

PI: MCCUNE, JOSEPH M	Title: Human immune system layering and the neonatal response to vaccines	
Received: 06/14/2010	FOA: PA10-069	Council: 01/2011
Competition ID: ADOBE-FORMS-B	FOA Title: NIH EXPLORATORY DEVELOPMENTAL RESEARCH GRANT PROGRAM (PARENT R21)	
1 R21 AI094009-01	Dual:	Accession Number: 3306088
IPF: 577508	Organization: UNIVERSITY OF CALIFORNIA SAN FRANCISCO	
Former Number:	Department: Medicine	
IRG/SRG: IHD	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 1: Year 2: 	Animals: N Humans: Y Clinical Trial: N Current HS Code: 30 HESC: N	New Investigator: N Early Stage Investigator: N
<i>Senior/Key Personnel:</i>	<i>Organization:</i>	<i>Role Category:</i>
Joseph McCune	The Regents of the University of California, San Francisco	PD/PI
Shannon Thyne MD	The Regents of the University of California, San Francisco	Co-Investigator

Additions for Review

Supplemental Material

Supplemental data

We selected these applications as sound examples of good grantsmanship. That said, time has passed since these grantees applied, and so the samples may not reflect the latest application format or rules. Therefore, always follow your funding opportunity's instructions for application format. We post new samples periodically.

Please note that the application text may be used only for nonprofit educational purposes provided the document remains unchanged and the PI, the grantee organization, and NIAID are credited.

See more samples online: <https://www.niaid.nih.gov/grants-contracts/sample-applications>.

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE

State Application Identifier

1. * TYPE OF SUBMISSION

☐ Pre-application ☒ Application ☐ Changed/Corrected Application

2. DATE SUBMITTED

06/14/2010

Applicant Identifier

4. a. Federal Identifier

b. Agency Routing Identifier

5. APPLICANT INFORMATION

* Organizational DUNS: [REDACTED]

* Legal Name: The Regents of the University of California, San Francisco

Department:

Division:

* Street1: 3333 California Street

Street2: Suite 315

* City: San Francisco

County / Parish: San Francisco

* State: CA: California

Province:

* Country: USA: UNITED STATES

* ZIP / Postal Code: 941186215

Person to be contacted on matters involving this application

Prefix: * First Name: Olive

Middle Name:

* Last Name: Giovannetti

Suffix:

* Phone Number: [REDACTED]

Fax Number: [REDACTED]

Email: [REDACTED]

6. * EMPLOYER IDENTIFICATION (EIN) or (TIN): [REDACTED]

7. * TYPE OF APPLICANT:

H: Public/State Controlled Institution of Higher Education

Other (Specify):

Small Business Organization Type

☐

Women Owned

☐

Socially and Economically Disadvantaged

8. * TYPE OF APPLICATION:

☒ New ☐ Resubmission☐ Renewal ☐ Continuation ☐ Revision

If Revision, mark appropriate box(es).

☐ A. Increase Award☐ B. Decrease Award☐ C. Increase Duration☐ D. Decrease Duration☐ E. Other (specify):* Is this application being submitted to other agencies? Yes ☐ No ☒ What other Agencies:

9. * NAME OF FEDERAL AGENCY:

National Institutes of Health

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER:

TITLE:

11. * DESCRIPTIVE TITLE OF APPLICANT'S PROJECT:

Human immune system layering and the neonatal response to vaccines

12. PROPOSED PROJECT:

* Start Date

* Ending Date

04/01/2011

03/31/2013

* 13. CONGRESSIONAL DISTRICT OF APPLICANT

CA-012

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: * First Name: Joseph

Middle Name: M.

* Last Name: McCune

Suffix:

Position/Title: Professor of Medicine

* Organization Name: The Regents of the University of California, San Francisco

Department: Medicine

Division: Experimental Medicine

* Street1: 1001 Potrero Avenue

Street2: Bldg. 3, Rm 601

* City: San Francisco

County / Parish: San Francisco

* State: CA: California

Province:

* Country: USA: UNITED STATES

* ZIP / Postal Code: 941103518

* Phone Number: [REDACTED]

Fax Number: [REDACTED]

* Email: [REDACTED]

15. ESTIMATED PROJECT FUNDING a. Total Federal Funds Requested <input style="width: 150px;" type="text"/> b. Total Non-Federal Funds <input style="width: 150px;" type="text" value="0.00"/> c. Total Federal & Non-Federal Funds <input style="width: 150px;" type="text"/> d. Estimated Program Income <input style="width: 150px;" type="text" value="0.00"/>	16. * IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS? a. YES <input type="checkbox"/> THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON: DATE: <input style="width: 100px;" type="text"/> b. NO <input checked="" type="checkbox"/> PROGRAM IS NOT COVERED BY E.O. 12372; OR <input type="checkbox"/> PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW
17. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001) <div style="text-align: center;"><input checked="" type="checkbox"/> * I agree</div> <small>* The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.</small>	
18. SFLLL or other Explanatory Documentation <div style="display: flex; align-items: center; margin-top: 5px;"><input style="width: 400px;" type="text"/><div style="margin-left: 10px;"><input type="button" value="Add Attachment"/> <input type="button" value="Delete Attachment"/> <input type="button" value="View Attachment"/></div></div>	
19. Authorized Representative <div style="display: flex; justify-content: space-between; margin-top: 5px;"><div>Prefix: <input style="width: 80px;" type="text"/></div><div>* First Name: <input style="width: 250px;" type="text" value="Joyce"/></div><div>Middle Name: <input style="width: 180px;" type="text"/></div></div> <div style="display: flex; justify-content: space-between; margin-top: 5px;"><div>* Last Name: <input style="width: 450px;" type="text" value="Abe"/></div><div>Suffix: <input style="width: 100px;" type="text"/></div></div> <div style="margin-top: 5px;">* Position/Title: <input style="width: 350px;" type="text" value="Contracts and Grants Officer"/></div> <div style="margin-top: 5px;">* Organization: <input style="width: 450px;" type="text" value="The Regents of the University of California, San Francisco"/></div> <div style="display: flex; justify-content: space-between; margin-top: 5px;"><div>Department: <input style="width: 200px;" type="text" value="Office of Sponsored Research"/></div><div>Division: <input style="width: 200px;" type="text" value="Contracts and Grants"/></div></div> <div style="margin-top: 5px;">* Street1: <input style="width: 400px;" type="text" value="3333 California Street"/></div> <div style="margin-top: 5px;">Street2: <input style="width: 400px;" type="text" value="Suite 315"/></div> <div style="display: flex; justify-content: space-between; margin-top: 5px;"><div>* City: <input style="width: 250px;" type="text" value="San Francisco"/></div><div>County / Parish: <input style="width: 200px;" type="text" value="San Francisco"/></div></div> <div style="display: flex; justify-content: space-between; margin-top: 5px;"><div>* State: <input style="width: 400px;" type="text" value="CA: California"/></div><div>Province: <input style="width: 150px;" type="text"/></div></div> <div style="display: flex; justify-content: space-between; margin-top: 5px;"><div>* Country: <input style="width: 400px;" type="text" value="USA: UNITED STATES"/></div><div>* ZIP / Postal Code: <input style="width: 150px;" type="text" value="941186215"/></div></div> <div style="display: flex; justify-content: space-between; margin-top: 5px;"><div>* Phone Number: <input style="width: 150px;" type="text"/></div><div>Fax Number: <input style="width: 150px;" type="text"/></div></div> <div style="margin-top: 5px;">* Email: <input style="width: 450px;" type="text"/></div> <div style="display: flex; justify-content: space-between; margin-top: 20px;"><div style="width: 45%;">* Signature of Authorized Representative <div style="border: 1px solid black; padding: 5px; text-align: center; margin-top: 5px;">Joyce Abe</div></div><div style="width: 45%;">* Date Signed <div style="border: 1px solid black; padding: 5px; text-align: center; margin-top: 5px;">06/14/2010</div></div></div>	
20. Pre-application <input style="width: 300px;" type="text"/> <div style="margin-left: 10px;"><input type="button" value="Add Attachment"/> <input type="button" value="Delete Attachment"/> <input type="button" value="View Attachment"/></div>	

424 R&R and PHS-398 Specific Table Of Contents

Page Numbers

SF 424 R&R Face Page-----	1
Table of Contents-----	3
Performance Sites-----	4
Research & Related Other Project Information-----	5
Project Summary/Abstract (Description)-----	6
Public Health Relevance Statement (Narrative attachment)-----	7
Facilities & Other Resources-----	8
Equipment-----	9
Research & Related Senior/Key Person-----	10
Biographical Sketches for each listed Senior/Key Person-----	11
PHS 398 Specific Cover Page Supplement-----	19
PHS 398 Specific Modular Budget-----	21
Personnel Justification-----	24
Additional Narrative Justification-----	25
PHS 398 Specific Research Plan-----	26
Specific Aims-----	27
Research Strategy-----	28
Human Subjects Sections-----	34
Protection of Human Subjects-----	34
Women & Minorities-----	36
Planned Enrollment Table-----	37
Children-----	38
Bibliography & References Cited-----	39
Letters of Support-----	41
Resource Sharing Plan-----	46
PHS 398 Checklist-----	47

Project/Performance Site Location(s)**Project/Performance Site Primary Location**☐ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: University of California, San Francisco

DUNS Number:

* Street1: 1001 Potrero Avenue

Street2: Bldg. 3, Room 627

* City: San Francisco

County: San Francisco

* State: CA: California

Province:

* Country: USA: UNITED STATES

* ZIP / Postal Code: 941103518

* Project/ Performance Site Congressional District: CA-012

Project/Performance Site Location 1☐ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:

DUNS Number:

* Street1:

Street2:

* City:

County:

* State:

Province:

* Country: USA: UNITED STATES

* ZIP / Postal Code:

* Project/ Performance Site Congressional District:

Additional Location(s)

Add Attachment

Delete Attachment

View Attachment

RESEARCH & RELATED Other Project Information1. * Are Human Subjects Involved? ☒ Yes ☐ No

1.a If YES to Human Subjects

Is the Project Exempt from Federal regulations? ☐ Yes ☒ NoIf yes, check appropriate exemption number. ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6If no, is the IRB review Pending? ☒ Yes ☐ NoIRB Approval Date: Human Subject Assurance Number: 2. * Are Vertebrate Animals Used? ☐ Yes ☒ No

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ☐ Yes ☐ NoIACUC Approval Date: Animal Welfare Assurance Number 3. * Is proprietary/privileged information included in the application? ☐ Yes ☒ No4.a. * Does this project have an actual or potential impact on the environment? ☐ Yes ☒ No4.b. If yes, please explain: 4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ☐ Yes ☐ No4.d. If yes, please explain: 5. * Is the research performance site designated, or eligible to be designated, as a historic place? ☐ Yes ☒ No5.a. If yes, please explain: 6. * Does this project involve activities outside of the United States or partnerships with international collaborators? ☐ Yes ☒ No6.a. If yes, identify countries: 6.b. Optional Explanation: 7. * Project Summary/Abstract 8. * Project Narrative 9. Bibliography & References Cited 10. Facilities & Other Resources 11. Equipment 12. Other Attachments ☐

PROJECT SUMMARY

The development of the mammalian immune system is typically thought to occur in a linear fashion, from immaturity to maturity as a function of antigen exposure. Previous findings in birds and in mice, however, indicate that this view is oversimplified. Thus, in these species, the developing immune system appears to be “layered” in a manner that is independent of antigen exposure, beginning as a multilineage fetal system that is replaced by an anatomically and biologically distinct multilineage system after birth. If so, then developmentally ordered and unique hematopoietic stem/progenitor cells (HSPC) could give rise to distinct lymphocyte lineages at different stages of development.

In ongoing experiments, we have found that such immune system “layering” occurs in humans. Our preliminary data show that a vigorous human fetal immune response to exogenous antigens can be actively suppressed by antigen-specific Tregs, that these fetal Tregs are derived from a fetal-specific lineage of T cells, and that this lineage is generated by an HSPC that is distinct from that found in adults. These data suggest that the human immune system is comprised of two distinct waves: one generated from a “fetal” HSPC that exists *in utero* in the fetal liver and bone marrow, and another generated from a superseding “adult” HSPC that resides in the bone marrow at later time points. The former gives rise to an immune system that is prone to deliver a tolerogenic response to foreign antigens. The latter gives rise to an immune system that is more likely to generate an immunoreactive responses (e.g., one including cytotoxic T cells and neutralizing antibodies).

Given these findings, we hypothesize that physiologic layering of immune system ontogeny leads to a normal range in the ratio of fetal- to adult-type T cells at birth, with some neonates exhibiting a higher fraction of fetal T cells than others; and that those with a high ratio of fetal/adult T cells will generate predominant Th2 responses to routine childhood immunizations. These hypotheses will be addressed in the experiments of the following Specific Aims: (1) to determine the normal range of fetal to adult T cells in the umbilical cord blood of the full term neonate; and (2) to determine whether those full term neonates with a high ratio of fetal/adult T cells are more likely to generate a Th2-polarized immune response to routine childhood vaccines.

Should this exploratory study reveal normal variation in the ratio of fetal to adult T cells at birth and should such variation be directly related to a Th2 skew after childhood vaccination, modalities aimed at changing this ratio more towards the adult lineage at birth may provide benefit to a substantial number of newborns.

PROJECT NARRATIVE

These exploratory studies are relevant to public health for two reasons. First, they may provide proof-of-concept evidence for the existence of a range in the extent of immune system “layering” in human neonates. Secondly, they may demonstrate that this range is itself related to (and possibly causal of) differences in the ability of neonates to withstand infections and to respond to vaccines.

FACILITIES & OTHER RESOURCES

Laboratory: The Division of Experimental Medicine (DEM) is located within laboratories built in 1991 on the San Francisco General Hospital (SFGH) campus of University of California at San Francisco (UCSF). This facility occupies 27,000 square feet of laboratory space arranged in multiple bays to promote scientific interaction and includes several specialized multi-user facilities: a customized 4-laser, air-launched, 19-parameter BD FACSaria cell sorter for the phenotypic analysis and purification of various HIV-infected and uninfected cells; two customized, 4-laser BD LSRII flow analyzers set up to read 14 or 19 parameters; a phosphorimager to facilitate rapid quantitative analysis of autoradiographic samples; confocal and electron immunofluorescent microscopes for cell analysis. Additional facilities within the Division include a 1,500 square foot biosafety level 2 (BSL2*) containment laboratory for the safe handling of HIV and other infectious agents, standard (BSL2) tissue culture facilities on each of the two research floors, and three large cold rooms. The facility also has a conference room/library with seating for more than 70 people and fully equipped for video relay of major seminars from the other UCSF campuses. Dr. McCune has a fully equipped laboratory with 15 benches/desks.

Clinical: The DEM is located next door to SFGH, a public hospital and regional trauma center fully equipped with modern diagnostic instruments, a clinical laboratory which processes routine as well as nonstandard laboratories on a round-the-clock basis, and ancillary services capable of handling most medical conditions on an out- and/or in-patient basis. The clinical resources of SFGH include the Clinical and Translational Science Institutes (CTSI) Clinical Research Center (CCRC) with 10 inpatient beds and an outpatient facility. Ancillary support for a variety of other important clinical and translational research needs have been established by the UCSF CTSI, including recharge services for statistical consultation for study design, data analysis, and database management; assistance in regulatory requirements for human subjects; and core laboratories offering sample processing and aliquoting, immunologic assays, virologic assays, DNA extraction, restriction fragment length polymorphism analysis, and special instruments, devices or services (including high performance liquid chromatography, dual energy x-ray absorptiometry, treadmill and exercise physiology, and indirect calorimetry).

Animal: Not applicable.

Computer: A completely networked computer system with hardware connections to the greater UCSF system is available to all personnel for scientific writing, editing, data nucleic acid and protein data analysis, and graphics illustration. It is equipped with a complete set of sequence analysis programs, including GeneWorks, DNA Strider, access to sequence databases, and an in-house reference library. This allows the DEM staff to access mainframe computers dedicated to sequence analysis and database storage in the international Internet network, which provides E-mail and file transfer capabilities as well. Access to the MEDLINE literature database is also available to every computer in the DEM via this Internet connection. Dr. McCune has a desktop computer networked to the University server.

Office: Dr. McCune has a furnished office in close proximity to his laboratory. In addition, the Division is staffed by four full time administrators.

Other: The DEM was created (as of 3/1/06) by the Department of Medicine at UCSF, with its laboratory space based at San Francisco General Hospital, not far from the new Mission Bay campus. The Division Chief is Dr. Joseph M. McCune and the Associate Chief is Dr. Doug Nixon. Other faculty members are Drs. Cheryl Stoddart, Elizabeth Sinclair, Mary Premenko-Lanier, and Maggie Feeney. The Division is currently accepting applications from physician scientists and PhD scientists engaged in hypothesis-driven, patient- or disease-oriented research on the immunology of chronic infectious diseases in humans. Over the course of the next several years, it is anticipated that 2-3 more appointments will be made at the Assistant to Full Professor level in the In Residence series, depending upon qualifications. Successful candidates will also become members of the UCSF Graduate Program in Biomedical Sciences. All the support services of a major University campus are available to this project: library, computer center, material management, accounting, business, and fiscal and personnel services.

EQUIPMENT

Major equipment available in the Division of Experimental Medicine includes: a customized 4-laser, air-launched, 19-parameter BD FACSAria cell sorter for the phenotypic analysis and purification of various HIV-infected and uninfected cells; two customized, 4-laser BD LSRII flow analyzers set up to read 14 or 19 parameters; a phosphorimager to facilitate rapid quantitative analysis of autoradiographic samples; confocal and electron immunofluorescent microscopes for cell analysis; DNA sequencing equipment (including an Applied Biosystems 373A fluorescence-based DNA sequencing system); ultracentrifuges; low- and high-speed centrifuges, immunofluorescence microscope with digital camera; autoclaves; X-ray processor; bacterial incubators/shakers; liquid scintillation counter; FPLC; spectrophotometer; thermal cyclers; and miscellaneous small equipment. The equipment is located either directly in the laboratory or in adjacent equipment rooms.

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix:		* First Name:	Joseph	Middle Name:	M.
* Last Name:	McCune	Suffix:			
Position/Title:	Professor of Medicine	Department:	Medicine		
Organization Name:	The Regents of the University of California, San Francisco	Division:	Experimental Medicine		
* Street1:	1001 Potrero Avenue				
Street2:	Bldg. 3, Rm 601				
* City:	San Francisco	County/ Parish:	San Francisco		
* State:	CA: California	Province:			
* Country:	USA: UNITED STATES	* Zip / Postal Code:	941103518		
* Phone Number:		Fax Number:			
* E-Mail:					
Credential, e.g., agency login:					
* Project Role:	PD/PI	Other Project Role Category:			
Degree Type:					
Degree Year:					
* Attach Biographical Sketch	McCuneSF424Bio1011440819.pdf	Add Attachment	Delete Attachment	View Attachment	
Attach Current & Pending Support		Add Attachment	Delete Attachment	View Attachment	

PROFILE - Senior/Key Person 1

Prefix:		* First Name:	Shannon	Middle Name:	M.
* Last Name:	Thyne	Suffix:	MD		
Position/Title:	Assistant Clinical Professor	Department:	Pediatrics		
Organization Name:	The Regents of the University of California, San Francisco	Division:	General Pediatrics		
* Street1:	SFGH, Dept. of Pediatrics				
Street2:	1001 Potrero Ave, Mail Stop 6D				
* City:	San Francisco	County/ Parish:	San Francisco		
* State:	CA: California	Province:			
* Country:	USA: UNITED STATES	* Zip / Postal Code:	941103518		
* Phone Number:		Fax Number:			
* E-Mail:					
Credential, e.g., agency login:					
* Project Role:	Co-Investigator	Other Project Role Category:			
Degree Type:					
Degree Year:					
* Attach Biographical Sketch	ThyneSF424Bio1011440775.pdf	Add Attachment	Delete Attachment	View Attachment	
Attach Current & Pending Support		Add Attachment	Delete Attachment	View Attachment	

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME McCune, Joseph M.		POSITION TITLE Professor of Medicine, University of California, San Francisco Chief, Division of Experimental Medicine	
eRA COMMONS USER NAME [REDACTED]			
EDUCATION/TRAINING <i>(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)</i>			
INSTITUTION AND LOCATION	DEGREE <i>(if applicable)</i>	YEAR(s)	FIELD OF STUDY
Harvard College	A.B.	1975	Biochemistry
The Rockefeller University	Ph.D.	1981	Cell Biology and Immunology
Cornell Medical College	M.D.	1982	Medicine

A. Personal Statement

The goal of this exploratory study is to determine whether the human immune system shows “layering” during ontogeny, with a tolerogenic fetal system that is superseded by an “adult-type” system that is more likely to respond against foreign antigens. If so, then we hypothesize that those neonates born with a larger component of the “fetal-type” system at birth will be less capable of responding to childhood immunizations with a strong Th1-type response and more likely to succumb to infections and/or atopic disorders. This hypothesis will be studied in the context of a cross-sectional analysis of 150 cord blood specimens and a prospective study of 50 cord blood specimens. These are studies that are consistent with the thrust of my research over the past 20 years and that will take advantage of existing collaborations, approved human subjects protocols, and standardized techniques. They will be conducted within the Division of Experimental Medicine, a new division in the UCSF Department of Medicine designed to foster a multidisciplinary environment for the pursuit of hypothesis-driven studies in human subjects.

B. Positions and Honors

Positions: Resident in Internal Medicine, University of California, San Francisco (UCSF) (1982-84); Infectious Disease Fellow, UCSF (1984-86); Postdoctoral Fellow, Department of Pathology, Stanford University School of Medicine (1985-88); Physician, UCSF AIDS Clinic, San Francisco General Hospital (SFGH) (1986-present); Scientific Director, SyStemix, Inc. (1988-91); Vice-President, New Enterprise Research Division, SyStemix, Inc. (1991-94); Director: SyStemix-Sandoz Antiviral Project & SyStemix-Sandoz HIV Gene Therapy Joint Venture (Progenesys, Inc) (1993-94); Associate Investigator, Gladstone Institute of Virology and Immunology (GIVI) (1995-1999); Senior Investigator, GIVI (1999-2006); Associate Professor of Medicine, UCSF (1995-1999); Associate Director, General Clinical Research Center, SFGH (1995-2006); Visiting Professor (on sabbatical), Institut Pasteur, Paris, France (2003-2004); Director and PI of the UCSF Clinical and Translational Science Institute (2006-2008); Senior Associate Dean of Clinical and Translational Research (2005-2008); Chief of the Division of Experimental Medicine (2006-present); Professor of Medicine, UCSF (1999-present)

Member: Project Inform Immune Restoration Think Tank (1992-present); Health Advisory Board, Pediatric AIDS Foundation (1994-present); National Board of Governors, Project Inform (1995-present); Councilor, The Henry Kunkel Society (1997-present); The Rockefeller University Board of Trustees (1998-2001); NIAID Study Section AIP (2000-present); UCSF Promotions Subcommittee (2000-2004); Chair, Scientific Advisory Board, Foundation of AIDS and Immunologic Research (2000-present); Chair, Gladstone Committee on Management and Mentoring Training (2001-present); Chair, UCSF Search Committee for Chair of GI, SFGH (2003); Chair, Translational Research Search Committee (2004-present); Elizabeth Glaser Pediatric AIDS Foundation Board of Directors (2006-present); Immune Tolerance Institute Board of Directors (2006-present)

Honors: National Merit Scholarship (1972-75); Harvard National Scholarship (1972-75); Michael C. Rockefeller Memorial Fellowship (1975); Pfizer Postdoctoral Fellowship (1986-89); Diplomate, Internal Medicine (1987); Elizabeth Glaser Scientist Award (1996-2000); Member, American Society for Clinical Investigation (1999); Member, American Association of Physicians (2000); Burroughs Wellcome Fund Clinical Scientist Award in Translational Research (2000-2005); NIH Merit Award (2001); NIH Director's Pioneer Award (2004); NIH

Clinical and Translational Science Institute Award (2006); Pediatric Fellows and Fellows Leadership and Advocacy Group Mentorship Award, nominee (2007); UCSF Postdoctoral Scholar's Association Outstanding Mentor Award (2007)

C. Selected Peer-reviewed Publications (from a total of 168)

1. Hellerstein M, Hanley MB, Cesar D, Siler S, Papageorgopoulos C, Wieder E, Schmidt D, Hoh R, Neese R, Macallan D, Deeks S, McCune JM. Directly measured kinetics of circulating T lymphocytes in normal and HIV-1-infected humans. *Nature Med.* 5:83-89, 1999.
2. McCune JM, Hanley MB, Cesar D, Halvorsen R, Hoh R, Schmidt D, Wieder E, Deeks S, Siler S, Neese R, Hellerstein M. Factors influencing T-cell turnover in HIV-1-seropositive patients. *J. Clin. Invest.* 105:R1-R8, 2000.
3. Huang SS, Barbour JD, Deeks SD, Huang JS, Grant RM, Ng VL, McCune JM. Reversal of Human Immunodeficiency Virus type 1-associated hematosuppression by effective antiretroviral therapy. *Clin. Infect. Dis.* 30:504-10, 2000.
4. Napolitano LA, Grant RM, Deeks SG, Schmidt D, De Rosa SC, Herzenberg LA, Herndier BG, Andersson J, McCune JM. Increased production of IL-7 accompanies HIV-1-mediated T-cell depletion: implications for T-cell homeostasis. *Nature Med.* 7:73-79, 2001.
5. Napolitano LA, Lo JC, Gotway MB, Mulligan K, Barbour JD, Schmidt D, Grant RM, Halvorsen RA, Schambelan M, McCune JM. Increased thymic mass and circulating naive CD4+ T cells in HIV-1-infected adults treated with growth hormone. *AIDS.* 16: 1103-1111, 2002.
6. Hellerstein MK, Hoh RA, Hanley MB, Cesar D, Lee D, Neese RA, McCune JM. Subpopulations of long-lived and short-lived T-cells in advanced HIV-1 infection. *J. Clin. Invest.* 112: 956-966, 2003.
7. Napolitano LA, Burt T, Bacchetti P, Barron Y, French A, Kovacs A, Anastos K, Young M, McCune JM, Greenblatt RM. Increased circulating interleukin-7 levels in HIV-1-infected women. *J. Acquir. Immune Defic. Syndr.* 40:581-84, 2005.
8. Hartigan-O'Connor D, Abel K, McCune JM. Suppression of SIV-specific CD4+ T cells by infant but not adult macaque regulatory T cells: implications for SIV disease progression, *J. Exp. Med.*, 204:2679-92, 2007.
9. Mold JE, Michaelsson J, Burt TD, Muench MO, Beckerman KP, Busch MP, Lee T-H, Nixon DF, and McCune JM. Maternal alloantigens promote the development of tolerogenic fetal regulatory T cells in utero. *Science*, 322:1562-65, 2008. PMID: PMC2648820.
10. Napolitano LA, Schmidt D, Gotway MB, Ameli N, Filbert EL, Ng MM, Clor JL, Epling L, Sinclair E, Baum PD, Li K, Killian ML, Bacchetti P, McCune JM. Growth hormone enhances thymic function in HIV-infected adults. *J. Clin. Invest.* 118:1085-98, 2008. PMID: PMC2248326.
11. Favre D, Lederer S,* Kanwar B,* Min Ma Z,* Proll S, Kasakow Z, Mold J, Swainson L, Barbour JD, Baskin CR, Palermo R, Pandrea I, Miller C, Katze M, McCune JM. Critical loss of the balance between TH17 and T regulatory cell populations in pathogenic SIV infection. *PLoS Pathogens*, 5(2):e1000295, 2009 (*co-equal authors). PMID: PMC2635016.
12. Loke P,* Favre D,* Hunt PW, Leung JM, Kanwar B, Martin JN, Deeks SG, McCune JM. Correlating cellular and molecular signatures of mucosal immunity that distinguish HIV controllers from non-controllers. *Blood*, Feb 16, 2010. PMID: PMC2858476 [Available on 2011/4/15]
13. L. Swainson, J. Mold, U. Bajpai, and M. McCune. 2010. Expression of the autoimmune susceptibility gene FcRL3 on human regulatory T cells is associated with dysfunction and high levels of programmed cell death-1. *J Immunol* 184: 3639-47. NIHMS # 207784.
14. Stoddart CA, Keir ME, McCune JM. IFN- α -mediated induction of CCR5 leads to expanded HIV-1 tropism in vivo. *PLoS Pathogens*, 6:e1000766, 2010. PMID: PMC2824759.
15. Favre D,* Mold J,* Hunt P, Kanwar B, Loke P, Seu L, Barbour J, Lowe MM, Jayawardene A, Aweeka F, Huang Y, Douek D, Brenchley JM, Martin JN, Hecht FM, Deeks SG, McCune JM. Tryptophan catabolism by indoleamine 2,3-dioxygenase 1 alters the balance of Th17 and regulatory T cells in HIV disease. *Science Translational Medicine*, 2:32ra36, 2010. (*co-equal authors).

D. Research Support

Current Research Support

DP1 OD000329 (PI: McCune)

09/30/04–07/31/10

NIH Director's Pioneer Award

This project will address the need to understand patterns of an effective immune response to HIV in humans, with the intent to inform the creation of an effective vaccine.

R37 AI40312 (PI: McCune)

04/01/96–02/28/11

NIH/NIAID: HIV Effects on the Central Hematolymphoid System *in vivo*

The major goal of this project is to test the hypothesis that HIV infection leads to destruction and/or dysfunction of the bone marrow and thymic microenvironments which enable multilineage and lineage-committed hematopoiesis.

P01 DA026134 (PI: Smith)

09/30/08-05/31/11

NIH/NIDA: HIV Proteomic Center for Host-Viral Response Characterization

The overall goals of this grant are to apply proteomics technologies to investigate changes in protein abundances resulting from the pathologic events associated with concomitant lentiviral infection and substance abuse as well as from pharmacologic interventions designed to treat these pathologies.

KL2 RR024130 (PI: Johnston)

09/30/06-06/30/11

UL1 RR024131

TL1 RR024129

NIH: Clinical and Translational Science Institute

The purpose of this grant is to forge a uniquely transformative, novel, and integrative academic home for Clinical and Translational Science (CTSI). Dr. McCune was PI of the grant until a successor was named on 12/22/08.

R01AI084109 (PI: McCune)

09/05/09-08/31/13

NIH/NIAID: Interruption of Maternal-Fetal Transmission of HIV

The specific aim of this study is to demonstrate that the in utero exposure of the fetal rhesus macaque to SIV epitopes will induce a tolerogenic immune response that will protect against pathogenic SIV infection after birth.

Completed Research Support

U01 AI43864 (PI: McCune)

04/31/03-03/31/10

NIH/NIAID

Thymic function in adults with HIV-1 disease

R01 AI47062 (PI: McCune)

07/01/03-12/31/08

NIH/NIAID

Assessing laboratory correlates of immune protection

R21AI068583 (PI: McCune)

07/01/06-06/30/09

NIH/NIAID

Gag/anti-Gag immune complexes and enhancement of lentiviral disease progression

AI05418 (PI: Stoddart)

08/01/00-07/31/07

NIH/NIAID

SCID-hu animal model for evaluating HIV therapies

McCune role on the project: Consultant.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME Thyne, Shannon M.	POSITION TITLE Associate Professor of Clinical Pediatrics		
eRA COMMONS USER NAME			
EDUCATION/TRAINING <i>(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)</i>			
INSTITUTION AND LOCATION	DEGREE <i>(if applicable)</i>	YEAR(s)	FIELD OF STUDY
Yale College, New Haven, CT	BA	05/89	Psychology
Brown University School of Medicine, Providence, RI (Brown-Dartmouth Program in Medicine)	MD	05/95	Medicine

A. Personal Statement

I am the Medical Director of a busy, urban, inner-city pediatric clinic based at San Francisco General Hospital, within the UCSF academic hospital network. This unit houses primary care, urgent care, and multiple subspecialty clinics providing care to a diverse and underserved patient population. This clinical setting has given me the opportunity to engage in clinical investigations in the areas of asthma, infectious diseases, adolescent health, child development, and improved systems of care. Over the past 10 years, I have collaborated with several well-established PIs on clinical research trials in these areas and have developed my own independent research projects in the area of pediatric asthma. Due to my ongoing connection to the clinical setting, I remain particularly adept at the design and implementation of feasible research projects, and am able to maintain consistent and close oversight of the research teams. The studies proposed by Dr. McCune in his R21 are exactly in line with the type of work that I've been able to carry out in my clinic in the past. I have no doubt but that I will be able to organize this effort going forward, should it be funded.

B. Positions and Honors**Positions**

1995-1998 Resident, UCSF Department of Pediatrics
 1998-1999 Chief Resident, UCSF Department of Pediatrics
 1999-2000 Clinical Instructor, Pediatrics, University of California, San Francisco
 2000-2006 Health Sciences Assistant Professor, UCSF Department of Pediatrics
 2001-present Medical Director, Child Protection Services, City of San Francisco
 2001-present Medical Director, Children's Health Center, San Francisco General Hospital
 2006-2009 Health Sciences Associate Professor, UCSF Department of Pediatrics
 2009-present Associate Professor of Clinical Pediatrics, UCSF Department of Pediatrics

Selected Honors and Awards

1995 American Medical Women's Association, Janet A. Glasgow Memorial Achievement Citation
 1995 New England Pediatric Society, Pediatric Prize
 1996 Julius Krevans Award for Clinical Excellence in Pediatrics, San Francisco General Hospital
 1997 Resident Teaching Award, UCSF Department of Pediatrics
 1998 Moses Grossman Award for Community Service, UCSF Department of Pediatrics
 2001 Hellman Family Award for Early Career Faculty, UCSF School of Medicine
 2002 Nominee, Kaiser Award for Excellence in Teaching, UCSF School of Medicine
 2003 Nominee, Medical Student Award for Excellence in Small Group Instruction, UCSF School of Medicine
 2004 UCSF Medical Student Award for Excellence in Small Group Instruction
 2005 AMA Hospital Recognition Program, "Innovative Approaches to Patient-Centered Communication" for Pediatric Asthma Clinic at San Francisco General Hospital

- 2006 UCSF Academy of Medical Educators Award for Excellence in Innovative Curricular Design and Program Development
- 2006 San Francisco Department of Public Health, Award of Excellence, for Best Practices in Cultural Competency Training, Advancing Cultural Competency in the San Francisco Department of Public Health
- 2007 Ambulatory Pediatric Association, Health Care Delivery Award (The *Yes We Can* Urban Asthma Partnership at UCSF/San Francisco General Hospital)
- 2008 San Francisco Health Plan, Award for Clinical Excellence in Pediatric Asthma Care
- 2009 San Francisco Health Plan, Overall Award for Excellence in Pediatric Primary Care (Fluoride Varnish Program)

C. Selected Peer-reviewed Publications

1. Tsai, HJ, Kho, JY, Shaikh N, Choudry S, Naqvi M, Navarro D, Matallana H, Castro R, Lilly CM, Watson HG, Meade K, Lenoir M, Thyne S, Ziv E, Burchard EG; Admixture-matched case-control: a practical approach for genetic association studies in admixed populations. *Human Genetics*. 2006 January; 118(5):626-39.
2. Tsai, HJ, Shaikh, N, Kho, JY, Battle N, Naqvi M, Navarro D, Matallana H, Lilly CM, Eng CS, Kumar G, Thyne S, Watson HG, Meade K, Lenoir M, Choudhry S, Burchard EG; from the Study of African Americans, Asthma, Genes Environments (SAGE); Beta(2)-Adrenergic receptor polymorphisms: pharmacogenetic response to bronchodilator among African American asthmatics. *Human Genetics*. 2006 June; 119(5):547-57.
3. Thyne SM, Rising J, Legion V and Love MB. The Yes We Can Urban Asthma Partnership: an innovative approach to childhood asthma management. *Journal of Asthma*, 2006 October, 43:667-673.
4. Cabana, MD and Thyne SM. An emergency department-based follow-up clinic can improve asthma outcomes [Invited Commentary]. *Journal of Pediatrics*, 2006 November, 149 (5), 725.
5. Thyne SM, Marmor AK, Madden N, Herrick G. Comprehensive Asthma Management for Underserved Children: The San Francisco General Hospital Experience. *Paediatric and Perinatal Epidemiology*. Volume 21, Supplement 3, 2007 November; 9-34(6).
6. *Thyne SM, *Navqi, M, Choudhry S, Tsai, H, Ziv E, et al. Ethnic specific differences in bronchodilator responsiveness and morbidity among African American, Puerto Rican, and Mexican Asthmatics. **These authors contributed equally to this manuscript*. *Journal of Asthma*. 2007 October; 44 (8), 639-48.
7. Naqvi M, Tsai HJ, Choudhry S, Thyne SM, Navarro D, Nazario S, Rodriguez-Santana JR, Casal J, Torres A, Chapela R, Watson HG, Meade K, Rodriguez-Cintrón W, LeNoir M, Avila PC, and Burchard EG. Association between IgE levels and asthma severity among African American, Mexican and Puerto Rican asthmatics. *Journal of Allergy, Asthma and Clinical Immunology*. 2007, 121 (1): 137-143.
8. Orrell-Valente JK, Jarlsberg LG, Tait MA, Thyne SM, Tubash T and Cabana, MD. Parents' specific concerns about daily asthma medications for children. *Journal of Asthma*, 2007, 44:385-390.
9. Cabana MD, Abu-Isa H, Thyne SM, Yawn B. Specialty differences in prescribing inhaled corticosteroids for children. *Clinical Pediatrics*, 2007, 46 (8): 698-705.
10. Cabana MD, Chaffin DC, Jarlsberg LG, Thyne SM, and Clark NM. Selective Provision of Asthma Self-Management Tools to Families. *Pediatrics*. 2008 April; 121(4):e900-925.
11. Bergman DA, Sharek PJ, Ekegren K, Thyne SM, Mayer M, Saunders S. The use of telemedicine access to schools to facilitate expert assessment of children with asthma. *International Journal of Telemedicine and Application*. 2008 January; 1: 1-7. PMID: PMC2271044.
12. Seibold MA, Wang B, Eng C, Kumar G, Beckman KB, Sen S, Choudhry S, Meade K, Lenoir M, Watson HG, Thyne S, Williams K, Kumar R, Weiss KB, Grammer LC, Avila PC, Schleimer RP, González Burchard E, and Brenner R. An african-specific functional polymorphism in *KCNMB1* shows sex-specific association with asthma severity. *Molecular Genetics*, 2008, 17(17):2681-2690. PMID: PMC2733805.
13. Naqvi M, Tcheurekdjian H, DeBoard JA, Williams LK, Navarro D, Castro RA, Rodriguez-Santana JR, Chapela R, Watson HG, Meade K, Rodriguez-Cintrón W, LeNoir M, Thyne SM, Avila PC, Choudhry S, Burchard EG. Inhaled corticosteroids and augmented bronchodilator responsiveness in Latino and African American asthmatic patients. *Ann Allergy Asthma Immunol*. 2008, Jun;100(6):551-7.
14. Tcheurekdjian H, Thyne SM, Williams LK, Via M, Rodriguez-Santana JR, Rodriguez-Cintrón W, Avila PC, Burchard EG. Augmentation of bronchodilator responsiveness by leukotriene modifiers in Puerto Rican and Mexican children. *Ann Allergy Asthma Immunol*. 2009;102:510-7.

15. Seibold MA, Reese TA, Choudhry , Muhammad Salam MT, Beckman K, Eng C, Atakilit A, Meade K, Lenoir M, H. Watson HG, Thyne S, Kumar R, Weiss KB, Grammer LC, Avila P, Schleimer RP, Fahy JV, Rodriguez-Santana J, Rodriguez-Cintron W, Boot RG, Sheppard D, Gilliland FD, Locksley RM, and Burchard EG. Differential Enzymatic Activity of Common Haplotypic Versions of the Human Acidic Mammalian Chitinase Protein. J Biol Chem, 2009; 284 (29): 19650-19658. PMCID: PMC2740590 [Available on 2010/7/17].

D. Research Support

Current Research Support

HHSN272200700031C (Chambers, PI)

09/15/07-09/14/12

NIH/NIAID

Clinical Trial for Community-Acquired Methicillin Resistant Staphylococcus Aureus (CA-MRSA) Infections

The purpose of this contract is to advance the field of clinical management for uncomplicated skin and soft tissue infection caused by community-acquired methicillin-resistant Staphylococcus aureus (CA-MRSA) by determining the optimal outpatient treatment strategy within multiethnic, urban underserved pediatric communities.

Role: Co-investigator

R01 HD053408 (Shafer, PI)

12/1/07-11/31/10

NIH/NICHHD

Parental Attitudes Toward Confidential STI Services in Teens

The purpose of this grant is to examine parental knowledge and attitudes towards confidential STI health services for teens. This research would help investigators to strengthen the role of parents and encourage partnerships between health care providers and parents to ultimately improve the health outcomes for teens, particularly those from underserved populations.

Role: Co-investigator

R01 ESO15794 (Gonzalez-Burchard, PI)

07/01/08-06/30/11

NIH/NIEHS

Gene-Environments & Admixture in Latino Americans (GALA 2)

The purpose of this project is to identify genetic, social and environmental risk factors for asthma among various Latino subgroups recruited throughout the US.

Role: Co-investigator

[REDACTED]

[REDACTED]

HL098107 (Boushey, H and Cabana, M)

09/30/09-06/30/15

NIH/NHLBI

[REDACTED]

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

1. Project Director / Principal Investigator (PD/PI)

Prefix: * First Name:
Middle Name:
* Last Name:
Suffix:

2. Human Subjects

Clinical Trial? ☒ No ☐ Yes
* Agency-Defined Phase III Clinical Trial? ☐ No ☐ Yes

3. Applicant Organization Contact

Person to be contacted on matters involving this application

Prefix: * First Name:
Middle Name:
* Last Name:
Suffix:
* Phone Number: Fax Number:
Email:

* Title:

* Street1:
Street2:
* City:
County/Parish:
* State:
Province:
* Country: * Zip / Postal Code:

PHS 398 Cover Page Supplement

4. Human Embryonic Stem Cells

* Does the proposed project involve human embryonic stem cells?

☒ No ☐ Yes

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: <http://stemcells.nih.gov/research/registry/>. Or, if a specific stem cell line cannot be referenced at this time, please check the box indicating that one from the registry will be used:

Cell Line(s):

☐ Specific stem cell line cannot be referenced at this time. One from the registry will be used.

PHS 398 Modular Budget, Periods 1 and 2

OMB Number: 0925-0001

Budget Period: 1				
Start Date: <input style="width: 100px;" type="text" value="04/01/2011"/>		End Date: <input style="width: 100px;" type="text" value="03/31/2012"/>		
A. Direct Costs				
* Direct Cost less Consortium F&A			* Funds Requested (\$) <input style="width: 100px;" type="text"/>	
Consortium F&A			<input style="width: 100px;" type="text"/>	
* Total Direct Costs			<input style="width: 100px;" type="text"/>	
B. Indirect Costs				
	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base (\$)	* Funds Requested (\$)
1.	<input style="width: 500px;" type="text" value="Sponsored Research _ On Campus"/>	<input style="width: 50px;" type="text" value="54.5"/>	<input style="width: 100px;" type="text"/>	<input style="width: 100px;" type="text"/>
2.	<input style="width: 500px;" type="text"/>	<input style="width: 50px;" type="text"/>	<input style="width: 100px;" type="text"/>	<input style="width: 100px;" type="text"/>
3.	<input style="width: 500px;" type="text"/>	<input style="width: 50px;" type="text"/>	<input style="width: 100px;" type="text"/>	<input style="width: 100px;" type="text"/>
4.	<input style="width: 500px;" type="text"/>	<input style="width: 50px;" type="text"/>	<input style="width: 100px;" type="text"/>	<input style="width: 100px;" type="text"/>
Cognizant Agency (Agency Name, POC Name and Phone Number)		<input style="width: 450px;" type="text" value="DHHS, (415) 437-7820"/>		
Indirect Cost Rate Agreement Date <input style="width: 100px;" type="text" value="08/28/2009"/>		Total Indirect Costs <input style="width: 100px;" type="text"/>		
C. Total Direct and Indirect Costs (A + B)				Funds Requested (\$) <input style="width: 100px;" type="text"/>

Budget Period: 2				
Start Date: <input style="width: 100px;" type="text" value="04/01/2012"/>		End Date: <input style="width: 100px;" type="text" value="03/31/2013"/>		
A. Direct Costs				
* Direct Cost less Consortium F&A			* Funds Requested (\$) <input style="width: 100px;" type="text"/>	
Consortium F&A			<input style="width: 100px;" type="text"/>	
* Total Direct Costs			<input style="width: 100px;" type="text"/>	
B. Indirect Costs				
	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base (\$)	* Funds Requested (\$)
1.	<input style="width: 500px;" type="text" value="Sponsored Research _ On Campus"/>	<input style="width: 50px;" type="text" value="54.5"/>	<input style="width: 100px;" type="text"/>	<input style="width: 100px;" type="text"/>
2.	<input style="width: 500px;" type="text"/>	<input style="width: 50px;" type="text"/>	<input style="width: 100px;" type="text"/>	<input style="width: 100px;" type="text"/>
3.	<input style="width: 500px;" type="text"/>	<input style="width: 50px;" type="text"/>	<input style="width: 100px;" type="text"/>	<input style="width: 100px;" type="text"/>
4.	<input style="width: 500px;" type="text"/>	<input style="width: 50px;" type="text"/>	<input style="width: 100px;" type="text"/>	<input style="width: 100px;" type="text"/>
Cognizant Agency (Agency Name, POC Name and Phone Number)		<input style="width: 450px;" type="text" value="DHHS, (415) 437-7820"/>		
Indirect Cost Rate Agreement Date <input style="width: 100px;" type="text" value="08/28/2009"/>		Total Indirect Costs <input style="width: 100px;" type="text"/>		
C. Total Direct and Indirect Costs (A + B)				Funds Requested (\$) <input style="width: 100px;" type="text"/>

PHS 398 Modular Budget, Periods 3 and 4

Budget Period: 3				
Start Date: <input style="width: 100px;" type="text"/>		End Date: <input style="width: 100px;" type="text"/>		
A. Direct Costs				
* Direct Cost less Consortium F&A				* Funds Requested (\$)
Consortium F&A				
* Total Direct Costs				
B. Indirect Costs				
	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base (\$)	* Funds Requested (\$)
1.	<input style="width: 500px;" type="text"/>	<input style="width: 50px;" type="text"/>	<input style="width: 100px;" type="text"/>	<input style="width: 100px;" type="text"/>
2.	<input style="width: 500px;" type="text"/>	<input style="width: 50px;" type="text"/>	<input style="width: 100px;" type="text"/>	<input style="width: 100px;" type="text"/>
3.	<input style="width: 500px;" type="text"/>	<input style="width: 50px;" type="text"/>	<input style="width: 100px;" type="text"/>	<input style="width: 100px;" type="text"/>
4.	<input style="width: 500px;" type="text"/>	<input style="width: 50px;" type="text"/>	<input style="width: 100px;" type="text"/>	<input style="width: 100px;" type="text"/>
Cognizant Agency (Agency Name, POC Name and Phone Number)		<input style="width: 450px; height: 50px;" type="text"/>		
Indirect Cost Rate Agreement Date <input style="width: 100px;" type="text"/>		Total Indirect Costs <input style="width: 100px;" type="text"/>		
C. Total Direct and Indirect Costs (A + B)				Funds Requested (\$)
Budget Period: 4				
Start Date: <input style="width: 100px;" type="text"/>		End Date: <input style="width: 100px;" type="text"/>		
A. Direct Costs				
* Direct Cost less Consortium F&A				* Funds Requested (\$)
Consortium F&A				
* Total Direct Costs				
B. Indirect Costs				
	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base (\$)	* Funds Requested (\$)
1.	<input style="width: 500px;" type="text"/>	<input style="width: 50px;" type="text"/>	<input style="width: 100px;" type="text"/>	<input style="width: 100px;" type="text"/>
2.	<input style="width: 500px;" type="text"/>	<input style="width: 50px;" type="text"/>	<input style="width: 100px;" type="text"/>	<input style="width: 100px;" type="text"/>
3.	<input style="width: 500px;" type="text"/>	<input style="width: 50px;" type="text"/>	<input style="width: 100px;" type="text"/>	<input style="width: 100px;" type="text"/>
4.	<input style="width: 500px;" type="text"/>	<input style="width: 50px;" type="text"/>	<input style="width: 100px;" type="text"/>	<input style="width: 100px;" type="text"/>
Cognizant Agency (Agency Name, POC Name and Phone Number)		<input style="width: 450px; height: 50px;" type="text"/>		
Indirect Cost Rate Agreement Date <input style="width: 100px;" type="text"/>		Total Indirect Costs <input style="width: 100px;" type="text"/>		
C. Total Direct and Indirect Costs (A + B)				Funds Requested (\$)

PHS 398 Modular Budget, Periods 5 and Cumulative

Budget Period: 5			
Start Date: <input style="width: 100px;" type="text"/>		End Date: <input style="width: 100px;" type="text"/>	
A. Direct Costs			* Funds Requested (\$)
* Direct Cost less Consortium F&A			<input style="width: 150px;" type="text"/>
Consortium F&A			<input style="width: 150px;" type="text"/>
* Total Direct Costs			<input style="width: 150px;" type="text"/>
B. Indirect Costs			
	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base (\$)
1.	<input style="width: 500px;" type="text"/>	<input style="width: 50px;" type="text"/>	<input style="width: 100px;" type="text"/>
2.	<input style="width: 500px;" type="text"/>	<input style="width: 50px;" type="text"/>	<input style="width: 100px;" type="text"/>
3.	<input style="width: 500px;" type="text"/>	<input style="width: 50px;" type="text"/>	<input style="width: 100px;" type="text"/>
4.	<input style="width: 500px;" type="text"/>	<input style="width: 50px;" type="text"/>	<input style="width: 100px;" type="text"/>
Cognizant Agency (Agency Name, POC Name and Phone Number) <input style="width: 450px;" type="text"/>			
Indirect Cost Rate Agreement Date <input style="width: 100px;" type="text"/>		Total Indirect Costs <input style="width: 100px;" type="text"/>	
C. Total Direct and Indirect Costs (A + B)			Funds Requested (\$) <input style="width: 100px;" type="text"/>
Cumulative Budget Information			
1. Total Costs, Entire Project Period			
*Section A, Total Direct Cost less Consortium F&A for Entire Project Period		\$	<input style="width: 150px;" type="text"/>
Section A, Total Consortium F&A for Entire Project Period		\$	<input style="width: 150px;" type="text"/>
*Section A, Total Direct Costs for Entire Project Period		\$	<input style="width: 150px;" type="text"/>
*Section B, Total Indirect Costs for Entire Project Period		\$	<input style="width: 150px;" type="text"/>
*Section C, Total Direct and Indirect Costs (A+B) for Entire Project Period		\$	<input style="width: 150px;" type="text"/>
2. Budget Justifications			
Personnel Justification	<input style="width: 230px;" type="text" value="PersonnelJustification10114407"/>	<input type="button" value="Add Attachment"/>	<input type="button" value="Delete Attachment"/>
Consortium Justification	<input style="width: 230px;" type="text"/>	<input type="button" value="Add Attachment"/>	<input type="button" value="Delete Attachment"/>
Additional Narrative Justification	<input style="width: 230px;" type="text" value="AdditionalNarrativeJustificati"/>	<input type="button" value="Add Attachment"/>	<input type="button" value="Delete Attachment"/>
		<input type="button" value="View Attachment"/>	<input type="button" value="View Attachment"/>

PERSONNEL JUSTIFICATION

Joseph M. McCune, MD, PhD, Principal Investigator, will devote 0.60 calendar months of his effort to this project during Years 01 and 02. Dr. McCune is a Professor of Medicine and the Chief of the Division of Experimental Medicine at UCSF. He has had over 28 years of experience in taking care of patients with HIV disease, 26 years of which have been devoted to research directly related to understanding the mechanisms of pathogenesis of disease. He will be involved in the design, implementation, and interpretation of all aspects of this study.

Shannon Thyne, MD, Co-Investigator, will devote 0.60 calendar months of effort to this project in Years 01 and 02. Dr. Thyne is an Associate Professor of Clinical Pediatrics and the Medical Director of the Children's Health Center at San Francisco General Hospital. She will assist with the collection of samples from patients who have been previously recruited by Dr. Burt.

Trevor Burt, MD, Assistant Adjunct Professor, will devote 2.40 calendar months of his effort to this project during Years 01 and 02. Dr. Burt has worked in Dr. McCune's lab on and off since 2000, when he first came as a Sarnoff Research Fellow from Harvard Medical School (2000-2001). After finishing medical school and completing an internship and residency at Children's Hospital/Harvard, he started to work again in Dr. McCune's lab as a postdoctoral fellow (in 2005). He is now a board-certified pediatrician with sub-specialty training in neonatal/perinatal medicine, has extensive clinical expertise in the care of newborns and infants, has been involved in recruiting subjects into clinical studies in the past, and is fully familiar with all of the techniques to be used in this project.

TBH, Staff Research Associate II, will devote 4.80 calendar months of effort to this project in Years 01 and 02. This individual will assist Dr. Burt with most of the data collection for this project, including work in Specific Aims 1 and 2. Tasks will include sample acquisition, logging, and preparation; analyses conducted by flow cytometry, ELISA, and PCR; data collection, analysis, and storage; and quality control measurements.

ADDITIONAL NARRATIVE JUSTIFICATION

So that we can finish this project in two years, it is necessary to budget more resources in FY01 for the work in Aims 1 and 2. Thus, more cord blood specimens will be analyzed in Year 01 than in Year 02, and all specimens will have been obtained halfway through Year 02. The remaining time period of the grant will be used for data analysis. These additional resources in FY01 will cost \$25,000 or one additional module.

PHS 398 Research Plan

1. Application Type:

From SF 424 (R&R) Cover Page. The response provided on that page, regarding the type of application being submitted, is repeated for your reference, as you attach the appropriate sections of the Research Plan.

*Type of Application:

☒ New ☐ Resubmission ☐ Renewal ☐ Continuation ☐ Revision

2. Research Plan Attachments:

Please attach applicable sections of the research plan, below.

1. Introduction to Application (for RESUBMISSION or REVISION only)	<input type="text"/>	Add Attachment	Delete Attachment	View Attachment
2. Specific Aims	<input type="text" value="SpecificAims1011440772.pdf"/>	Add Attachment	Delete Attachment	View Attachment
3. *Research Strategy	<input type="text" value="ResearchStrategy1011440814.p"/>	Add Attachment	Delete Attachment	View Attachment
4. Inclusion Enrollment Report	<input type="text"/>	Add Attachment	Delete Attachment	View Attachment
5. Progress Report Publication List	<input type="text"/>	Add Attachment	Delete Attachment	View Attachment

Human Subjects Sections

6. Protection of Human Subjects	<input type="text" value="ProtectionHumanSubjects1011"/>	Add Attachment	Delete Attachment	View Attachment
7. Inclusion of Women and Minorities	<input type="text" value="InclusionWomenMinorities101"/>	Add Attachment	Delete Attachment	View Attachment
8. Targeted/Planned Enrollment Table	<input type="text" value="TPETForm1011440815.pdf"/>	Add Attachment	Delete Attachment	View Attachment
9. Inclusion of Children	<input type="text" value="InclusionChildren1011440810"/>	Add Attachment	Delete Attachment	View Attachment

Other Research Plan Sections

10. Vertebrate Animals	<input type="text"/>	Add Attachment	Delete Attachment	View Attachment
11. Select Agent Research	<input type="text"/>	Add Attachment	Delete Attachment	View Attachment
12. Multiple PD/PI Leadership Plan	<input type="text"/>	Add Attachment	Delete Attachment	View Attachment
13. Consortium/Contractual Arrangements	<input type="text"/>	Add Attachment	Delete Attachment	View Attachment
14. Letters of Support	<input type="text" value="LOSThyme1011440726.pdf"/>	Add Attachment	Delete Attachment	View Attachment
15. Resource Sharing Plan(s)	<input type="text" value="ResourceSharingPlan10114406"/>	Add Attachment	Delete Attachment	View Attachment

16. Appendix [Add Attachments](#) [Remove Attachments](#) [View Attachments](#)

SPECIFIC AIMS

The development of the mammalian immune system is typically thought to occur in a linear fashion, from immaturity to maturity as a function of antigen exposure. Previous findings in birds and in mice, however, indicate that this view is oversimplified. Thus, in these species, the developing immune system appears to be “layered”¹ in a manner that is independent of antigen exposure, beginning as a multilineage fetal system that is replaced by an anatomically and biologically distinct multilineage system after birth.^{2,3} If so, then developmentally ordered and unique hematopoietic stem/progenitor cells (HSPC) could give rise to distinct lymphocyte lineages at different stages of development.

In ongoing experiments, we have found that such immune system “layering” occurs in humans. Our preliminary data show that: (1) the human fetus can mount a vigorous immune response to exogenous antigens;⁴ (2) the human fetal immune response to exogenous antigens can be actively suppressed by antigen-specific Tregs;^{4,5} (3) fetal Tregs are derived from a fetal-specific lineage of T cells; (4) fetal and adult HSPCs give rise to distinct populations of T cells; and (5) fetal HSPC-derived T cells show an enhanced ability to generate Tregs during thymic maturation and upon exposure to foreign antigens *in vitro*.⁶ These data suggest that the human immune system is comprised of two distinct waves: one generated from a “fetal” HSPC that exists *in utero* in the fetal liver and bone marrow, and another generated from a superseding “adult” HSPC that resides in the bone marrow at later time points. The former gives rise to an immune system that is prone to deliver a tolerogenic response to foreign antigens. The latter gives rise to an immune system that is more likely to generate an immunoreactive responses (e.g., one including cytotoxic T cells and neutralizing antibodies).

Given these findings, we hypothesize that physiologic layering of immune system ontogeny leads to a normal range in the ratio of fetal- to adult-type T cells at birth, with some neonates exhibiting a higher fraction of fetal T cells than others; and that those with a high ratio of fetal/adult T cells will generate predominant Th2 responses to routine childhood immunizations.

These hypotheses will be addressed in the experiments of the following Specific Aims:

Specific Aim 1. To determine the normal range of fetal to adult T cells in the umbilical cord blood of the full term neonate.

In these experiments, comprehensive phenotypic, transcriptional, and functional analyses will be carried out on umbilical cord blood (UCB) mononuclear cells from a total of 200 normal full-term deliveries, obtained over an 18-month time frame from the Human Cord Blood Bank of the UCSF Clinical and Translational Sciences Institute, from Dr. Elizabeth Shpall of the University of Texas M.D. Anderson Cancer Center, and as part of a prospective study to be carried out with Dr. Shannon Thyne of the Child Health Center at San Francisco General Hospital (SFGH). Naïve T cells in these samples will be studied to determine the ratio of fetal/adult T cells (T_F/T_A) and the relationship of this ratio to naïve T cell function.

Specific Aim 2. To determine whether those full-term neonates with a high ratio of fetal/adult T cells are more likely to generate a Th2-polarized immune response to routine childhood immunizations.

Under the auspices of an existing protocol that has been approved by the UCSF Committee on Human Research protocol and in collaboration with Dr. Shannon Thyne, 50 full-term infants will be followed from birth through 12 months. Cord blood samples obtained from each of these newborns will be examined for the T_F/T_A ratio and this ratio will be related to the response of the newborn to hepatitis B vaccination.

We anticipate that this study will reveal normal variation in the ratio of fetal to adult T cells at birth and that such variability in this ratio will be directly related to – and possibly causal of – a Th2 skew that results in a poor response to childhood vaccines *and* a heightened predisposition to childhood infections and to atopic disorders. If so, then modalities aimed at changing this ratio more towards the adult lineage at birth may provide benefit to a substantial number of newborns.

RESEARCH STRATEGY

(a) SIGNIFICANCE The developing human immune system faces a balancing act that must be carefully timed. On the one hand, it must tolerate the presence of the surrounding mother and her non-inherited maternal alloantigens (NIMA) or otherwise risk the potential of engaging a fatal “graft vs. host” disease. On the other hand, novel antigens must be recognized as foreign when encountered after birth, triggering a vigorous adaptive immune response (e.g., with cytolytic T cells and neutralizing antigens) against them. Otherwise, the newborn will be susceptible to diseases caused by multiple infectious agents.

In ongoing experiments, we have obtained preliminary data (see below) indicating that this switch from a fetal-type to an adult-type immune response is dependent upon the stage-specific appearance of distinct multilineage hematopoietic stem/progenitor cells (HSPC).⁶ Thus, *in utero*, hematopoiesis in the first and second trimester is largely sustained by a fetal-type HSPC that gives rise to tolerogenic Tregs; later (and perhaps as early as the third trimester), an adult-type HSPC instead gives rise to immunoreactive T cells. The timing of this switch coincides with birth and normally allows the newborn to move from a stance of tolerance to one of active defence against all foreign antigens. In this manner, the “immune privileged” aspect of mammalian pregnancy is preserved while the ability of the newborn to fight infections is also permitted.

Nonetheless, infection remains a leading cause of death and morbidity in newborns. Not only are neonates susceptible to more severe forms of disease caused by human pathogens such as herpes simplex virus 1, respiratory syncytial virus, *Bordetella pertussis* and *Staphylococcus aureus*, they are also subject to serious infection by microbial entities that are commensal flora in adults. For example, even after implementation of intensive screening and prevention practices, the estimated rate of Group B Streptococcal sepsis in the first week of life is 0.34 per 1000 live births, resulting in 60-70 deaths per year.⁷ In addition to the immediate impact of neonatal illness and death, the long-term disability resulting from these infections represents a profound public health burden.⁸ Premature infants, in particular, are predisposed to more severe infections from all pathogens and can also succumb to fatal infection by microbes that infrequently cause severe disease in adults, such as *Staphylococcus epidermidis*.⁹ Compared with adults and older children, newborns produce less, and generally less effective, antibody in response to most immunizations. They are also less able to generate T cells that mediate effective antimicrobial responses.¹⁰⁻¹⁵ Together, these deficiencies render the neonate a vulnerable target for a host of invading pathogens.

If the switch to an “adult-type” immune system is incomplete or overly slow after birth, two other problems may also arise. First, the neonate may respond less well to immunizations provided during the first months of life, either generating low levels of an effective response or polarized features of a non-effective response.^{10,11,16-25} Secondly, those neonates that are most likely to develop atopic disorders after birth are also those who are most likely to generate suboptimal (and/or strong Th2-type) response to vaccination.^{21,26} Since fetal Tregs may suppress Th1-type (or other) immune responses to vaccines in a manner that is different than adult Tregs, we speculate that strong Th2 polarization of childhood responses to vaccines may in part be due to a higher than normal proportion of fetal Tregs at birth.

In the studies of this proposal, we hypothesize that the immune system “layering” that is necessary for effective *in utero* development and postnatal protection of the human fetus occurs at a dissimilar pace in different individuals, predisposing some at birth to less effective immune responses to childhood immunizations.

(b) INNOVATION Previous experiments have demonstrated that similar “layering” of the immune system can occur in avian and murine models.¹⁻³ In these species, however, the timing and/or anatomic constraints are entirely different. In particular, the murine immune system develops at a markedly different pace than does the human immune system, e.g., with very few Tregs detectable until three days after birth²⁷ as compared to the late 1st trimester in the human. This study is innovative in two respects: this is the first time that human immune system layering has been studied *in utero* and at birth; in addition, we have identified and validated a set of genes that are uniquely expressed in fetal or adult T cells, allowing us to quantitatively and qualitatively study the kinetics of the two populations as a function of time. The proposed research has the potential to improve prevention (through improved vaccine strategies) and treatment of neonatal infection (by providing a better understanding of normal human fetal immune development), and should teach us how the developmental state of the fetus and newborn affects their ability to respond to pathogens or vaccines.

(c) RESEARCH PLAN We propose to test the hypothesis that the immune system of the human newborn is comprised of two distinct hematopoietic lineages, one derived from a multilineage HSPC that resides in the fetal liver and bone marrow, and another from an HSPC that begins to function later in pregnancy and that supplants the fetal lineage thereafter. The former lineage is endowed with tolerogenic T cells that allow the fetus to co-exist with the mother (including her NIMA and other foreign antigens circulating with her); the latter lineage is instead comprised of T cells that are more likely to develop effector functions against novel antigens. The basis for this hypothesis is found by precedent in avian and murine models as well as by way of our largely unpublished Preliminary Data:

The human fetus can mount a vigorous immune response to exogenous antigens. Although the human fetal and neonatal adaptive immune systems are often described as “immature” (*i.e.*, dysfunctional or ineffective at mounting a response to antigenic challenge), there is substantial evidence that immune responses can develop at or before birth in species such as sheep and nonhuman primates (but, interestingly, not mice) (reviewed in refs.16 and 28). To determine whether human fetal T cells are responsive against alloantigens, fetal (~20 g.w.) lymphocytes from spleen or lymph node (LN) were labeled with carboxyfluorescein succinimidyl ester (CFSE) and co-cultured with irradiated antigen-presenting cells (APCs) that had been isolated from the peripheral blood of a single healthy adult donor. After five days substantive proliferative responses were observed for both CD4⁺ and CD8⁺ fetal T cells (Fig. 1). This finding raised the question: if fetal T cells respond so vigorously to alloantigens *in vitro*, do they not also respond to NIMA expressed by maternal cells that have moved into fetal LNs *in utero*?

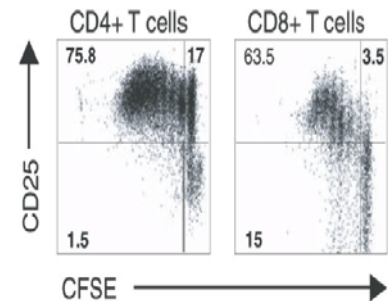


Figure 1. Representative plot of CD4⁺ and CD8⁺ fetal T cell proliferation after stimulation with allogeneic APCs from an unrelated donor for 5 days (3:1 ratio of fetal lymphocytes: allogeneic APCs)

The human fetal immune response to exogenous antigens can be actively suppressed by antigen-specific Tregs. We recently demonstrated that human fetal secondary lymphoid tissues contain significantly

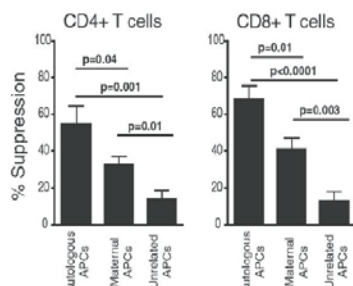


Figure 2. Comparison of fetal T_{Reg} cell suppression against autologous, maternal, or unrelated APCs determined based on the following calculation: % Suppression = 1 - [(%CFSElow (total LN cells)) / (%CFSElow (CD25-depleted LN cells))]

higher frequencies of CD4⁺CD25^{high} Tregs than those of adults.⁴ Because Tregs have been shown to regulate maternal immunity to fetal alloantigens,²⁹ we reasoned that fetal Tregs might also play a role in suppressing fetal immune responses against invading maternal cells. To test this hypothesis, fetal LN cultures were either depleted or “mock-depleted” of Tregs before stimulation with self (autologous), maternal, or unrelated allogeneic APCs. Depletion of Tregs resulted in a highly significant increase in proliferation of CD4⁺ and CD8⁺ T cells responding to autologous or maternal APCs, but only a slight (yet statistically significant) increase in proliferation of those responding to unrelated APCs (Fig. 2).

These data indicate that fetal T cells are

not inherently deficient at responding to maternal alloantigens; rather, they are actively suppressed by fetal Tregs.

The frequency of Tregs in peripheral lymphoid organs changes markedly during the course of gestation, falling from ~15–20% of total CD4⁺ T cells at 12–20 g.w. to ~3–7% at birth.³⁰ To test the hypothesis that such a change in frequency reflects a greater propensity of fetal naive CD4⁺ and CD8⁺ T cells to differentiate into Tregs in response to stimulation, fetal LN cells were depleted of CD25^{high} Tregs and stimulated *in vitro*. After a five-day primary mixed lymphocyte reaction (MLR), a significant fraction of fetal, but not adult, CD4⁺ and CD8⁺ T cells had divided and upregulated FoxP3 expression to high levels (Fig. 3A, B).

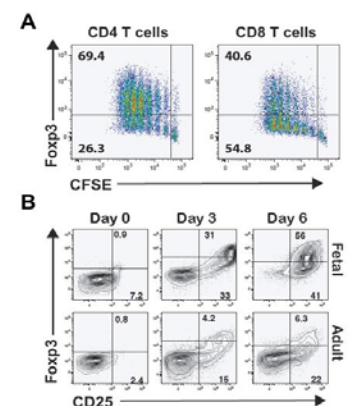


Figure 3. (A) Fetal T cells depleted of CD25⁺Foxp3⁺ cells were stimulated for 5 days with unrelated APCs and Foxp3 expression measured. (B) CD25 and Foxp3 upregulation by adult and fetal T cells depleted of CD25⁺Foxp3⁺ cells at day 0, as a function of time after stimulation.

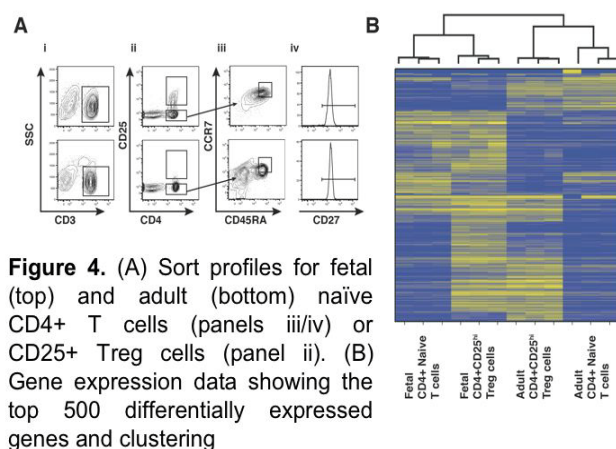


Figure 4. (A) Sort profiles for fetal (top) and adult (bottom) naive CD4⁺ T cells (panels iii/iv) or CD25⁺ Treg cells (panel ii). (B) Gene expression data showing the top 500 differentially expressed genes and clustering

Fetal Tregs are derived from a fetal-specific lineage of T cells. The above studies revealed profound differences in function between fetal and adult T cells that had otherwise indistinguishable phenotypes. To determine whether such differences are intrinsic to the T cell lineages found during these stages of ontogeny, CD4⁺CD25^{high} Tregs and CD4⁺CD45RA⁺CCR7⁺CD27⁺ naïve T cells from fetal and adult samples were sorted with a FACSDiVA (Fig. 4A). Microarray analysis (Fig. 4B) identified thousands of genes whose expression levels in adult and fetal CD4⁺ naïve T cell populations differed significantly ($P < 0.05$) and in a highly consistent manner between donors, including NOG, GZMA, and RGS1 were highly expressed (20-55 fold greater) by fetal cells whereas NAP1L2, NR3C2, and SYT4A were highly expressed by adult cells.

Fetal and adult HSPCs give rise to distinct populations of T cells. In avian and mouse models, there is strong evidence that fetal HSPCs give rise to unique subsets of lymphocytes that cannot be generated from adult HSPCs and that immune system “layering”¹ occurs during ontogeny.^{2,3} To test whether a similar situation exists in humans, we performed a series of experiments in which fetal HSPCs from fetal liver and BM (18-22 g.w.) and adult BM samples were injected directly into the human Thy/Liv organ of the SCID-hu Thy/Liv mouse.³¹ Following a 7-8 week maturation period, we were able to identify mature thymocyte populations derived from each HSPC population, based on the expression of a unique HLA type (typically HLA-A2 or A3) expressed by the donor (source of HSPCs) but not by the recipient thymic implant.^{31,32} We isolated mature CD3⁺CD4⁺CD8⁻CD25⁻ thymocytes from thymic implants injected with fetal liver, fetal BM, or adult BM-derived HSPCs by FACS (FACS Aria), and performed microarray analysis on each population (Fig. 5A). We found that both HSPC populations from fetal liver and BM gave rise to identical populations of CD4⁺ thymocytes on the basis of gene expression, with no differentially-expressed genes between them (Fig. 5B). By contrast, adult BM-derived HSPCs gave rise to CD4⁺ thymocytes that showed substantial differences in gene expression patterns compared to each population of fetal HSPC-derived thymocytes (Fig. 5B: 1243 and 1162 differentially-expressed genes versus fetal liver and fetal BM, respectively). These data are consistent with the hypothesis that the developmental stage of HSPCs is in part responsible for the differences seen in peripheral T cell compartments in the fetus and adult, and that layering occurs during the ontogeny of the human immune system.

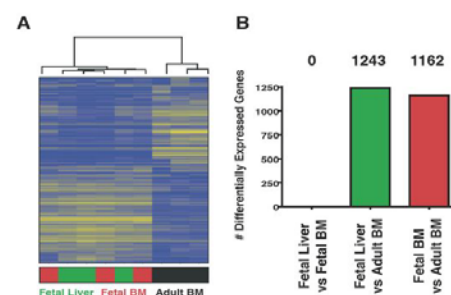


Figure 5. (A) Clustering of the top differentially expressed (DE) genes by CD4⁺ thymocytes from fetal liver HSPC (green), fetal BM HSPC (red), and adult BM HSPC (black). (B) Total number of DE genes (>2-fold difference expression difference and $p < 0.05$) for each thymocyte population.

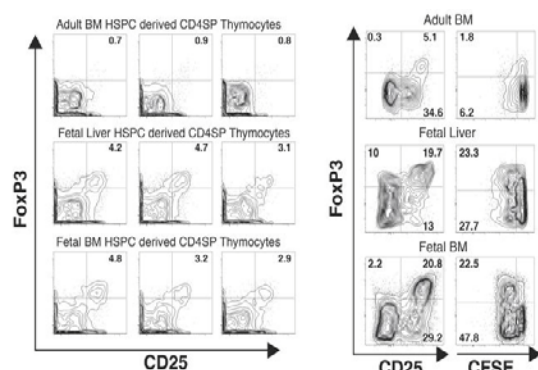


Figure 6. (A) CD25 and Foxp3 expression by CD3⁺CD4⁺CD8⁻ thymocytes from adult BM, fetal liver, or fetal BM (3 SCID-hu thymic implants/group). (B) Expression of CD25 and Foxp3 (left) and proliferation (CFSE-dilution, right) following stimulation of CD3⁺CD4⁺CD8⁻ thymocytes with allogeneic APCs for 7 days *in vitro*.

Fetal HSPC-derived T cells show an enhanced ability to generate Tregs during thymic maturation and upon exposure to foreign antigens *in vitro*. CD4⁺CD25⁺Foxp3⁺ Tregs can be generated during thymic maturation or following activation of peripheral T cells. Some evidence indicates that Tregs may arise from a committed progenitor that is distinct from conventional T cell precursors.³³ We observed that fetal HSPC-derived thymocyte populations contained significantly greater frequencies of Tregs than those derived from adult HSPCs (Fig. 6A). In accordance with what we observed in peripheral fetal and adult T cell populations, we also noted that fetal HSPC-derived thymocytes were highly responsive to stimulation with allogeneic APCs and showed a propensity to differentiate into FoxP3⁺ Tregs (Fig. 6B).

In sum, the above Preliminary Data indicate that the fetal

immune system is derived from a HSPC that gives rise to tolerogenic Tregs while the adult HSPC is more likely to give rise to immunoreactive T effector cells. At this point, we have very little information about the relative balance of these two compartments at birth. It is also not clear whether and to what extent variations in this balance may impact upon the response of the neonatal immune system to novel antigens, including those associated with routine vaccines or with environmental allergens. The experiments of this proposal are designed to explore these questions.

Specific Aim 1. To determine the normal range of fetal to adult T cells in the umbilical cord blood of neonates at birth

Hypothesis. Physiologic layering of the human immune system during ontogeny leads to a normal range in the ratio of fetal to adult-type T cells at the time of birth, with some neonates born with a more tolerogenic immune system than others.

Rationale. As described in the above Preliminary Data, the human fetal immune system is poised to generate a tolerogenic Treg response upon stimulation, an attribute that is conferred by an HSPC that resides within the fetal liver and bone marrow. After birth, bone marrow-derived HSPC give rise instead to immunoreactive T cells with a reduced propensity to generate Tregs. Teleologically, such “layering” of the immune system would appear to be consistent with, and possibly necessary for, maintenance of the semi-allogeneic state of pregnancy and, reciprocally, for the generation of an active immune response against foreign (e.g., infectious agents) after birth. Similar stage-specific waves of distinct hematopoietic progenitors have also been described in avian and murine models.¹⁻³ A key question that remains unanswered is the following: is there inter-individual variation in the rate at which the fetal-type hematopoietic system is replaced by the adult-type system over time? In this Aim, we propose to determine whether and to what extent such variability may exist at the time of birth. Given known transcripts that uniquely identify tolerogenic fetal T cells (T_F) and immunoreactive adult T cells (T_A), the normal range of these two T cell subpopulations in the umbilical cord blood will be determined.

Experimental Approach. Comprehensive phenotypic, transcriptional, and functional analyses will be carried out on umbilical cord blood (UCB) mononuclear cells from a total of 200 normal full-term deliveries. Over an 18-month time frame, 75 of these samples will be obtained on a recharge basis from the Human Cord Blood Bank of the UCSF Clinical and Translational Sciences Institute (see attached letter from Dr. William Balke), 75 will be obtained on a collaborative basis from Dr. Elizabeth Shpall of the University of Texas M.D. Anderson Cancer Center (see attached letter), and 50 will be obtained as part of a prospective study to be carried out with Dr. Shannon Thyne of the Child Health Center at SFGH (see attached letter). Initial studies will focus on naive T cells obtained by a combination of ficoll hypaque gradient enrichment and FACS sorting; excess cells will be viably cryopreserved in liquid nitrogen for future experiments that may interrogate other subpopulations of cells. The following assays will be carried out:

1. Phenotypic analysis of T cell populations. The frequency of various T cell populations in the cord blood will be analyzed using standard markers of naive $CD4^+$ T cells ($CD45RA^+CD27^+CCR7^+$), memory/effector T cells ($CD4^+CD45RO^+CD95^+HLA-DR^+$), and Tregs ($CD4^+CD25^{high}FoxP3^+CD127^{low}$).⁵ Absolute numbers will be quantified using TRUcount tubes (BD).
2. Transcriptional analysis of naïve T cell populations. Phenotypically-pure naïve $CD4^+$ T cells will be obtained by sort purification on a FACS Aria (BD) and subjected to qRT-PCR assay to detect transcripts (transcript specific to cell-type Z denoted as t_Z) that are unique to fetal naive T cells (e.g., NOG, GZMA, and RGS1; t_F) or to adult naïve T cells (e.g., NAP1L2, NR3C2, and SYT4A; t_A) as well as transcripts for house-keeping genes that are equivalently expressed in fetal and adult naive T cells (e.g., the β chain of the T cell receptor or HPRT; denoted by t_X). Each transcript will be quantitated in replicate and three standardized ratios of fetal/adult T cell transcripts (F/A-T) will be calculated based on the formula $(t_F/t_X)/(t_A/t_X) = (t_F/t_A)$. The three ratios will be F/A-T1 (NOG/NAP1L2), F/A-T2 (GZMA/NR3C2), and F/A-T3 (RGS1/SYT4A), and the mean of these ratios will be used to represent the fetal/adult T cell ratio (T_F/T_A).
3. Functional analysis of T cell populations. To test whether UCB T cells upregulate FoxP3 and adopt a Treg phenotype upon activation with alloantigens,⁵ naïve $CD4^+$ T cells will be isolated by FACS and stimulated with allogeneic adult APCs plus or minus concurrent stimulation with cross-linking antibodies against CD3 and CD28. This type of stimulation reliably leads to Treg differentiation from naive $CD4^+$ T_F (see Figure 3, Preliminary Data). Prior to stimulation, the cells will be labeled with CFSE for determination of proliferation. After six days of stimulation, the cells will be harvested and each T cell subpopulation will

be measured for proliferation (CFSE dilution) and for Treg differentiation (FoxP3 upregulation) by flow cytometry. Standard types of cytokine response (Th1, Th2, Th17, and Th22) will be measured by cytokine production after six days of differentiation *in vitro* by carrying out intracellular cytokine flow cytometry (CFC) for the following cytokines: IL-2, INF- γ , TNF- α , IL-4, IL-17, and IL-22. To test whether cord blood Tregs are better able to suppress Th1 vs. Th2 responses, CD4⁺ naïve T cells will be sort-purified from a healthy adult (or cord blood) donor and cultured under Th1 or Th2 conditions in the presence of anti-CD3/anti-CD28. Th1 and Th2 polarization will be measured by cytokine production after six days of differentiation *in vitro*. These cells will subsequently be labeled with CFSE and cultured in the presence of different quantities of sort-purified CD4⁺CD25⁺ Treg from allogeneic adult blood or cord blood in the presence of anti-CD3/CD28. Suppression of Th1 or Th2 cells will be measured by inhibition of proliferation (by CFSE dilution) and suppression of cytokine secretion (by cytokine flow cytometry) after a six-day culture period.

Interpretation of Results. The experiments described in this Aim extend observations that we have made in human fetal and adult samples to a much larger number of samples of human cord blood. We anticipate that, at term, there will be a normative range of fetal- and adult-type Tregs and HSPCs in cord blood, representing variable kinetics by which layering of immune system ontogeny proceeds in different individuals. The relative frequency of fetal versus adult T cell-specific transcripts (tT_F/tT_A) will be taken as a measurement of the actual fetal/adult T cell ratio (T_F/T_A). Thus, we will make the assumption that $tT_F/tT_A = T_F/T_A$. In those cases in which the fetal/adult T cell ratio (T_F/T_A) is high, it is predictable that cord blood naïve T cells will be more likely to upregulate FoxP3 upon stimulation and that a predominant tolerogenic response to antigen will ensue.

Potential Problems and Alternative Approaches. Given our existing data sets and the techniques that have already been established in the lab, the experiments of this Aim should be relatively straightforward. Though it is highly unlikely, it is possible that there will be no significant variability in the T_F/T_A ratio in full-term newborns. This would be an interesting finding, suggesting that the fetal-to-adult T cell transition occurs earlier during the third trimester of pregnancy and is complete at birth. Should this be the case, we will shift out attention to human premature infants and to nonhuman primates, each of which can be studied during the timeframe of the third trimester.

Specific Aim 2. To determine whether those full-term neonates with a high ratio of fetal/adult T cells are more likely to generate a Th2-polarized immune response to routine childhood immunizations.

Hypothesis. Infants with a high ratio of fetal/adult T cells will generate predominant Th2 responses to routine childhood vaccinations.

Rationale. In human neonates, T cell responses are often characterized by deficient Th1 responses,¹⁶⁻¹⁹ a reduced capacity to induce T cell memory,²⁰ a high frequency of IgE and IgG4 production,²¹ a skewed Th2 response,²²⁻²⁴ and even the induction of hyporesponsiveness.²⁵ Such responsiveness to routine childhood vaccines has been found to vary within populations, possibly as a result of genetic and/or environmental factors^{26,34,35} and is in part magnified by the formulation of vaccines with the Th2-polarizing adjuvant, alum.³⁶ In addition, those neonates that are most likely to develop atopic disorders after birth are also those who are most likely to generate suboptimal (and/or strong Th2-type) response to vaccination.^{21,26,37} Since fetal Tregs may suppress Th1-type (or other) immune responses to vaccines in a manner that is different than adult Tregs, we speculate that strong Th2 polarization of childhood responses to vaccines may in part be due to a higher than normal proportion of fetal T cells at birth.

Recombinant Hepatitis B (HepB) vaccine is routinely given at birth in the United States and provides an ideal opportunity to investigate an *in vivo* response to antigenic stimulation in the newborn. The immune response to HepB vaccine is well studied and is characterized by a meager (10%) seroconversion rate with the first dose at birth.³⁸ Subsequent vaccine doses are then observed to yield a substantial antibody response.³⁹ The primary neonatal CD4⁺ T cell response to HepB vaccine is characterized by both Th1 and Th2 cytokine production; interestingly, however, the HepB-specific memory CD4⁺ recall response consists of robust Th2 cytokine production at one year of age.⁴⁰

Experimental Approach. The experiments of this Aim will be carried out under the auspices of a study that has been approved by the UCSF Committee on Human Research protocol (H6325-26775. The effects of regulatory T cells on the development of the pediatric immune system; McCune PI). In a prospective study design, 50 pregnant women will be enrolled prior to delivery. Exclusion criteria will include previous/current

HepB infection (HepBsAg⁺), other immunomodulatory infections detected by prenatal screening (HepC, HIV), or plans for the use of cord blood for alternative purposes (e.g., banking). With assistance from collaborators in the SFGH Child Health Center (see attached letter of collaboration from Dr. Shannon Thyne), cord blood will be obtained from these deliveries and each of the 50 infants will be followed with blood draws at 6 and 12 months. In all cases, infants will have received routine childhood immunizations, including those against HepB at birth, 1-2, and 6-12 months of age. UCB and infant peripheral blood will be processed by ficoll hypaque and concurrent purification of CD4⁺ cells by negative selection (RosetteSep, StemCell). These cells will be tested for the following parameters over time:

1. The fraction of T_F vs. T_A in the umbilical cord blood. This will be carried out using the qRT-PCR-based approach described in Aim 1 to determine the ratio of fetal/adult T cells (T_F/T_A).
2. Analysis of the cellular immune response against HepB vaccine. Established flow cytometric assays will be used to assess the fraction of CD4⁺CD25^{high}FoxP3⁺CD127^{low} Tregs among CD3⁺CD4⁺ T cells at each time point. These fractions will be converted to absolute numbers using TruCount tube (BD) analysis of whole blood counts prior to CD4⁺ cell isolation, as described.⁴¹ In addition to quantitative phenotyping, the ability of circulating Tregs to suppress the proliferation of HepB-specific responder CD4⁺CD25⁻ T cells will be assessed using well-established methods.^{5,41,42} UCB or peripheral mononuclear cells that have been depleted of CD25⁺ cells (or mock depleted) will be stimulated with (1) polyclonal activators (cross-linking antibodies against CD3 and CD28), (2) soluble HepB antigen, and (3) peptides corresponding to HepB. Cytokine production in CD4⁺ cells will be assessed by CFC, as described above in Aim 1, to determine whether antigen-specific stimulation yields a response that is predominantly Th1 or Th2 in type.
3. Levels of circulating Immunoglobulin (Ig) isotypes generated against HepB vaccine. Established ELISAs will be used to quantitate circulating levels of vaccine-induced IgG1, IgG2, IgG3, IgG4, and IgE relative to total Ig.

Interpretation of Results. This study will relate the ratio of fetal to adult naïve T cells (T_F/T_A) to a number of immune parameters associated with routine childhood immunizations. In particular, it will be of interest to know whether a high T_F/T_A ratio is associated with a higher propensity towards a less effective “immature” response (e.g., a Th2-predominant cytokine response to vaccine antigens and the predominance of less-mature, antigen-specific IgG1, IgG3, and IgE compared to IgG2). Infants will be studied both cross-sectionally as well as prospectively, and we anticipate that the T_F/T_A ratio and measures of immaturity (especially a bias towards a Th2 response) will decrease with age in tandem. The ability of Tregs to suppress antigen-specific responses against HepB will also be measured. Given the hypothesis that T_F in the newborn generate tolerance to antigens they encounter by becoming Tregs, more robust HepB-specific Treg suppression would be expected in those children with a higher T_F/T_A ratio.

Statistical Analysis. These data will be analyzed in consultation with biostatisticians in the Biostatistics Consultation Service, associated with the UCSF CTSI (see attached letter of support from Dr. Peter Bacchetti). The sample size that has been chosen will detect a 35% difference in cytokine production, with a study power of 0.8 and significance level of 0.05. The statistical approach will use an ANCOVA-type linear model approach for each outcome variable. Some data may be longitudinal as well as cross-sectional and we will include random effects to account for within-subject correlation. We will explore approaches that treat age as continuous, in particular, the Laird-Ware repeated measures model. If the measures exhibit strong skewness or outlying values, we will attempt to transform the outcome (e.g., log-transformation) to mitigate these issues. If this is not adequate, we will compare the groups using non-parametric (e.g., rank based) methods.

Potential Pitfalls and Alternative Approaches. The primary challenge in this study is that of recruiting and retaining 50 patients over an 18-month period. Given the experience of our collaborator, Dr. Shannon Thyne, we believe that this goal is attainable. The Birth Center at SFGH delivers 1250-1300 infants yearly, all of whom are under the care of the Division of Neonatology, in which Dr. Burt (an MD investigator in the PI's lab who will be working on this study) is an attending physician. He will therefore have the ability to recruit patients (under our CHR-approved protocol) as they are admitted to the hospital in labor. Furthermore, in previous studies carried out through the Birth Center, members of the McCune lab have successfully recruited similar numbers of patients in a period of approximately one year. Approximately 80% of infants born at SFGH receive their primary care in the Child Health Center at SFGH. If by three months of recruitment, we are not meeting expected goals, this study will be extended to the Birth Center at UCSF, which has a similar delivery rate and where we also have CHR approval to recruit.

PROTECTION OF HUMAN SUBJECTS

a. Human subjects involvement and characteristics

In Aim 1, human cord blood specimen will be obtained under the auspices of CHR/IRB approval from the UCSF CTSI Human Cord Blood Bank (n=75), from Dr. Elizabeth Shpall of UT M.D. Anderson Cancer Center (n=75), and from the Child Health Center of San Francisco General Hospital (SFGH) (n=50). The latter population will be followed in a longitudinal study in Aim 2 but will be recruited antenatally because a cord blood collection is required. For this reason, pregnant women will be approached for recruitment and consenting purposes, and the children will be followed from birth until 12 months of age.

For Aim 2, pregnant women will be approached for recruitment, consent, and the collection of a focused medical history. At birth, a cord blood sample will be obtained. From six months to 12 months of age, each subject will have a focused medical history and peripheral blood collection every six months. All pregnant women with a gestational age greater than 20 weeks are eligible. Exclusion criteria include certain infections (e.g., Hepatitis B or C, and HIV) that can affect the study results. There are no exclusion criteria based on gender or race.

Pregnant women and children will be involved in this study, as described above, because this study is evaluating the development of immune function from birth until 12 months of age. Their involvement will be in providing medical history, and acting as guardian of the children involved in decision-making, including consent. All aspects of this study will be performed at San Francisco General Hospital, which is a University of California, San Francisco affiliated hospital.

b. Sources of materials:

Information and materials collected during this study will be used for this study only. Viably frozen umbilical cord blood cells will be kept for further study related to this project involving markers relevant to fetal and newborn immunity that may be discovered in the future. This point will be made clear during the consent process, and included in written consent forms. All subjects will have cord blood collected at birth and/or peripheral blood collected at each study visit. Peripheral blood will be collected by standard venipuncture. Cord blood will be collected from the placenta after delivery of the subject. The volume of peripheral blood to be collected is 2 mL per kilogram. The volume of cord blood to be collected is about 20-40 mL depending on the size and vascularity of the placenta.

Demographic and medical data will be collected from all subjects and their parents. Demographic data will include age, sex, and race which will be used for data analysis. Medical data will include the presence or absence of inflammatory conditions (infections, autoimmune disorders, etc.), atopic conditions (asthma, allergic rhinoconjunctivitis, etc.), exposures that can modify inflammatory/atopic conditions (breast feeding, tobacco smoke, pets, etc.), and immunization history. Information will be stored in an encrypted (meeting mandated IT security standards) password-protected database and will contain subject identifiers such as name, address, date of birth, and medical record numbers. This information is necessary to maintain contact with the subjects during the full year of the study. Only the primary investigator and members of the research team that will contact the subjects will have access to information linked to subject identifiers. Cells isolated and stored from collected blood samples will not have any subject identifiers and will be linked to subjects with a number generated specifically for this study.

c. Potential risks:

1. Venipuncture: There may be discomfort associated with obtaining peripheral blood via venipuncture. Venipuncture also carries the risk of developing a hematoma at the site of the puncture and a <1% risk of developing an infection at the site of puncture.

2. Confidentiality: Certain aspects of the subjects' medical history and demographics will be collected for this study. Loss of privacy may lead to problems with insurability or social stigmatization. Also, some of the

laboratory evaluations being performed may potentially indicate an immune dysfunction. Loss of privacy in regards to this information may also lead to problems with insurability.

Adequacy of protection against risks

a. Recruitment and informed consent: For the longitudinal study described in Aims 1 and 2, subjects will be recruited from the SFGH Child Health Center. Pregnant women, upon admission to the Child Health Center (typically, in early stages of labor), will be asked by their obstetrician if they would be willing to speak with a member of the research team regarding this study; if they agree, a research assistant will be immediately available to discuss the details of the study. If, after discussion with the research assistant, the parents would like their child to participate in this study, informed consent will be obtained. Informed consent forms will be signed by the parents, who will be supplied with a copy of the form. All information will be explained in a non-technical fashion, ample time will be available for the subjects' parents to ask any questions, and the parents will also be asked if they have any specific questions or concerns about the study. Parents will be informed of the risks associated with venipuncture and loss of confidentiality of collected information. Since there are many Spanish-monolingual patients who deliver at the SFGH Child Health Center, research associates will be required to be fluent in English and Spanish. If a fluent Spanish-speaking research associate is not available, hospital interpreter services will be used to interpret all aspects of the consent. Consent forms translated into Spanish (and approved by the UCSF Committee on Human Research) will be used if subjects are Spanish-monolingual, or indicate that this is their preference. Hospital interpreter services will be engaged to translate for other non-English or non-Spanish speaking patients who indicate interest in participating. The most common other language spoken by patients in the SFGH Child Health Center is Mandarin Chinese. CHR-approved consent forms translated into Chinese will also be available.

b. Protection against risks:

1. Venipuncture: Blood draws will be coordinated, as much as feasible, with blood draws obtained during routine medical care to minimize the number of times venipuncture is performed. Venipuncture will be performed via standard techniques and skin preparation (with alcohol) to minimize the risks of developing a hematoma or infection at the site of puncture. A topical anesthetic will be made available for use prior to venipuncture if desired by the subjects' parents. Venipuncture will be performed by a phlebotomist, physician or nurse who is experienced in drawing blood from small children to minimize risk of discomfort and to ensure provision of appropriate distraction techniques for developmental stage.

2. Confidentiality: Data will be handled in a confidential manner to prevent loss of privacy. Paper files will be kept in a locked cabinet in a secure laboratory. Electronic files will be stored in an encrypted and password protected database on secure university server. Only the primary investigator and members of the research team who contact the subjects will have access to information linked to subject identifiers. Cells isolated and stored from collected blood samples will not have any subject identifiers and will be linked to subjects with a number generated specifically for this study.

INCLUSION OF WOMEN AND MINORITIES

This study has a prospective component in Aims 1 and 2 that will recruit 50 children antenatally, and monitor them from birth until 12 months of age. It is anticipated that the number of female and male infants recruited will be approximately the same. There are no inclusion/exclusion criteria based on sex or race. Subject recruitment will occur at San Francisco General Hospital, a UCSF-affiliated hospital that has a patient population reflective of the diversity of the general population of San Francisco, and is demonstrated in our expected enrollment table (please see Target/Planned Enrollment Table for details).

Targeted/Planned Enrollment Table

Study Title: Human immune system layering and the neonatal response to vaccines

Total Planned Enrollment: 200

TARGETED/PLANNED ENROLLMENT: Number of Subjects			
Ethnic Category	Sex/Gender		
	Females	Males	Total
Hispanic or Latino	40	40	80
Not Hispanic or Latino	60	60	120
Ethnic Category: Total of All Subjects *	100	100	200
Racial Categories			
American Indian/Alaska Native	5	5	10
Asian	16	16	32
Native Hawaiian or Other Pacific Islander	16	16	32
Black or African American	16	16	32
White	47	47	94
Racial Categories: Total of All Subjects *	100	100	200

* The "Ethnic Category: Total of All Subjects" must be equal to the "Racial Categories: Total of All Subjects."

INCLUSION OF CHILDREN

This study focuses primarily on better understanding the immune system of the human fetus and newborn. Aims 1 and 2 involve collection of umbilical cord blood from the placentas of 200 newborns, of whom 50 will be recruited before birth and then monitored from birth to 12 months of age. This age range has been chosen because: (a) the purpose of this study is to investigate the effects of the state of the immune system at birth on the response to immunization; (b) such responses should be discernible within one year; and (c) the duration of the granting period is only two years. The research team has significant expertise in working with children and in performing pediatric research studies. Dr. Shannon Thyne is a board-certified pediatrician who has been the Medical Director of the SFGH Children's Health Center since 2001 and who has been involved in many research studies emanating from this clinic. Dr. Burt, who will be the primary link with Dr. McCune's lab, is a board-certified pediatrician with sub-specialty training in neonatal/perinatal medicine. He has extensive clinical expertise in the care of newborns and infants, and has also been involved in recruiting subjects into clinical studies in the past. Dr. McCune, the PI, has over 28 years of experience in clinical investigation, with a focus throughout this time on issues related to the fetal/neonatal immune system.

Phlebotomy will be carried out in an environment tailored to, and with methods appropriate for, the child's developmental stage. A topical anesthetic will be made available for use prior to venipuncture if desired by the subjects' parents. Venipuncture will be performed by a phlebotomist, physician, or nurse who is experienced in drawing blood from small children to minimize risk of discomfort and to ensure provision of appropriate distraction techniques for developmental stage.

BIBLIOGRAPHY & REFERENCES CITED

1. Herzenberg, L.A., and L.A. Herzenberg. 1989. Toward a layered immune system. *Cell* 59:953-954.
2. Coltey, M., R.P. Bucy, C.H. Chen, J. Cihak, U. Losch, D. Char, N.M. Le Douarin, and M.D. Cooper. 1989. Analysis of the first two waves of thymus homing stem cells and their T cell progeny in chick-quail chimeras. *J Exp Med* 170:543-557.
3. Ikuta, K., T. Kina, I. MacNeil, N. Uchida, B. Peault, Y.H. Chien, and I.L. Weissman. 1990. A developmental switch in thymic lymphocyte maturation potential occurs at the level of hematopoietic stem cells. *Cell* 62:863-874.
4. Michaelsson, J., J.E. Mold, J.M. McCune, and D.F. Nixon. 2006. Regulation of T cell responses in the developing human fetus. *J Immunol* 176:5741-5748.
5. Mold, J., J. Michaelsson, T.D. Burt, M.O. Muench, K.P. Beckerman, M. Busch, T.H. Lee, D.F. Nixon, and J.M. McCune. 2008. Maternal alloantigens promote the development of tolerogenic fetal regulatory T cells in utero *Science* 322:1562-1565.
6. Mold, J., S. Venkatasubrahmanyam, T. Burt, J. Rivera, S.A. Galkina, K. Weinberg, A.J. Butte, C.S. Stoddart, and J.M. McCune. 2010. Identification of a unique T cell lineage derived from fetal hematopoietic stem cells. *in preparation*
7. Phares, C.R., R. Lynfield, M.M. Farley, J. Mohle-Boetani, L.H. Harrison, S. Petit, A.S. Craig, W. Schaffner, S.M. Zansky, K. Gershman, K.R. Stefonek, B.A. Albanese, E.R. Zell, A. Schuchat, and S.J. Schrag. 2008. Epidemiology of invasive group B streptococcal disease in the United States, 1999-2005. *JAMA* 299:2056-2065.
8. Koenig, J.M., and W.J. Keenan. 2009. Group B streptococcus and early-onset sepsis in the era of maternal prophylaxis. *Pediatr Clin North Am* 56:689-708, Table of Contents.
9. Khadilkar, V., D. Tudehope, and S. Fraser. 1995. A prospective study of nosocomial infection in a neonatal intensive care unit. *J Paediatr Child Health* 31:387-391.
10. Siegrist, C.A. 2000. Vaccination in the neonatal period and early infancy. *Int Rev Immunol* 19:195-219.
11. Siegrist, C.A. 2001. Neonatal and early life vaccinology. *Vaccine* 19:3331-3346.
12. Garcia, A.M., S.A. Fadel, S. Cao, and M. Sarzotti. 2000. T cell immunity in neonates. *Immunol Res* 22:177-190.
13. Adkins, B. 2000. Development of neonatal Th1/Th2 function. *Int Rev Immunol* 19:157-171.
14. Adkins, B. 2005. Neonatal T cell function. *J Pediatr Gastroenterol Nutr* 40 Suppl 1:S5-7.
15. Zaghouani, H., C.M. Hoeman, and B. Adkins. 2009. Neonatal immunity: faulty T-helpers and the shortcomings of dendritic cells. *Trends Immunol* 30:585-591.
16. Ridge, J.P., E.J. Fuchs, and P. Matzinger. 1996. Neonatal tolerance revisited: turning on newborn T cells with dendritic cells. *Science* 271:1723-1726.
17. Goriely, S., B. Vincart, P. Stordeur, J. Vekemans, F. Willems, M. Goldman, and D. De Wit. 2001. Deficient IL-12(p35) gene expression by dendritic cells derived from neonatal monocytes. *J Immunol* 166:2141-2146.
18. Goriely, S., C. Van Lint, R. Dadkhah, M. Libin, D. De Wit, D. Demonte, F. Willems, and M. Goldman. 2004. A defect in nucleosome remodeling prevents IL-12(p35) gene transcription in neonatal dendritic cells. *J Exp Med* 199:1011-1016.
19. White, G.P., E.M. Hollams, S.T. Yerkovich, A. Bosco, B.J. Holt, M.R. Bassami, M. Kusel, P.D. Sly, and P.G. Holt. 2006. CpG methylation patterns in the IFN γ promoter in naive T cells: variations during Th1 and Th2 differentiation and between atopics and non-atopics. *Pediatr Allergy Immunol* 17:557-564.
20. Thornton, C.A., J.W. Upham, M.E. Wikstrom, B.J. Holt, G.P. White, M.J. Sharp, P.D. Sly, and P.G. Holt. 2004. Functional maturation of CD4⁺CD25⁺CTLA4⁺CD45RA⁺ T regulatory cells in human neonatal T cell responses to environmental antigens/allergens. *J Immunol* 173:3084-3092.
21. Dannemann, A., R. van Ree, M. Kulig, R.L. Bergmann, P. Bauer, J. Forster, I. Guggenmoos-Holzmann, R.C. Aalberse, and U. Wahn. 1996. Specific IgE and IgG4 immune responses to tetanus and diphtheria toxoid in atopic and nonatopic children during the first two years of life. *Int Arch Allergy Immunol* 111:262-267.
22. Rowe, J., C. Macaubas, T.M. Monger, B.J. Holt, J. Harvey, J.T. Poolman, P.D. Sly, and P.G. Holt. 2000. Antigen-specific responses to diphtheria-tetanus-acellular pertussis vaccine in human infants are initially Th2 polarized. *Infect Immun* 68:3873-3877.
23. Ota, M.O., J. Vekemans, S.E. Schlegel-Haueter, K. Fielding, H. Whittle, P.H. Lambert, K.P. McAdam, C.A. Siegrist, and A. Marchant. 2004. Hepatitis B immunisation induces higher antibody and memory Th2 responses in new-borns than in adults. *Vaccine* 22:511-519.
24. Vekemans, J., M.O. Ota, E.C. Wang, M. Kidd, L.K. Borysiewicz, H. Whittle, K.P. McAdam, G. Morgan, and A. Marchant. 2002. T cell responses to vaccines in infants: defective IFN γ production after oral polio vaccination. *Clin Exp Immunol* 127:495-498.

25. Belloni, C., P. Orsolini, M. Martinetti, G. Chirico, R.M. Cerbo, G. Comolli, U. Maccarini, C. Barlassina, C. Togni, F. Polatti, and et al. 1993. Control of hepatitis B: evaluation of two different vaccinal schedules in newborns from HBsAg negative mothers. *New Microbiol* 16:237-244.
26. Rowe, J., C. Macaubas, T. Monger, B.J. Holt, J. Harvey, J.T. Poolman, R. Loh, P.D. Sly, and P.G. Holt. 2001. Heterogeneity in diphtheria-tetanus-acellular pertussis vaccine-specific cellular immunity during infancy: relationship to variations in the kinetics of postnatal maturation of systemic th1 function. *J Infect Dis* 184:80-88.
27. Asano, M., M. Toda, N. Sakaguchi, and S. Sakaguchi. 1996. Autoimmune disease as a consequence of developmental abnormality of a T cell subpopulation. *J Exp Med* 184:387-396.
28. Silverstein, A.M. 1964. Ontogeny of the Immune Response. *Science* 144:1423-1428.
29. Aluvihare, V.R., M. Kallikourdis, and A.G. Betz. 2004. Regulatory T cells mediate maternal tolerance to the fetus. *Nat Immunol* 5:266-271.
30. Takahata, Y., A. Nomura, H. Takada, S. Ohga, K. Furuno, S. Hikino, H. Nakayama, S. Sakaguchi, and T. Hara. 2004. CD25+CD4+ T cells in human cord blood: an immunoregulatory subset with naive phenotype and specific expression of forkhead box p3 (Foxp3) gene. *Exp Hematol* 32:622-629.
31. McCune, J.M., R. Namikawa, H. Kaneshima, L.D. Shultz, M. Lieberman, and I.L. Weissman. 1988. The SCID-hu mouse: murine model for the analysis of human hematolymphoid differentiation and function. *Science* 241:1632-1639.
32. Namikawa, R., K.N. Weilbaecher, H. Kaneshima, E.J. Yee, and J.M. McCune. 1990. Long-term human hematopoiesis in the SCID-hu mouse. *J Exp Med* 172:1055-1063.
33. Pennington, D.J., B. Silva-Santos, T. Silberzahn, M. Escorcio-Correia, M.J. Woodward, S.J. Roberts, A.L. Smith, P.J. Dyson, and A.C. Hayday. 2006. Early events in the thymus affect the balance of effector and regulatory T cells. *Nature* 444:1073-1077.
34. Marchant, A., V. Appay, M. Van Der Sande, N. Dulphy, C. Liesnard, M. Kidd, S. Kaye, O. Ojuola, G.M. Gillespie, A.L. Vargas Cuero, V. Cerundolo, M. Callan, K.P. McAdam, S.L. Rowland-Jones, C. Donner, A.J. McMichael, and H. Whittle. 2003. Mature CD8(+) T lymphocyte response to viral infection during fetal life. *J Clin Invest* 111:1747-1755.
35. Brustoski, K., M. Kramer, U. Moller, P.G. Kremsner, and A.J. Luty. 2005. Neonatal and maternal immunological responses to conserved epitopes within the DBL-gamma3 chondroitin sulfate A-binding domain of Plasmodium falciparum erythrocyte membrane protein 1. *Infect Immun* 73:7988-7995.
36. Lindblad, E.B. 2004. Aluminium compounds for use in vaccines. *Immunol Cell Biol* 82:497-505.
37. Sigurs, N., G. Hattevig, B. Kjellman, N.I. Kjellman, L. Nilsson, and B. Bjorksten. 1994. Appearance of atopic disease in relation to serum IgE antibodies in children followed up from birth for 4 to 15 years. *J Allergy Clin Immunol* 94:757-763.
38. Belloni, C., G. Chirico, A. Pistorio, P. Orsolini, C. Tinelli, and G. Rondini. 1998. Immunogenicity of hepatitis B vaccine in term and preterm infants. *Acta Paediatr* 87:336-338.
39. Andre, F.E., and A.J. Zuckerman. 1994. Review: protective efficacy of hepatitis B vaccines in neonates. *J Med Virol* 44:144-151.
40. Ota, M.O., J. Vekemans, S.E. Schlegel-Haueter, K. Fielding, M. Sanneh, M. Kidd, M.J. Newport, P. Aaby, H. Whittle, P.H. Lambert, K.P. McAdam, C.A. Siegrist, and A. Marchant. 2002. Influence of Mycobacterium bovis bacillus Calmette-Guerin on antibody and cytokine responses to human neonatal vaccination. *J Immunol* 168:919-925.
41. Hartigan-O'Connor, D.J., C. Poon, E. Sinclair, and J.M. McCune. 2007. Human CD4+ regulatory T cells express lower levels of the IL-7 receptor alpha chain (CD127), allowing consistent identification and sorting of live cells. *J Immunol Methods* 319:41-52.
42. Hartigan-O'Connor, D.J., K. Abel, and J.M. McCune. 2007. Suppression of SIV-specific CD4+ T cells by infant but not adult macaque regulatory T cells: implications for SIV disease progression. *J Exp Med* 204:2679-2692.

UNIVERSITY OF CALIFORNIA, SAN FRANCISCO

BERKELEY • DAVIS • IRVINE • LOS ANGELES • RIVERSIDE • SAN DIEGO • SAN FRANCISCO



SANTA BARBARA • SANTA CRUZ

Shannon Thyne, MD
San Francisco General Hospital, Department of Pediatrics
1001 Potrero Avenue, Mail Stop 6E
SAN FRANCISCO, CALIFORNIA 94110



May 31, 2010

Joseph M. McCune, MD, PhD
Division of Experimental Medicine
San Francisco General Hospital
University of California, San Francisco
1001 Potrero Ave, Building 3, Rm. 601
San Francisco, CA 94110

Dear Mike,

I am writing to confirm that I would be delighted to collaborate with you on the research you are proposing in your R21 application, entitled "Human immune system layering and the neonatal response to vaccines." Specifically, as Medical Director of Children's Health Center at San Francisco General Hospital, I will assist with collection of samples from patients who have been previously recruited into your vaccine response study.

As you know, our clinic follows approximately 80% of the children born at SFGH. Given our successes and experience with similar follow-up studies in the past, I am confident that we will be able to work together to insure excellent study retention and follow-up rates. I will facilitate the process of identifying and collecting samples from these patients when they come for their routine follow up visits. I will also educate clinic staff about these protocols and monitor compliance with protocol details. These activities will all be carried out in accordance with our CHR-approved protocols.

I look forward to working with you on this exciting and innovative project.

Sincerely,

A handwritten signature in purple ink that reads "Shannon Thyne".

Shannon Thyne, MD
Associate Professor of Clinical Pediatrics
Medical Director, Children's Health Center
San Francisco General Hospital
University of California, San Francisco

University of California
San Francisco



Epidemiology & Biostatistics
Division of Biostatistics

185Berry Street, Lobby 5
Suite 5700
San Francisco, CA 94107-1762

May 28, 2010

J. M. McCune, MD, PhD
UCSF Box 1234
Bldg, 3, Rm. 601
Division of Experimental Medicine
San Francisco General Hospital
1001 Potrero Ave.
San Francisco, CA 94110

Dear Mike,

I am writing to say that my colleagues and I of the Biostatistics Unit of the UCSF Clinical and Translational Science Institute would be very happy to provide the statistical guidance that you need for your R21 proposal ("Human immune system layering and the neonatal response to vaccines"). We can advise you about experimental design, proper interpretation of resulting data, and the design of further studies, as necessary.

It has been a pleasure to work with you in the past. Given the design of the exploratory experiments that you wish to perform as well as the relatively large number of samples that you will be examining, I am sure that your studies will provide much useful information about the issues you wish to address.

Please let me know when and how I can help with the proposal or with the design or analysis of your studies.

Best wishes,

A handwritten signature in black ink that reads "Peter Bacchetti".

Peter Bacchetti, Ph.D.
Professor of Biostatistics



Clinical and Translational Science Institute

at the University of California, San Francisco

Bringing better health to more people more quickly!

ctsi.ucsf.edu

June 1, 2010

Joseph M. McCune, MD, PhD
Division of Experimental Medicine
San Francisco General Hospital
University of California, San Francisco
1001 Potrero Ave, Building 3, Rm. 601
San Francisco, CA 94110

Dear Mike:

As Program Director of the UCSF Clinical and Translational Science Institute-Clinical Research Services (CTSI-CRS), I am delighted to offer the support of the CTSI-CRS to you and your colleagues to pursue research proposed in your R21 application, "Human immune system layering and the neonatal response to vaccines".

The primary mission of the CTSI-CRS is to provide first-rate infrastructure and services for the clinical and translational research programs of UCSF investigators. The CRS targets investigators with research funding from peer-reviewed sources such as the National Institutes of Health (NIH) and investigators in the early stages of their career development, and is supported in part by an NIH Clinical and Translational Science Award (CTSA) grant (UL1RR024131) from the National Center for Research Resources.

For your study needs, CTSI-CRS nurses collect cord blood samples for the CTSI-CRS Human Cord Blood Bank that allows UCSF investigators to obtain full-term, de-identified human cord blood specimens for their research. Under the auspices of a protocol approved by the UCSF Committee on Human Research, we will enroll with informed consent, women who are undergoing an elective C-section, and then obtain the clamped cord and placenta so that fresh human cord blood (typically 25- 80 mls) can be withdrawn. We will provide an expedient process by which whole cord blood, anti-coagulated to your specifications, can be delivered to you for further processing.

Per our approved protocol, there will be no patient identifiers sent with the specimen, although we will be able to give you information about the gender and ethnicity of the newborn. All of this will take place with a fee-for-service recharge mechanism, which we anticipate to be approximately \$55 per specimen. We anticipate that at least one cord blood sample per week, and potentially more, will be available for your project.



Clinical and Translational Science Institute

at the University of California, San Francisco

Bringing better health to more people more quickly!

ctsi.ucsf.edu

I am pleased to be able to offer this service to you – and hope that it will enable you to answer the important questions that you are addressing.

Yours sincerely,

C. William Balke, MD
Program Director
CTSI-Clinical Research Services



Elizabeth J. Shpall, M.D.
Ashbel Smith Professor of Medicine
Medical Director, Cell Therapy Laboratory
Director, Cord Blood Therapy

Department of Stem Cell Transplantation and Cellular Therapy
Unit 423
1515 Holcombe Boulevard
Houston, Texas 77030

June 2, 2010

Joseph M. McCune, M.D., Ph.D.
Division of Experimental Medicine
San Francisco General Hospital
University of California, San Francisco
1001 Potrero Ave, Building 3, Rm. 601
San Francisco, CA 94110

Dear Mike,

I am writing to say that my colleagues and I would be happy to collaborate with you on your analysis of "layering" during the development of the human immune system. For the purposes of the work in Aim 1 on your R21 application ("Human immune system layering and the neonatal response to vaccines"), we should be able to send you up to more than 100 cryopreserved cord blood specimens during the course of the first 18 months of this project. These would be transferred to you under the auspices of our existing MTA (MT2009-5827) and corresponding laboratory research protocols. Each is likely to have a volume of 25 mls and we routinely find that the viability of the cells upon thawing is > 85%.

Given our interests in the use of such cord blood for the purposes of transplantation, the information that you gather about the normative ranges of layering at birth will be quite helpful.

I wish you the best of luck with your grant submission. Please let me know when you are ready to begin these experiments.

Sincerely,

A handwritten signature in black ink that reads "Elizabeth J. Shpall MD".

Elizabeth Shpall, M.D.

RESOURCE SHARING PLAN(S)

The University of California, San Francisco, is committed to the open and timely dissemination of research outcomes. Investigators in the proposed activity recognize that promising new methods, technologies, and immunological insights may arise during the course of the research. The Investigators are aware of and agreed to abide by the principles for sharing research resources as described by NIH in "Principles and Guidelines for Recipients of NIH Research Grants and Contracts on Obtaining and Disseminating Biomedical Research Programs." The data generated in this grant will be presented at national or international conferences and published in a timely fashion. All final peer-reviewed manuscripts that arise from this proposal will be submitted to the digital archive PubMed Central.

PHS 398 Checklist

OMB Number: 0925-0001

1. Application Type:

From SF 424 (R&R) Cover Page. The responses provided on the R&R cover page are repeated here for your reference, as you answer the questions that are specific to the PHS398.

* Type of Application:

☒ New ☐ Resubmission ☐ Renewal ☐ Continuation ☐ Revision

Federal Identifier:

2. Change of Investigator / Change of Institution Questions

☐ Change of principal investigator / program director

Name of former principal investigator / program director:

Prefix:

* First Name:

Middle Name:

* Last Name:

Suffix:

☐ Change of Grantee Institution

* Name of former institution:

3. Inventions and Patents (For renewal applications only)

* Inventions and Patents: Yes ☐ No ☐

If the answer is "Yes" then please answer the following:

* Previously Reported: Yes ☐ No ☐

4. * Program Income

Is program income anticipated during the periods for which the grant support is requested?

☐ Yes ☒ No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

*Budget Period *Anticipated Amount (\$)

*Source(s)

<input type="text"/>	<input type="text"/>	<input type="text"/>
<input type="text"/>	<input type="text"/>	<input type="text"/>
<input type="text"/>	<input type="text"/>	<input type="text"/>
<input type="text"/>	<input type="text"/>	<input type="text"/>
<input type="text"/>	<input type="text"/>	<input type="text"/>

5. * Disclosure Permission Statement

If this application does not result in an award, is the Government permitted to disclose the title of your proposed project, and the name, address, telephone number and e-mail address of the official signing for the applicant organization, to organizations that may be interested in contacting you for further information (e.g., possible collaborations, investment)?

☒ Yes ☐ No