted Diseases*. chapter 154, in Hoffman R, Benz EJ, Shattil SJ, Furie B, Silberstein LE, McGlave P, Heslop H, eds. *Hematology: Basic Principles and Practice*, 5th ed. Elsevier / Churchill-Livingstone. Philadelphia, 2009. pp2277-2290.
2. **Busch M**, Walderhaug M, Custer B, Allain J-P, Reddy R, McDonough B. Risk Assessment and Cost-Effective / Utility Analysis. presented as "Decision Analysis and Health Economics in Transfusion Medicine" at the International Assn for Biologicals Standards (IABS) meeting, Sao Paulo, Brazil, Oct 2007. *Biologicals (ex-Devel Biologics)*, 37(2):78-87, 2009 (accepted 9/30/08 // 1/9/09)
3. **Busch MP**, Kleinman SH. Hepatitis C Infection: Recent Insights Relevant to Transfusion Safety. [invited speaker's paper, presented at ISBT European/Eastern Mediterranean Regional Meeting, Cairo Egypt, March 2009] *ISBT Science Series*, 4(1):72-79, Mar 2009 (accepted 12/10/08)
4. **Busch MP**, Glynn SA. Use of Blood-Donor and Transfusion-Recipient Biospecimen Repositories to Address Emerging Blood Safety Concerns and Advance Infectious Disease Research: The National Heart, Lung, and Blood Institute Biologic Specimen Repository. [editorial / invited report] *J Infect Dis*, 199(11):1564-6, 2009 June 1. (accepted 1/26/09).
5. Widell A, **Busch MP**. Exposed or Not Exposed – That is the Question: Evidence for Resolving and Abortive HCV Infections in Blood Donors. [editorial] *Transfusion* 49(7):1277-1281, July 2009 (accepted 4/16/09).

in press" - will be published in 2009

1. Kuhns MC, McNamara AL, Holzmayer V, Lou SC, **Busch MP**. Frequency of Diagnostically Significant Hepatitis B Surface Antigen Mutants. (presented at 3rd Annual Hepatitis / Retrovirus Symposium, Sept 24-26, 2006, Washington, DC). J Med Virol, (suppl, Symposium Proc), 2007-2008 (in press).
2. Schito ML, D'Souza MP, Owen SM, **Busch MP**. Challenges for Rapid Molecular HIV Diagnostics. J Infect Dis, 2009 (subm 6/29/09, accepted 7/31/09, MS # 43847).
3. Kleinman SH, Lelie PN, **Busch MP**. Infectivity of Human Immunodeficiency Virus-1 (HIV-1), Hepatitis C Virus (HCV), and Hepatitis B Virus (HBV), and Risk of Transmission by Transfusion. Transfusion, 2009 (submitted 3/19/09, accepted 5/8/09, MS # 2009-0165.R1).
3. Petersen LR, **Busch MP**. Transfusion-Transmitted Arboviruses. [review] Vox Sang, 2009-2010 (submitted 9-14-09, accepted 10/09, MS # VOX-09.0219).

Letters to Editors (Professional Journals):

Published in 2009

1. Edlin BR, Shu MA, Winkelstein E, Des Jarlais DC, **Busch MP**, Rehermann B, O'Brien TR, Talal AH, Tobler LH, Zeremski M, Beeder AB. More Rare Birds, and the Occasional Swan. *Gastroenterology*, 136(7):2412-2414, June 2009 (accepted 3/13/09)

Footnote Authorship:

Published in 2009

1. Bennett B, Branson B, Delaney K, Owen M, Pentella M, Werner B. HIV Testing Algorithms. A Status Report. A Publication from The Association of Public Health Laboratories and the Centers for Disease Control & Prevention. APHL, Silver Spring MD. April 2009. [www.cdc.gov/aphl.org](http://www.cdc.gov/aphl)

* Laboratory & Point-of-Contact Algorithm Workgroup Members: Bennett B, Boromisa B, Branson B, **Busch M**, Butera S, Cadoff E, Campbell S, Cowan E, Cross D, Delaney K, Dowling T, Ethridge S, Facente S, Heffelfinger J, Hodinka R, King J, Liska S, Louie B, Martin EG, Mei J, Meyer W, Myers R, O'Connell R, Owen M, Pandori M, Patel P, Paul S, Peel S, Pentella M, Randall L, Rayfield M, Stramer S, Werner B.

2. Klein HG, Glynn SA, Ness PM, Blajchman MA, for the NHLBI Working Group * on Research Opportunities for the Pathogen Reduction/Inactivation of Blood Components. Research Opportunities for Pathogen Reduction / Inactivation of Blood Components: Summary of an NHLBI Workshop. *Transfusion*, 49(6):1261-1268, June 2009 (accepted 3/6/09)

* NHLBI 2008 Working Group participants: Alter H, Atreya C, Bianco C, Blajchman MA, **Busch M**, Cardo L, Custer B, Dodd R, Gajic O, Gropper MA, Josephson N, Klein HG, Kleinman SH, Looney MR, Lowell C, McCullough J, Matthay M, Ness PM, Norris PJ, Reems J, Slichter S, Snyder EL, Toy P, Triulzi D, Vostal J, Wagner S. Invited Speakers: Corash LM, Goodrich RP. NHLBI Participants: Barbosa L, Fain K, Glynn SA, Nemo G, Shurin SP.