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SUMMARY STATEMENT
(Privileged Communication)

Release Date: 12/10/2013

Application Number: 1 F30 ES024045-01

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Review Group: ZRG1 F07-K (20)
Center for Scientific Review Special Emphasis Panel
Fellowship: Immunology

Meeting Date: 11/13/2013

Council: JAN 2014

PCC: 6W87L69Z

Requested Start:

Dual IC(s): AI, HL

Project Title: RAGE as an upstream activator of the Th2 inflammatory immune response in
asthma

Requested: 3 years

Sponsor: Oury, Tim D

Department: Pathology

Organization: UNIVERSITY OF PITTSBURGH AT PITTSBURGH

City, State: PITTSBURGH PENNSYLVANIA

SRG Action: Impact Score: 30 Percentile: 29

Next Steps: Visit http://grants.nih.gov/grants/next_steps.htm

Human Subjects: 10-No human subjects involved

Animal Subjects: 30-Vertebrate animals involved - no SRG concerns noted

1F30ES024045-01 OCZYPOK, ELIZABETH

RESUME AND SUMMARY OF DISCUSSION: This is an F30 predoctoral fellowship application from an MD/PhD student, who proposes to examine the role of the pro-inflammatory receptor for advanced glycation end products (RAGE) in stimulating allergic responses after allergen exposure. There is general consensus among the reviewers that this is an excellent application that addresses an important issue and will provide a good training vehicle for the applicant to become an independent scientist. Main strengths include the applicant herself who has an outstanding scholastic record and years of research experience that is highlighted by multiple publications. The training potential is high as both the applicant and sponsor are committed to training and have provided a detailed training plan. The institutional environment is outstanding for trainee development. The sponsor is an expert in asthma research and is qualified to train MD/PhD students, while there are mild concerns about whether the sponsor will have sufficient resources to allow the trainee to effectively pursue the proposed studies. Inclusion of a co-sponsor partially mitigates these concerns, although the extent of training and support by the co-sponsor is unclear. The research plan is well-written and supported by preliminary data. There are concerns with the potential problems with the RAGE knockout mice and the role of IL-33 in the proposed model, as well as the limited scope of the research plan. A technical aspect of discussion is the feasibility of the flow cytometry in the genetically altered mouse strain. Overall, in consideration of the strengths and noted minor weaknesses, the review panel has fairly strong enthusiasm for this application.

DESCRIPTION (provided by applicant): Allergens are the most common cause of acute asthma exacerbations, leading to inflammation and constriction of airway smooth muscle cells. Allergens trigger the release of interleukin(IL)-33 from various cell types in the lung. IL-33 stimulates both type 2 innate lymphoid cells (ILC2s) and T-helper 2 (Th2) cells to secrete large amounts of IL-5 and IL-13, which are important mediators of the Th2 inflammatory response. IL-5 and IL-13, along with IL-4, are responsible for the classic pathology associated with asthma: airway hyper-responsiveness, eosinophilic inflammation, and mucus hyper-secretion. Notably, these pathological findings are not present in mice lacking the receptor for advanced glycation end products (RAGE) in the house dust mite (HDM) or ovalbumin (OVA) models of asthma/allergic airway inflammation(AAI). These data suggest that RAGE, a pro-inflammatory, multi-ligand, immunoglobulin-like receptor that is highly expressed in the lung, is necessary for the development of an allergen-induced asthmatic Th2 response. Both RAGE knockout (KO) and wild-type (WT) mice demonstrate normal increases in IL-4, immunoglobulin(Ig)-G, and IgE in response to HDM or OVA, however, only WT mice demonstrate increases in cytokines IL-5 and IL-13. The inability to mount an IL-5 and IL-13 response after allergen exposure in the absence of RAGE could be due to defects in the adaptive immune system (Th2 cells), the innate immune system (ILC2s), or both. Furthermore, these deficits in Th2 cytokine production may be secondary to an inability of RAGE KO mice to increase levels of IL-33, which was also abnormally not increased in RAGE KO mice as compared to WT mice treated with HDM. This investigation seeks to study the upstream signaling events that control the induction of the Th2 response following allergen exposure. First, flow cytometry will be used to determine if absence of RAGE signaling impairs cellular recruitment and/or cytokine production in Th2 cells, ILC2s, or both. Next, cell culture studies will determine if RAGE signaling increases IL-5 and IL-13 production indirectly by promoting IL-33 secretion and/or through direct RAGE signaling on Th2 cells, ILC2s, or both. Understanding the mechanisms by which loss of RAGE signaling prevents asthma pathogenesis may lead to the discovery of new therapeutic targets for the treatment of asthma or the prevention of allergen-induced exacerbations of the disease.

PUBLIC HEALTH RELEVANCE: Asthma is one of the leading causes of morbidity worldwide, and exacerbations of the disease, leading to bronchoconstriction and difficulty breathing, are commonly triggered by environmental particulates such as allergens. This project aims to understand the mechanism by which the receptor for advanced glycation end products (RAGE) promotes inflammation and airway hyper-responsiveness in allergen-induced asthma. Understanding this molecular process

may lead to novel therapeutics that (1) prevent asthma exacerbations or disease development, or (2) help to treat patients whose asthma does not respond to current bronchodilator or corticosteroid therapies.

CRITIQUE 1:

Fellowship Applicant: 1

Sponsors, Collaborators, and Consultants: 2

Research Training Plan: 4

Training Potential: 2

Institutional Environment & Commitment to Training: 1

Overall Impact/Merit: This is an excellent to outstanding candidate for an F30 award. The mentor and institution are also outstanding. The mentor has experience in training MSTP students. The only fly in the ointment is the funding for the mentor; however, there is a letter of financial commitment from the Department Chair. The candidate has a well written proposal that is feasible and the topic (allergy) is clinically important. There are some issues with the research plan including possible problems with the RAGE KO mice and the role of IL-33 in the proposed model. Overall, however, training potential is excellent and this individual should do well in the lab setting.

1. Fellowship Applicant:

Strengths

- Research experience with 2 first author papers. There are also a number of meeting abstracts.
- Excellent academic record.
- Reference letters are strong. There was one anomaly where the candidate was given all 5's (lower 40%) but the respondent spoke highly of her. It is suspected (but not known for sure) that this person reversed the scale and meant to give all 1's.

2. Sponsors, Collaborators, and Consultants:

Strengths

- Sponsor (Dr. Oury) is qualified in this area.
- Dr. Oury has experience training MSTP students.
- The co-Mentor, Dr. Anuradha has funding and is well qualified in this research area.

Weaknesses

- The mentor is between funding according to an attached letter, but a financial commitment from the Department Chair is included.

3. Research Training Plan:

Strengths

- Training plan is well described.
- Asthma is an important medical issue.
- Well written proposal and many alternatives and pitfalls have been considered.

Weaknesses

- Although the source of the RAGE KO mice is not listed, a paper from this group used a strain generated by Dr. A. Bierhaus. The way this strain was generated, it has EGFP inserted in (or near) the RAGE locus such that many cells from the KO in the spleen and elsewhere express EGFP making flow cytometric analysis almost impossible. This may be the reason for the odd looking FACS plots in Figure 4B. However, the candidate should examine this independently (e.g. FACS without antibody staining). If this is the case flow cytometry studies will have to be re-evaluated.
- The use of HMGB1 to stimulate cells can be tricky as many preps are contaminated with LPS.
- Although the hypothesis is that RAGE stimulation actively induces cytokine production, the candidate should pay more attention to the passive release of IL-33 that can occur from cell damage. Perhaps it's the damage from the allergen that initiates the disease via IL-33.
- The candidate should more directly address the role of IL-33 in the models. There are no experiments to do this. Putting RAGE ligand on isolated cells and measuring IL-13 does not necessarily mean that IL-33 is involved. IL-33 does increase after HMD, but it is not obvious that it is involved in the pathogenesis of asthma in this model.
- The candidate might consider a bone marrow chimera to determine where the relevant RAGE is expressed.
- Studies examining cells after administration of IL-33 are difficult to understand. If IL-33 provokes asthma in WT, but not RAGE KO, it is not obvious how RAGE and IL-33 are connected.

4. Training Potential:

Strengths

- Candidate and mentor are committed to training and this makes the training potential excellent.

Weaknesses

- Somewhat confusing research plan lessens the training potential.

5. Institutional Environment & Commitment to Training:

Strengths

- Outstanding environment.

Vertebrate Animals: Acceptable.

Training in the Responsible Conduct of Research: Acceptable

Comments on Format (Required): None noted

Comments on Subject Matter (Required): None noted

Comments on Faculty Participation (Required): None noted

Comments on Duration (Required): None noted

Comments on Frequency (Required): None noted

Budget and Period of Support: Recommend as Requested.

Additional Comments to Applicant:

- Although the RAGE KO are on the C57BL/6 background as stated by the candidate, it is not noted which C57BL/6 strain B6J or B6N. Control mice will be purchased for Taconic farms, which sell C57BL/6N (NIH) line. B6N and B6J are not congenic. Standardizing the background might be considered as sometime differences between in-house bred and vendor mice are noted that are unrelated to the gene of interest.

CRITIQUE 2:

Fellowship Applicant: 2

Sponsors, Collaborators, and Consultants: 3

Research Training Plan: 4

Training Potential: 2

Institutional Environment & Commitment to Training: 2

Overall Impact/Merit: This proposal is from an outstanding candidate who has excellent to outstanding academic achievement and 2 first-author original papers and 1 review paper. The sponsor is a pioneer of the field. The proposal is relatively well designed, though it is somewhat simple and can be deepened for a 3 year project. Overall, training potential is high.

1. Fellowship Applicant:

Strengths

- Two first author original articles and one first author review article. Various presentation experiences form undergraduate research.
- Outstanding range of academic record

2. Sponsors, Collaborators, and Consultants:

Strengths

- Dr. Tim D. Oury, is an expert in asthma research and identified RAGE, a major topic of this proposal.
- Co-sponsor Dr. Ray is an established investigator and well-funded through 2017.

Weaknesses

- Limited funding (bridge fund till 2015).
- Not clear how co-sponsor and the main sponsors play their roles. (ie Is the co-sponsor's presence merely for funding purposes? The training plan does not specify this point.)

3. Research Training Plan:

Strengths

- The preliminary data on RAGE function in IL5/13 production is profound. (previously published data form the sponsors' laboratory) The significance of the project is high.
- Aim 1 Cytokine production by T cells and IC2 cells after HDM stimulation from WT and RAGE KO mice - feasible and simple experiments.

Weaknesses

- Induction setting is very artificial (intranasal inoculation of antigen for 5 days a week for 5 weeks). It is unclear why this prolonged treatment is necessary if an innate cell response is being tested.
- Aim 2 RAGE KO effect on IL-33. The hypothesis is to test if RAGE ligand induces IL-33 expression from myeloid/lymphoid cells. Before this test, hematopoietic vs non-hematopoietic tissue effect of RAGE ligand should be tested.
- Both aims are feasible but are rather too simple for a 3 year project.

4. Training Potential:

Strengths

- Strong candidate and good preliminary data.

Weaknesses

- None noted.

5. Institutional Environment & Commitment to Training:

Strengths

None noted.

Vertebrate Animals: Acceptable.

Training in the Responsible Conduct of Research: Acceptable

Comments on Format (Required):

- Yes.

Comments on Subject Matter (Required):

- Yes.

Comments on Faculty Participation (Required):

- Yes.

Comments on Duration (Required):

- Yes.

Comments on Frequency (Required):

- Yes.

Budget and Period of Support: Recommend as Requested.

CRITIQUE 3:

Fellowship Applicant: 1

Sponsors, Collaborators, and Consultants: 2

Research Training Plan: 3

Training Potential: 1

Institutional Environment & Commitment to Training: 2

Overall Impact/Merit: This research MD/PhD training proposal is by an applicant who has a good academic record and have years of research experience. The candidate has received very good research training in the past. This candidate presents an excellent research training program and sponsoring team of investigators appears to have the experience and resources to support the training. The applicant presents proposal to address the role of pro-inflammatory receptor for advanced glycation end products (RAGE) in Th2 response and asthma. It is a well written and focused proposal and sufficient amount of preliminary data is presented to support the hypothesis. Understanding the molecular mechanisms associated with asthma will help develop effective therapies.

1. Fellowship Applicant:

Strengths

- The candidate has a strong academic record and received high grades in her undergraduate and graduate (course work) years. In addition, the candidate has few years of research experience and expected to complete the study in a timely fashion.
- The candidate has received very strong recommendation letters towards this training fellowship. Presented training and career goals are strong and adequate.
- Proposal is reasonably well-written and considerable amounts of preliminary data have been presented suggesting the productivity during the initial period in the Lab.

2. Sponsors, Collaborators, and Consultants:

Strengths

- Primary sponsor is a physician scientist and demonstrated strong research interest and expertise in the field through publications and funding record.
- The primary sponsor has published on RAGE and has good background in training the fellow. Co-sponsor is also well known in the field.

Weaknesses

- It is not clear if the primary sponsor has sufficient funding to support the proposed research.

3. Research Training Plan:

Strengths

- Observations that RAGE is acting upstream of IL-33 and plays a critical role in IL-5 and IL-13 secretion from adaptive Th2 cells and ILC2s is significant. Therefore, further understanding the role RAGE in asthma is important.
- Highly focused experiments are proposed. Specific aim 1 will examine the role of RAGE in IL-15 and IL-13 production using RAGE deficient mice in house dust mite model. Specific aim 2 will examine direct and indirect role of RAGE in inducing Th2 response. Experiments are clearly described; anticipated results and alternatives have been presented well.

Weaknesses

- Does RAGE have a role in IL-17 and IL-23 induction? Th17 cells and their cytokines orchestrate the feature of asthma especially in double allergen challenge model. It may be important to examine how other types of immune cells are influenced by RAGE signaling and contributing to

production of various cytokines and recruitment of effector cells. Use of double allergen challenge model may be beneficial.

- Data presentation has to be improved and figure legends lack clarity.

4. Training Potential:

Strengths

- This trainee appears to have excellent training potential and the sponsors are established and capable of training this candidate.

5. Institutional Environment & Commitment to Training:

Strengths

- Excellent environment and well suited for the proposed training program.

Vertebrate Animals: Acceptable.

Training in the Responsible Conduct of Research: Acceptable

Comments on Format (Required): None noted

Comments on Subject Matter (Required): None noted

Comments on Faculty Participation (Required): None noted

Comments on Duration (Required): None noted

Comments on Frequency (Required): None noted

Resource Sharing Plans: Acceptable.

Budget and Period of Support: Recommend as Requested.

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

COMMITTEE BUDGET RECOMMENDATIONS: The budget is recommended 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. Some applications also receive a percentile ranking. For details on the review process, see http://grants.nih.gov/grants/peer_review_process.htm#scoring.

MEETING ROSTER

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CENTER FOR SCIENTIFIC REVIEW
ZRG1 F07-K (20) L
October 24, 2013

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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.