SUMMARY STATEMENT

PROGRAM CONTACT: **(Privileged Communication)** ***Release Date:*** **03/04/2011**
Mercy Prabhudas
[redacted]

Application Number: **1 R21 AI095831-01**

Principal Investigator

PETRIE, HOWARD T. PHD

Applicant Organization: SCRIPPS FLORIDA

Review Group: **CMIB**
Cellular and Molecular Immunology - B Study Section

Meeting Date: **02/03/2011**
Council: **MAY 2011**
Requested Start: **07/01/2011**

RFA/PA: **PA10-069**
PCC: **I2H**

Dual IC(s): **HD, HL**

Project Title: **Lymphoid signals for stromal growth and organization in the thymus.**

SRG Action: **Impact/Priority Score: 10**
Human Subjects: 10-No human subjects involved
Animal Subjects: 30-Vertebrate animals involved - no SRG concerns noted

Project Year	Direct Costs Requested	[redacted]ated Cost
1		
2		
TOTAL	[redacted]	[redacted]

ADMINISTRATIVE BUDGET NOTE: The budget shown is the requested budget and has not been adjusted to reflect any recommendations made by reviewers. If an award is planned, the costs will be calculated by Institute grants management staff based on the recommendations outlined below in the COMMITTEE BUDGET RECOMMENDATIONS section.

1R21AI095831-01 PETRIE, HOWARD

RESUME AND SUMMARY OF DISCUSSION: This is a new Exploratory/Development application from a very creative and outstanding leader who has made seminal contributions to the field of T cell biology. The application proposes to study lymphoid signals that stimulate stromal growth and organization of the thymus. The intercommunication between thymocytes and thymic epithelial cells has been a long difficult journey although this investigator is well poised to accomplish significant progress. The strengths of this application are the outstanding investigator, highly innovative and well written experimental plan, state-of-the-art techniques (i.e. microarray-based gene profiling), and the very high significance of more fully understanding the correlation between the thymocytes and the other cells in the thymus. While this is an ambitious application which is not without risk, this is certainly well within this investigator's capabilities. Thus the overall impact is extremely high and likely to significantly advance the field.

DESCRIPTION (provided by applicant): Like all blood cells, T lymphocytes are constantly lost during life, and must be continuously replaced. The thymus is the primary site of de novo T lymphopoiesis. Microenvironmental conditions unique to the thymus induce a complex series of developmental events in multipotent, marrow-derived progenitors, including positive and negative control of proliferation, T lineage specification, functional T lineage asymmetry, self-restriction, self-tolerance, and cell death/survival signals. Significant progress has been made in understanding the signals the thymus provides to lymphoid progenitors to induce and control these events. However, the proliferation, differentiation, and/or survival of the non-lymphoid (stromal) components of the thymus are equally dependent on lymphoid cells, and the absence of lymphoid cells (e.g., congenital immunodeficiency diseases) results in athymia, even though the nascent stromal cells are functionally competent. The signals that lymphoid cells provide to induce stromal growth, differentiation, and/or survival are completely unknown. In this proposal, we propose to address this process in a comprehensive fashion. Using our recently devised method for global characterization of stromal gene expression in situ, we will analyze the dynamic response of stromal cells to the presence of lymphoid progenitors in an inducible model of thymic growth. We will then identify stromally-expressed genes that encode receptors, and thus may respond to the presence of lymphoid cells, with particular emphasis on those that change during specific phases of the growth response (induction, log phase, termination, steady-state). We will verify the presence of the cognate ligands for these receptors in lymphoid cells, using informatic methods as well as manual means of curation. Cognate receptor:ligand pairs will then be prioritized using a variety of criteria, including dynamic patterns of expression, known functional relevance (growth, differentiation, survival) in other tissues, the availability of existing genetic models, etc. Finally, the biological relevance of a few high-priority candidates will be tested, using appropriate stromal-receptor or lymphoid-ligand mutant mouse models. This project is expected to provide novel insights into an unexplored area of biology that will not only fuel a better understanding of this process, but will facilitate the development of more mechanistic studies to characterize the unique, two-way interactions that occur between lymphoid progenitors and stromal cells in the thymus.

PUBLIC HEALTH RELEVANCE: The thymus is the primary organ where T lymphocytes are made, and thus is critical for normal immune function. Specialized cells inside the thymus (stromal cells) are responsible for instructing stem-like cells recruited from the bone marrow to undergo development into T lymphocytes. However, early in development, these stromal cells themselves require signals from immature lymphocytes to induce their own growth and development. In the absence of lymphocytes, for instance, as occurs in many pediatric immunodeficiency disorders, the thymus does not form. The goals of this project are to perform an in depth analysis of this very poorly understood two-way interplay between lymphocytes and stromal cells in the thymus, and how these processes shape the development of the immune system.

CRITIQUE 1:

Significance: 1
Investigator(s): 1
Innovation: 1
Approach: 2
Environment: 3

Overall Impact: This is a very strong proposal from a talented and experienced investigator who has provided key conceptual insights underlying the generally accepted paradigm that the thymus is a series of linked environments, and for the ordered sequence of migration events undertaken by T lymphocyte progenitors. The PI proposes to characterize the response of the IL7R deficient thymic stromal compartment to IL7R+ hematopoietic progenitor cells, using an elegant and exhaustive approach detailed in a recent publication in Immunity. In response to IL7R+ cells, it is known the thymus grows, with large numbers of proliferating stromal cells responding to the influx of competent progenitors. However, the signals delivered by progenitors to stromal cells are not well understood. PI proposes to isolate thymic stromal cells at different times after reconstitution by laser capture, subject them to microarray-based gene profiling, and subtract the signature simultaneously obtained of the lymphoid components of the thymus. The question is outstanding; the approach is state of the art and very likely to provide important insights.

1. Significance:

Strengths

- Highly significant general question of how the thymic stromal compartment senses and responds to competent T cell progenitors.
- Knowledge important for studies of thymic organogenesis, homeostasis, involution, and T cell positive and negative selection.

Weaknesses

2. Investigator(s):

Strengths

- Long history of interest in thymic development
- Great expertise has consistently pushed this field forward.
- Recent accomplishments include a key paper in Immunity that is expected to energize the field of thymic development, function, and thymic aging.

3. Innovation:

Strengths

- The innovation of this proposal is the focus on signaling into thymic stromal cells during thymic growth, together with the recent development of key technical breakthroughs that make this study possible.

4. Approach:

Strengths

- Builds on published work recently appearing in Immunity, in which gene expression of specific regions of the thymus are determined by first isolating these areas by laser capture, and microarraying them, and revealing the stroma-specific expression by subtracting out the lymphoid signature. The PI proposes to extend this to describe gene expression in cortical and

medullary IL7R-/- stromal cells when responding to an influx of competent progenitors. The stromal cells must sense and respond to IL7R+ progenitors because they increase cell division. In mice with profound blocks in T cell development, medullary epithelial compartments are almost entirely absent, however, some of the signals here are already know, as RANK: RANKL interactions between mature T cells and medullary TEC are known to drive expansion of mTEC. However, signals driving cortical growth are largely unknown.

Weaknesses

- The chief potential weakness of this proposal is that it is unclear whether the key signaling pairs that are important for signaling from lymphoid cells into stromal cells will be uncovered. Indeed, given that the IL7R-/- thymus can respond to incoming progenitors, the essential signaling molecules must be already expressed by IL7R-/- stromal cells. For these specific molecules to be captured by this exploratory approach, their expression will need to be further upregulated during thymic reconstitution, or unique signals downstream of these molecules that are upregulated will need to identify that allow the upstream triggers to be deduced. However, this is an acceptable level of risk for this potentially high reward R21 proposal.

5. Environment:

Strengths

- Adequate for these studies.

Weaknesses

Protections for Human Subjects: Not Applicable (No Human Subjects)

Vertebrate Animals: Acceptable

- Adequate justification provided was included.

Biohazards: Acceptable

Resource Sharing Plans: Acceptable

Budget and Period of Support: Recommend as Requested

CRITIQUE 2:

Significance: 1
Investigator(s): 1
Innovation: 2
Approach: 1
Environment: 3

Overall Impact: This is a very strong application from a first rate investigator who proposes to characterize changes in gene expression in thymic stromal cells in a model of thymic organ growth, then, using sophisticated computer algorithms, to define possible important receptor-ligand pairs that could control thymic stroma-developing thymocytes cross-talk, and test a few of these resulting signaling axes in vivo. The investigator has previous experience with the biological and informatics

approaches, so feasibility is high. Given how little we know about thymic stroma, the results are very likely to have a big impact in the field. The investigator is uniquely qualified to perform this project.

1. Significance:

Strengths

- The cross-talk between developing thymocytes and the stroma that supports them is known to be critical for development of the immune system, but its molecular basis are not well-defined. If successful this application would dramatically expand our knowledge in this area.

Weaknesses

2. Investigator(s):

Strengths

- Leader in the field.
- Experience with these kind of approaches
- Longstanding bioinformatics collaborator

Weaknesses

3. Innovation:

Strengths

- We know very little about how developing lymphoid cells influence stromal cell growth, differentiation and survival. The area of research is therefore innovative.
- The research approach is relatively novel, although it does not break new ground in respect to the investigator's previous publication.

Weaknesses

4. Approach:

Strengths

- Good experimental model.
- Well designed strategy for the analysis and validation of the profiling results.

Weaknesses

5. Environment:

Strengths

- Strong bioinformatics community.

Weaknesses

- Small immunology community.
- Little institutional support

Vertebrate Animals: Acceptable

- There are no concerns .

Resource Sharing Plans:

- For an application likely to generate large amount of data of interest for the general community the resource sharing plan is too vague. A plan to make a database of the results accesible to other investigator would strenghthen this aspect of the application.

Budget and Period of Support: Recommended budget

- Appropriate

CRITIQUE 3:

Significance: 1
Investigator(s): 1
Innovation: 1
Approach: 2
Environment: 1

Overall Impact: This R21 application from an experienced senior investigator seeks to identify the signals produced by developing T cells that induce the maturation of stromal elements in the thymus. These studies are highly significant as essentially nothing is known about how developing T cells signal stroma. Understanding how lymphoid cells induce thymic stromal responses has been a subject of contemplation for many laboratories for many years. However, given the difficulties associated with thymic stromal cell isolation, and the further limitation imposed by the small number of cells present in the immunodeficient thymus, it has been a very difficult issue to address. This application has established a novel, comprehensive, bioinformatics approach that will enable the identification of receptor-ligand pairs expressed by thymocytes and stroma without disrupting thymic structure, which has been shown to alter the expression of Notch ligands expressed by stroma. Therefore this proposal is innovative – both regarding focus and methods. The only caveat is that testing the function of stromal molecules can only be done by making conditional knockout mice which is costly and time-consuming. Nevertheless, this approach fits perfectly with the high-risk, high-reward mandate of the R21 mechanism.

THE FOLLOWING RESUME SECTIONS WERE PREPARED BY THE SCIENTIFIC REVIEW OFFICER TO SUMMARIZE THE OUTCOME OF DISCUSSIONS OF THE REVIEW COMMITTEE ON THE FOLLOWING ISSUES:

VERTEBRATE ANIMAL (Resume): ACCEPTABLE

MODEL ORGANISM SHARING PLAN: New model organisms will be generated during the project period and the application includes an adequate model organism sharing plan.

COMMITTEE BUDGET RECOMMENDATIONS: The budget was recommended for time and amount as requested.

10-080.html.
The impact/priority score is calculated after discussion of an application by averaging the overall scores (1-9) given by all voting reviewers on the committee and multiplying by 10. The criterion scores are submitted prior to the meeting by the individual reviewers assigned to an application, and are not discussed specifically at the review meeting or calculated into the overall impact score. For details on the review process, see http://grants.nih.gov/grants/peer_review_process.htm#scoring.

MEETING ROSTER

Cellular and Molecular Immunology - B Study Section
Immunology Integrated Review Group
CENTER FOR SCIENTIFIC REVIEW
CMIB
February 03, 2011 - February 04, 2011

CHAIRPERSON

PILLAI, SHIV SUBRAMANIAM, MBBS, PHD
ASSOCIATE PROFESSOR
DEPARTMENT OF MEDICINE
MASSACHUSETTS GENERAL HOSPITAL CANCER CENTER
HARVARD MEDICAL SCHOOL
BOSTON, MA 02129

MEMBERS

ALBEROLA-ILA, JOSE , MD, PHD *
ASSOCIATE PROFESSOR
IMMUNOBIOLOGY AND CANCER RESEARCH PROGRAM
OKLAHOMA MEDICAL RESEARCH FOUNDATION
OKLAHOMA CITY, OK 73104

BHANDOOLA, AVINASH , PHD
ASSOCIATE PROFESSOR
DEPARTMENT OF PATHOLOGY AND LABORATORY MEDICINE
UNIVERSITY OF PENNSYLVANIA SCHOOL OF MEDICINE
PHILADELPHIA, PA 19104

COLLINS, EDWARD JOHN, PHD
ASSOCIATE PROFESSOR
DEPARTMENT OF MICROBIOLOGY AND IMMUNOLOGY
UNIVERSITY OF NORTH CAROLINA
CHAPEL HILL, NC 27599

COVEY, LORI R, PHD
PROFESSOR
DEPARTMENT OF CELL BIOLOGY
AND NEUROSCIENCE
RUTGERS UNIVERSITY
PISCATAWAY, NJ 08854

DENT, ALEXANDER L, PHD *
ASSOCIATE PROFESSOR
DEPARTMENT OF MICROBIOLOGY AND IMMUNOLOGY
WALTHER ONCOLOGY CENTER
INDIANA UNIVERSITY SCHOOL OF MEDICINE
INDIANAPOLIS, IN 46202

FOWELL, DEBORAH J, PHD *
ASSOCIATE PROFESSOR
DEPARTMENT OF MICROBIOLOGY AND IMMUNOLOGY
SCHOOL OF MEDICINE AND DENTISTRY
UNIVERSITY OF ROCHESTER
ROCHESTER, NY 14642

FUGMANN, SEBASTIAN D, PHD *
INVESTIGATOR
BIOMEDICAL RESEARCH CENTER
NATIONAL INSTITUTE OF AGING
NATIONAL INSTITUTES HEALTH
BALTIMORE, MD 21224

GU, HUA , DSC *
IRENE DIAMOND ASSOCIATE PROFESSOR
DIVISION OF HEALTH SCIENCES
DEPARTMENT OF MICROBIOLOGY AND IMMUNOLOGY
COLUMBIA UNIVERSITY
NEW YORK, NY 10032

GUMPERZ, JENNY E, PHD *
ASSOCIATE PROFESSOR
DEPARTMENT OF MEDICAL MICROBIOLOGY AND IMMUNOLOGY
UNIVERSITY OF WISCONSIN MEDICAL SCHOOL
MADISON, WI 53706

HAGMAN, JAMES R, PHD
PROFESSOR
INTEGRATED DEPARTMENT OF IMMUNOLOGY
NATIONAL JEWISH MEDICAL AND RESEARCH CENTER
DENVER, CO 80206

HE, YOU-WEN , MD, PHD
ASSOCIATE PROFESSOR OF IMMUNOLOGY
DEPARTMENT OF IMMUNOLOGY
DUKE UNIVERSITY MEDICAL CENTER
DURHAM, NC 27710

JACOB, JOSHY , PHD
ASSOCIATE PROFESSOR
EMORY VACCINE CENTER
EMORY UNIVERSITY
ATLANTA, GA 30329

JAMESON, STEPHEN C, PHD
PROFESSOR
DEPARTMENT OF LABORATORY MEDICINE
UNIVERSITY OF MINNESOTA MEDICAL SCHOOL
MINNEAPOLIS, MN 55414

JUSTEMENT, LOUIS B, PHD *
PROFESSOR
DEPARTMENT OF MICROBIOLOGY
UNIVERSITY OF ALABAMA AT BIRMINGHAM
BIRMINGHAM, AL 35294

KEEGAN, ACHSAH D, PHD
PROFESSOR
DEPARTMENT OF MICROBIOLOGY AND IMMUNOLOGY
UNIVERSITY OF MARYLAND SCHOOL OF MEDICINE
BALTIMORE, MD 21201

LENTSCH, ALEX B, PHD *
PROFESSOR AND VICE CHAIRMAN FOR RESEARCH
DEPARTMENT OF SURGERY
UNIVERSITY OF CINCINNATI COLLEGE OF MEDICINE
CINCINNATI, OH 452670558

LORENZ, ULRIKE , PHD
ASSOCIATE PROFESSOR
DEPARTMENT OF MICROBIOLOGY
UNIVERSITY OF VIRGINIA HEALTH SYSTEM
CHARLOTTESVILLE, VA 22908

NEMAZEE, DAVID , PHD *
PROFESSOR
DEPARTMENT OF IMMUNOLOGY
THE SCRIPPS RESEARCH INSTITUTE
LA JOLLA, CA 92037

SANTAMBROGIO, LAURA , MD, PHD *
ASSOCIATE PROFESSOR
DEPARTMENT OF PATHOLOGY
ALBERT EINSTEIN COLLEGE OF MEDICINE
BRONX, NY 10461

SCHAEFER, BRIAN , PHD *
ASSOCIATE PROFESSOR
DEPARTMENT OF MICROBIOLOGY AND IMMUNOLOGY
UNIFORMED SERVICES UNIVERSITY
OF HEALTH SCIENCES
BETHESDA, MD 20814

SLECKMAN, BARRY P, MD, PHD
PROFESSOR
DEPARTMENT OF PATHOLOGY AND IMMUNOLOGY
SCHOOL OF MEDICINE
WASHINGTON UNIVERSITY
ST. LOUIS, MO 63110

SUN, ZUOMING , PHD *
ASSOCIATE PROFESSOR
DIVISION OF IMMUNOLOGY
BECKMAN RESEARCH INSTITUTE OF THE
CITY OF HOPE
DURATE, CA 91010

VAN KAER, LUC , PHD *
PROFESSOR
DEPARTMENT OF MICROBIOLOGY
AND IMMUNOLOGY
VANDERBILT UNIVERSITY SCHOOL OF MEDICINE
NASHVILLE, TN 37232

WANG, CHYUNG-RU , PHD *
PROFESSOR
DEPARTMENT OF MICROBIOLOGY AND IMMUNOLOGY
FINEBERG SCHOOL OF MEDICINE
NORTHWESTERN UNIVERSITY
CHICAGO, IL 60611

WIEST, DAVID L, PHD
PROFESSOR
DIVISION OF BASIC SCIENCES
FOX CHASE CANCER CENTER
PHILADELPHIA, PA 19111

ZUNIGA-PFLUCKER, JUAN CARLOS , PHD
PROFESSOR AND CHAIR
DEPARTMENT OF IMMUNOLOGY
SUNNYBROOK RESEARCH INSTITUTE
UNIVERSITY OF TORONTO
TORONTO, ON M4N 3M5
CANADA

SCIENTIFIC REVIEW ADMINISTRATOR

HAYDEN, BETTY , PHD
SCIENTIFIC REVIEW OFFICER
CENTER FOR SCIENTIFIC REVIEW
NATIONAL INSTITUTES OF HEALTH
BETHESDA, MD 20892

GRANTS TECHNICAL ASSISTANT

WASHINGTON, WENDY M, MBA
LEAD GRANTS TECHNICAL ASSISTANT
CENTER FOR SCIENTIFIC REVIEW
NATIONAL INSTITUTES OF HEALTH
BETHESDA, MD 20892

* Temporary Member. For grant applications, temporary members may participate in the entire meeting or may review only selected applications as needed.

Consultants are required to absent themselves from the room during the review of any application if their presence would constitute or appear to constitute a conflict of interest.