PROGRAM CONTACT:

(Privileged Communication)   *Release Date:*   03/04/2011

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   *RFA/PA:* PA10-069
Council: MAY 2011   *PCC:* I2H
Requested Start: 07/01/2011

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

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

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

Project Year	Direct Costs Requested	▇ated Cost
1		
2		
TOTAL	▇	▇

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 reconstition 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.

NIH has modified its policy regarding the receipt of resubmissions (amended applications). See Guide Notice NOT-OD-10-080 at http://grants.nih.gov/grants/guide/notice-files/NOT-OD-

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.