Targeting CTLA4 and PD-1/PD-L1 for treatment of allergic asthma

2016 ATS Foundation Research Grants

Jinghong Li

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Application Form

Project Name*

Name of Project

Targeting CTLA4 and PD-1/PD-L1 for treatment of allergic asthma

Geographic Area*

Geographic Area of Request

United States

Resubmission?

Is this a resubmission of a prior complete grant to the ATS Research Program?

Yes

Resubmission Continued

If yes, in what year was the original submission and what was the project title?

2015. Interaction between CTLA4 and PD-1 in allergic asthma.

Are you an ATS member?*

Yes

ATS Member Continued

If yes, what is your ATS Member ID number?

00126663

Please select the category that best describes the proposed research.*

Translational

Highest Degree?*

(Example: MD, DO, PhD, etc)

MD, PhD

Position Title*

Assistant Professor

Signing Official Name

A signing official is any individual at your institution with authority to sign contracts.

Susanna Pastell

Signing Official Telephone

A signing official is any individual at your institution with authority to sign contracts.

8585344896

Applicant's Current Institution*

University of California San Diego

Current Division Chief

Atul Malhotra, MD

Academic Rank*

Professor

Division*

Pulmonary and Critical Care Medicine

Department*

Medicine

ATS Primary Assembly/Research Field of Interest

Which field of research listed below most strongly aligns with your research interests?

Allergy, Immunology, and Inflammation

Primary Grant Category*

Please select the grant program that you are applying for (1st choice).

Partnered: Breathe California of LA

Institutional Assurances

Clinical Trials*

Is your experiment a clinical trial?

No

Human Subjects*

Will you be using human subjects?

No

Human Subjects

If you are using human subjects, has your institution's IRB approved this project?

Human Subjects

If your project has been IRB approved, what is its Human Subjects Assurance number?

Animals*

Will you use animals to conduct your research?

Yes

Animals

If yes, have you received IACUC approval for your proposal?

Approved

Animals

What is your project's Animal Welfare Assurance number?

A3033-01

Key Personnel

Personnel and Effort*

Please a) list the key personnel on your project b) list each of their roles on the project (mentor, consultant, research technician, etc.) c) approximate the percentage of total effort that the person will contribute to the project.

Jinghong Li, MD, PhD (PI), (50% effort, no salary), Assistant Professor of Medicine, is an experienced physician-scientist in the fields of inflammatory lung diseases. She will be responsible for administration and scientific progress of this grant, overall direction of the project. She will design the experiments, coordinate with Collaborators. She will apply her Molecular Biology expertise to the analysis of gene regulations both in vitro and in vivo. She will analyze the data and write the papers.

Patricia W. Finn, MD (Collaborator), (10% effort, no salary), Professor and Chair, Department of Medicine, University of Illinois at Chicago. Dr. Finn is an expert in CTLA4 and T cell activation in pulmonary inflammatory disorders. She will collaborate with Dr. Li on the proposed project, with her expertise in T cell activation and pulmonary inflammatory disorders animal models including allergic asthma models.

Gen-Sheng Feng, PhD (Collaborator), (10% effort, no salary), Professor of Pathology and Biology, UCSD. Dr. Feng is an expert in CTLA4 and SH2 domain-containing tyrosine phosphatase (Shp2) pathway. CTLA4 was found to bind to Shp2, and they both play important roles in T cell activation. Dr. Feng has been working on Shp2 airway knockout mouse in OVA induced asthma model to analyze Shp2 function in asthma. He will be responsible to help with the related assays required for the proposal and provide the Shp2 knockout mouse if needed.

TBN, Research Assistant (part time), (25% effort, with salary support), will be responsible to assist the PI in performing experiments. We have two new graduates from UCSD (Biology major, graduated in June 2016) working at lab. They both have experience in molecular biology experiments. If funded, will hire one of them as part time research assistant.

Biosketches

Applicant Biosketch*

Please complete the new NIH biosketch template on the ATS website: Click here. Biosketches that do not follow the directions on the template will be unsubmitted.

Jinghong_Li_Biosketch_ATS_2016.pdf

Mentor Biosketch

Please complete the new NIH biosketch template which can be found on the ATS website: Click here.

Co-Investigator 1 Biosketch

Please complete the new NIH biosketch template on the ATS website: Click here.

Finn_Biosketch_2016.pdf

Co-Investigator 2 Biosketch

Please complete the new NIH biosketch template on the ATS website: Click here

Feng_Bio_2016.pdf

Lay Summary

Synopsis*

USING TERMS THAT CAN BE UNDERSTOOD BY A NON-SCIENTIFIC AUDIENCE, please briefly describe what you are investigating, the techniques you will use to support your hypothesis, and how your project will contribute to scientific understanding of your disease of interest.

Note that this General Audience Summary will become public information; therefore, do not include proprietary/confidential information.

Our research aims at finding more effective treatment for asthma. Asthma has become an epidemic affecting 300 million people in the world. In Los Angeles/Southern California, the incidence of asthma is relatively high, partially due to the local weather condition and air pollution status. T cells play important roles in asthma. Many clinical trials were conducted with candidate drugs that would inhibit or neutralize T cells function, but without clinical significant success. Recently, the roles of bronchial epithelial cells in asthma were re-defined. The bronchial epithelial cells are not bystanders, they are actually involved in many steps of asthma.

We therefore propose to target both the T cells and bronchial epithelial cells for treatment of asthma. We propose to find the mechanism of the interaction between T cells and bronchial epithelial cells in asthma. Then we will target both simultaneously.

Our research has found that one of the FDA approved drugs for kidney transplantation, CTLA4-Ig has favorable therapeutic effects in mouse allergic asthma model. However, the effects are not strong enough. We will perform the studies in cellular level and in animal allergic asthma model. We propose to optimize the treatment of asthma by targeting both CTLA4 in T cells and PD-L1 in bronchial epithelial cells.

Our goal is to develop a new class of therapeutics for asthma. Of note, both therapeutic agents in our proposal are available on the market. CTLA4-Ig is a FDA approved drug for kidney transplantation. PD-L1 antibody is one of the most promising cancer immunotherapy drugs just approved by FDA in May 2016. We hope the study results from our proposal will help to move forward to clinical trials.

Patient Summary*

Please explain how your research may lead to improved patient outcomes.

We have learned a lot in the past decades about how allergic asthma is developed and what are the risk factors for asthma. In Los Angeles/Southern California, the incidence of asthma is relatively high, partially due to the local weather condition and air pollution status. Many clinical trials were conducted with the hope of finding more effective treatment for asthma, especially for the severe asthma patients. However, no new drug has made to the market in the past decades. This is very out of proportion compare with the knowledge we have gained, indicating the complexity of the disease. Recently, the roles of lung structural cells in asthma were re-defined. We propose to combine our previous research in asthma and the most updated knowledge in immunology, to target both immune cells and lung structural cells, in order to develop more effective treatment for asthma. If successful, these treatments will likely to become candidates for novel treatment.

Technical Abstract

Summary for SAC Reviewers*

Please provide a technical abstract of your proposal, keeping your response to 5000 characters or less.

Asthma is an inflammatory disorder characterized by increased antigen-dependent immune responses. The incidence of asthma is increasing worldwide in the past decades. In the pathogenesis of asthma, T cell activation and Th2 cytokines secretion play critical roles. Inhibition of the Th2 cytokines was the focus of most current therapeutic approaches. On the other hand, some Th1 cytokines, such as interferon-γ (IFN-γ), was increased in asthma patients. Only limited studies were done to address IFN-γ, as a therapeutic target for asthma. Recently, the roles of bronchial epithelial cells in asthma were re-defined. Under various stimuli or cellular damages, the bronchial epithelial cells release TSLP, IL-25 and IL-33 and other cytokines result in airway remodeling and pathological changes resembling asthma.

Complete T cell activation requires two signals: an antigen specific signal mediated by the T cell receptor (TCR), and a costimulatory signal mediated by CD28 and CD80/CD86. CD28 family members include CD28, cytotoxic T lymphocyte antigen 4 (CTLA4), and programmed death-1 (PD-1). CD28 binds to CD80/CD86 and positively mediates the costimulatory signal leads to complete T cell activation. CTLA4 binds to CD80/CD86 and blocks the costimulatory signal, leads to inhibition of T cell activation. Interestingly, PD-1 has two ligands, PD-1 ligand 1 (PD-L1) and PD-1 ligand 2 (PD-L2). PD-L1 and PD-L2 have opposite functions in mouse asthma model. PD-L1 knockout mice have reduced airway hyperresponsiveness (AHR). PD-L2 knockout mice developed higher AHR. Therefore, the role of PD-1 in asthma depends on the availability of ligands.

Taken together, CD28 contributes to T cell activation and PD-L1 contributes to increased AHR, whereas CTLA4 contributes to T cell inhibition and PD-L2 contributes reduced AHR. We speculate that IFN-γ, the Th1 cytokine increased in asthma and the best-known stimulator of PD-L1 expression, may contribute to the induction of PD-L1 in bronchial epithelial cells. PD-L1 is known to contribute to increased AHR. Therefore, the IFN-γ induced upregulation of PD-L1 in bronchial epithelial cells promotes severe inflammation result in airway remodeling and AHR.

We previously reported that CTLA4-Ig (FDA approved drug for renal transplant), a fusion protein known to inhibit the engagement of CD28 and CD80/CD86 showed therapeutic effects in mouse asthma model. We hypothesize CTLA4-Ig inhibits T cell activation, Th2 cytokines production and more importantly, IFN-γ production, the major inducer of PD-L1 expression. We hypothesize that the combination of CTLA4-Ig and anti-PD-L1 antibody will have synergistic therapeutic effects in allergic asthma model. CTLA4-Ig inhibits the T cell activation and IFN-γ production. Meanwhile, anti-PD-L1 will further block the function of PD-L1 in bronchial epithelial cells.

We have two specific aims. In Aim 1: We will determine the effects of CTLA4-Ig on IFN-γ and PD-L1 expression in spleen T cells and bronchial epithelial cells. We expect CTLA4-Ig will inhibit cell proliferation and decrease Th2 cytokine secretion in T cells. We expect CTLA4-Ig will also inhibit the IFN-γ secretion in T cells and PD-L1 mRNA and protein expression in bronchial epithelial cells. In Aim 2: We will determine the effects of combination of CTLA4-Ig and anti-PD-L1 antibody on OVA-induced mouse allergic asthma model in vivo. We will examine the effects of CTLA4-Ig, anti-PD-L1 antibody, and in combination of the two. We will examine the AHR, inflammation parameters and cytokines in bronchoalveolar lavage (BAL), serum IgE to evaluate the therapeutic effects.

Future Plans*

Where would you take this research after funding ends, and how will this support contribute to your research career development?

Results from this proposal will support at least two peer-reviewed publications. The first publication will focus on the mechanism of the interaction between CTLA4 and PD-1/PD-L1 in a cellular level. The second publication will focus on the therapeutic effects in animal models. Data from this research will form the basis of preliminary studies of a NIH R01 proposal. I would like to point out that both therapeutic agents we plan to use are already available on the market. CTLA4-Ig is a FDA approved drug for renal transplantation. Anti-PD-L1 antibody is one of the most promising cancer immunotherapy drugs that it was just approved by FDA in May 2016. The well-known profiles of these drugs will provide the likelihood that once our preclinical studies are done with promising results, these drugs may be on the fast track to clinical studies.

Healthcare Equality

Promoting healthcare equality*

How will your proposal promote healthcare equality?

For example, for clinical studies, please reflect how your patient recruitment strategy is likely to draw participants that reflect the burden of disease in the general population. Thus, for diseases like sarcoidosis, which affects mostly African Americans, the bulk of patients recruited should be African Americans, which will in turn help ensure that any therapeutic advances arising from the studies will be helpful to the ethnic/racial groups most affected.

The incidence of allergic asthma is increasing worldwide in the past decades. Allergic asthma has become an epidemic affecting 300 million people in the world. In Los Angeles/Southern California, the incidence of asthma is relatively high, partially due to the local weather condition and air pollution status. In particular, asthma has become the disease in which low socioeconomic status people and minority people suffer from a higher prevalence and more severe symptoms, especially in children and women. Many basic research and clinical trials have been performed to develop more effective treatment without much success in the past 20 years. A significant proportion of asthmatic patients continue to have symptoms and lifestyle restrictions with considerable loss of schooldays and workdays. The current proposal will investigate and develop more effective treatment for asthma, based on our previous research and the new knowledge gained in the recent years. We believe we have a better idea now on how to treat asthma in a more specific and effective way. This will benefit for the asthmatic patients, especially for the patients who have more severe symptoms that are not controlled under current available treatments, such as in children and women.

Project Proposal

Research Plans*

Please upload your Research Plans here.

Please note that the proposal must not exceed 10 pages, including pictures.

Please organize your Research Plans according to the guidelines in the Application Instructions "Research Plans" Section. **Proposals that do not follow this format will be unsubmitted.** The Application Instructions document is available on the ATS Foundation Research Program website.

Note that your research plan will not be uploaded until you save your application.

RESEARCH PLANS 9-11-16.pdf

Resubmission Statement

Resubmission of Prior Application

A resubmission is an application that was reviewed in a previous cycle, but was not funded. Please note that an application is not considered a resubmission if it has major changes. Only complete this section if your application is a resubmission.

In the statement you must:

1. Explain how you addressed the scientific critiques of the original application.
2. Explain how the application has been strengthened and modified since the original submission.

We appreciate reviewers' comments. The comments are very helpful. We have made the following improvement.

Reviewer 1's comments:
…Aim 1 is weak…a fast and easy exp…
We expanded our Aim 1 to include the PD-L1 promoter reporter assay. We obtained the full length PD-L1 promoter and we have generated truncated constructs for reporter assay. The IFN-γ responsive region, interferon regulatory factor-1 (IRF-1), at the PD-L1 promoter will be studied.

…I do not see letters from department…a letter from her division chief, Atul Malhotra, MD.
A letter from Dr. Dillmann, Chair of Department Medicine, and a letter from Dr. Malhotra, Division Chief of Pulmonary Critical Care Medicine, are included.

…all studies are in mice…
For the 2-year project, the studies are in mice. A paragraph of potential clinical translation was discussed in "Significance". Both drugs are FDA approved medications.

…no mention…affect CA residents…
A paragraph was added in the "Statement of problem" about the impact of Southern California local weather condition and air pollution status on asthma (with references).

Reviewer 2's comments:
…asthma subphenotypes…current role of existing biologics…
A paragraph was added at the "STATEMENT OF PROBLEM".

…Potential side effects of anti-PDL1...
Discussion was added to "Significance".

…CTLA-Ig appears to be quite effective…
CTLA-Ig appears to be quite effective in mouse model, but the airway resistance and BAL neutrophils are still high. We hope the addition of anti-PD-L1 will be more effective.

…Collaborator in another institution…
First of all, although Dr. Finn is located at UIC, we still have regular discussion (approximately once a month) over the phone or emails. Second, I have a new collaborator at UCSD, Dr. Gen-Sheng Feng, who is an expert in T cell immunology, specifically CTLA4 and Shp2. He is experienced in OVA induced asthma models by using Shp2 knockout mouse generated by his lab. Shp2 binds to CTLA4 in T cells and mediates CTLA4 function.

Bibliography

Literature Cited*

This section has no page limit. Please list citations by number in the order in which they appear in the text of the research plan.

Use the following format shown below ("New England Journal of Medicine" style in EndNote and "Annals of Internal Medicine" in Reference Manager).

1. Kajikawa O, Frevert CW, Lin SM, Goodman RB, Mongovin SM, Wong V, Ballman K, Daubeuf B, Elson G, Martin TR. Gene expression of toll-like receptor-2, toll-like receptor-4 and MD2 is differentially regulated in rabbits with Escherichia coli pneumonia. Gene 2005;3:193-202.

2. Altemeier WA, Matute-Bello G, Frevert CW, Kawata Y, Kajikawa O, Martin TR, Glenny RW. Mechanical ventilation with moderate tidal volumes synergistically increases lung cytokine response to systemic endotoxin. Am J Physiol Lung Cell Mol Physiol 2004;164:1949-1958.

References.pdf

Budget

Budget Form*

Please complete and upload the budget form for your proposal. This form can be found on the ATS website: Click here.

Budget-2016_Jinghong_Li.pdf

Resources

Lab Resources and Equipment*

List the resources that you have available to you that will be needed to complete your project. Describe these resources, and explain how each will contribute to the successful completion of your project.

Please note that the limit for this section is two pages.

This document should be divided into six sections:

Laboratory
Clinical
Animal
Computer
Office
Major Equipment
Other

Resources.pdf

Letters of Recommendation

You must submit two letters of support. These letters should highlight your accomplishments and describe your potential to become an independent investigator.

Your recommenders will be contacted using the information you supply below. They can upload the letters directly to Foundant once you have saved your application, and they will still be able to add the letters to your application after you have submitted it. Please note that you will not be able to read the letters.

The letters of support are due by the deadline for the full application: Wednesday, September 14th at 11:59pm ET.

First Letter of Support

Name of person providing letter of support*

Atul Malhotra, MD

How do you know this person?*

Dr. Malhotra is my Division Chief.

Second Letter of Support

Name of person writing second letter of support*

Wolfgang Dillmann, MD

How do you know this person?*

Dr. Dillmann is the Chair of Department of Medicine.

Letters from Collaborators

This section is optional. You may upload as many letters from collaborators as you would like. All letters should be included in a single PDF file.

Jinghong_ATS_Letters.pdf

Disclosure of Conflict of Interests

Many individuals have competing interests that may cause conflicts of interest. Disclosure and review of a potential "conflict of interest" (COI) that might affect an ATS research award doesn't imply that an "outside interest", or a person with such a relationship or interest, is wrong or inappropriate. Nor does it imply criticism of the character or actions of the individual, or of any

commercial or non-commercial entities with which an individual may be involved. Rather, ATS COI policies and procedures exist to assure the public of appropriate levels of transparency of the outside interests of participants in official ATS activities, and that official ATS activities are managed in a manner that maintains scientific rigor and independence.

• Disclosure of a real or potential COI will not automatically invalidate an application.

• All personal financial relationships with commercial entities (such as pharmaceutical companies and medical device manufacturers), and/or with non-commercial, non-governmental funders (such as private foundations), that are relevant to the application's subject matter must be disclosed. Such involvement with relevant commercial entities (companies) includes employment or ownership; consultancy(ies); Board or advisory committee service; service on speakers' bureaus or other acceptance of lecture fees directly paid by the company; expert testimony on behalf of the company; research grants; patents received or pending; royalties; and stock ownership or options (excluding mutual funds unless a sector fund concentrated in an industry or industries relevant to the activity).

• The ATS "Policy on Tobacco Relationships" requires that individuals with a current tobacco industry relationship not hold certain ATS roles, including that of research awardee. (Note: if the relationship is limited to personal holdings of tobacco stocks or options, divestiture of such holdings. See the policy for details.)

COI Question 1*

Have you within the past 12 months had a financial interest in a commercial entity (i.e., a "commercial interest") that could be considered broadly relevant to the subject matter of this proposal, or do you expect to have one during the duration of the grant award period?

No

COI Question 1b

If yes, please provide the company name(s) and the type of relationship:

COI Question 2*

Have you within the past 12 months received financial support from a non-commercial, non-governmental source (such as a private foundation or other non-profit source) that could be perceived as a real or potential conflict of interest relevant to the subject matter of this proposal, and/or do you expect to receive such before the end of the grant award period?

No

COI Question 2b

If yes, please provide the organization name(s) and the type of relationship:

COI Question 3*
Have you within the past 12 months had any non-financial affiliation or interest (i.e., a personal, professional or other affiliation or interest) that might be perceived to be a real or potential conflict of interest relevant to the subject matter of this proposal, and/or do you expect to receive such before the end of the grant award period?

No

COI Question 3b
If yes, please provide the company or organization name(s) and the type of relationship:

COI Question 4*
Have you within the past 12 months had a professional relationship with a tobacco entity or expect to have one before the end of the grant award period?

No

COI Question 4b
If yes, please provide the company or organization name(s) and the type of relationship:

Signature

Signature Form*
Please sign and upload the signature form available on the ATS website: Click here.

SIGNATURE_FORM_LI_JINGHONG.pdf

Email Address for Person Writing Letter of Support*
Please enter the email address of the person providing your letter of support. Then click on the "Compose Email" button to message your referrer to write a letter of support. Once you have saved this message, Foundant will automatically send your referrer a link to your application and instructions to upload this letter. Please confirm with your referrer that s/he has received this message.

emnebel@gmail.com

Letter of Recommendation*
Please upload a letter on official institution stationary that highlights the candidate's accomplishments and limitations as a researcher. Please describe the candidate's preparedness to

complete the project proposed in this application, and his or her ability to transition to an independent researcher in the near future.

The letter of support is due by the full application deadline: Wednesday, September 14th at 11:59pm.

jinghong-los.pdf

Email Address of Person Writing Second Letter*

Please follow the directions for contacting your first recommender.

wdillmann@ucsd.edu

Letter of Recommendation*

Please upload a letter on official institution stationary. In the letter, please describe the candidate's highlights and limitations as a researcher. Also explain his or her preparedness to undertake the research proposed in this application and comment on his or her ability to transition to an independent research in the near future.

The letter of support is due by the full application deadline: Wednesday, September 14th at 11:59pm ET.

Department_Chair_Letter_ATS_2016.pdf

File Attachment Summary

Applicant File Uploads

- Jinghong_Li_Biosketch_ATS_2016.pdf
- Finn_Biosketch_2016.pdf
- Feng_Bio_2016.pdf
- RESEARCH PLANS 9-11-16.pdf
- References.pdf
- Budget-2016_Jinghong_Li.pdf
- Resources.pdf
- jinghong-los.pdf
- Department_Chair_Letter_ATS_2016.pdf
- Jinghong_ATS_Letters.pdf
- SIGNATURE_FORM_LI_JINGHONG.pdf

Supporting Documents

No files were uploaded

BIOGRAPHICAL SKETCH

Provide the following information for all key personnel.
Follow the sample format for each person found in **Biosketch Sample**. **DO NOT EXCEED FOUR PAGES.**

NAME	**POSITION TITLE**
Li, Jinghong	Assistant Professor of Medicine
eRA COMMONS USER NAME (credential, e.g., agency login) JINGHONGLI	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Peking University Health Science Center (aka Beijing Medical University), Beijing, China	MD	08/84-07/90	Medicine
Peking University Health Science Center, Beijing, China	PhD	08/93-09/96	Molecular Biology
Tokyo Medical and Dental University, Tokyo, Japan	Postdoctoral	11/96-11/98	Molecular Biology
Harvard University	Postdoctoral	12/98-12/01	Molecular Genetics
INSTITUTION AND LOCATION	**Certificate** *(if applicable)*	**YEAR(s)**	**FIELD OF STUDY**
Peking University Health Science Center, Beijing, China	Residency	08/90-07/93	Internal Medicine
Unity Health System, Rochester, NY	Residency	07/06-06/09	Internal Medicine
University of California San Diego, San Diego, CA	Clinical Fellowship	07/09-06/12	Pulmonary and Critical Care Medicine

A. Personal Statement

My long-term research interest is to identify more effective therapeutic targets for pulmonary diseases. I studied pulmonary fibrosis animal models during my PhD thesis research and developed an interest in signaling transduction, specifically TGF-beta signaling pathway. I subsequently did postdoctoral training in TGF-beta and JAK/STAT signaling transduction in cancer cells and in *Drosophila* model. I have revealed several negative regulators of TGF-beta signaling pathway with further characterization of the eukaryotic initiation factor-4A (eIF4A), which mediates SMAD degradation to suppress DPP/BMP/TGF-beta function. Our research also demonstrated a non-canonical mode of JAK/STAT signaling. In 2006, I decided to return to clinical medicine, with the goal of better translating my research to novel therapeutics for the patients. After completing the Residency in Internal Medicine, I went on to pursue a fellowship in Pulmonary and Critical Care Medicine at University of California San Diego. I joined Dr. Patricia Finn's laboratory to work on the innate and adaptive regulation of cytotoxic T lymphocyte antigen 4 (CTLA4) in pulmonary inflammatory disease models, including asthma and acute lung injury (ARDS). Currently, I focus on the interaction between CTLA4 and PD-1/PD-L1 signaling by using T cells, lung epithelial cells and mouse models for asthma and lung cancer.

B. Research and/or Professional Experience:

Positions and Employment

08/90- 07/93 Medical Residency, Internal Medicine, Peking University First Hospital, Beijing, China
08/93- 09/96 Graduate Research Assistant, Peking University
11/96- 11/98 Postdoctoral Fellow, Tokyo Medical and Dental University, Tokyo, Japan
12/98- 12/01 Postdoctoral Fellow, Harvard University, Cambridge, MA

12/01- 06/06 Research Associate, University of Rochester, Rochester, NY
07/06- 06/09 Medical Residency, Internal Medicine, Unity Health System, Rochester, NY
07/09- 06/12 Clinical Fellowship, Pulmonary and Critical Care Medicine, University of California San Diego
07/12- 09/13 Clinical Instructor, Pulmonary and Critical Care Medicine, University of California San Diego
10/13- Assistant Professor, Pulmonary and Critical Care Medicine, University of California San Diego

Honors

1996 Outstanding Poster Award, The 4th Asian Pacific Society of Respirology Congress, Beijing
1996-1998 Postdoctoral Fellowship, Japan Society for the Promotion of Science
1997 ASBMR Young Investigator Award, The 19th Annual Meeting of the American Society for Bone and Mineral Research (ASBMR), Cincinnati, OH
2002-2005 Wilmot Cancer Research Fellowship, University of Rochester Wilmot Cancer Center
2008 Finalist, National Associates Abstract Competition, American College of Physician Internal Medicine 2008, Washington, DC
2010 Finalist, The Seventh Respiratory Disease Young Investigators' Forum, San Antonio, TX
2013 California Thoracic Society Scholar Award Winner, Carmel, CA
2013 Henry Christian Award, American Federation for Medical Research, Washington, DC
2013 Finalist, The Ninth Respiratory Disease Young Investigators' Forum, Austin, TX
2014 American Thoracic Society (ATS) Young Investigator, The 54th Annual Meeting of The Japanese Respiratory Society, Osaka, Japan

Professional Societies

1990-1996 Chinese Medical Association (CMA)
1996-1998 American Society for Bone and Mineral Research (ASBMR)
1999-2006 Genetics Society of America (GSA)
1999-present American Chinese Medical Association (ACMA)
2007-present American College of Physicians (ACP)
2009-present American Thoracic Society (ATS)
2011-present American College of Chest Physicians (ACCP)
2012-present California Thoracic Society (CTS)
2012-present American Federation for Medical Research (AFMR)

C. Contribution to Science (from 27 Publications)

1. My early publications focused on TGF-beta signaling pathway in pulmonary fibrosis and cancer. We demonstrated that TGF-beta antibody had therapeutic effect in rat pulmonary fibrosis model by inhibiting collagen production, not the cell proliferation. In mouse osteosarcoma cells, we dissect the regulation of TGF-beta signaling by differentially expressed Smad1, 2 and the inhibitory Smad4. We found CBFA1 gene is one of the downstream targets of Smad2. These work contributed to the understanding the function and downstream of TGF-beta pathway.

    a. **Jinghong Li**, Bing He and Baoying Weng. The therapeutic effect of TGF-b monoclonal antibody to bleomycin-induced pulmonary fibrosis in rats. ***Chinese Journal of Tuberculosis and Respiratory Diseases.*** 1997;20:347-349.
    b. **Jinghong Li**, Kunikazu Tsuji, Toshihisa Komori, Kohei Miyazono, Jeffrey L. Wrana, Yoshiaki Ito, Akira Nifuji, and Masaki Noda. Smad2 overexpression enhances Smad4 gene expression and suppresses CBFA1 gene expression in osteoblastic osteosarcoma ROS17/2.8 cells and primary osteoblastic osteosarcoma. ***J. Biol. Chem.*** 1998;273(47):31009-31015. PMID: 9812998.

2. I then continued my research on TGF-beta signaling pathway to identify new components involved in the pathway. I utilized *Drosophila* model, for which many of the components of the pathway were first identified by genetic screens. I performed a large-scale genetic screen to identify new negative regulators of DPP/BMP/TGF-beta pathway. Several negative regulators were found with further characterization of the

eukaryotic initiation factor-4A (eIF4A) as one of them. eIF4A acts synergistically with the ubiquitin E3 ligase, which regulates the abundance of SMAD protein by promoting its degradation. We speculate that eIF4A may function as an adaptor to link SMAD proteins to the protein degradation machinery. This work was featured in "News and Views" by Editor in ***Nature Cell Biology*** and was widely reported in scientific news and media. In another genetic screen, I found overexpression of receptor tyrosine kinase (RTK) could trigger Dpp and STAT pathway independently. These work contributed to the understanding of the interaction between DPP/BMP/TGF-beta pathway and translation machinery. This is a novel idea because transcription factor have long been regarding as the "master" of regulation.

a. **Jinghong Li**, Willis X. Li, and William M. Gelbart. A genetic screen for maternal effect suppressors of *decapentaplegic* identifies eukaryotic translation initiation factor 4A in *Drosophila*. ***Genetics*** 2005;171:1629-41. PMID: 15972466.
b. **Jinghong Li** and Willis X. Li. A novel function of Drosophila eIF4A as a negative regulator of Dpp/BMP signaling that mediates SMAD degradation. ***Nature Cell Biology*** 2006;8:1407-14. **Featured in *"News and Views"* by Editor.** PMID: 17115029.
c. **Jinghong Li** and Willis X. Li. Drosophila gain-of-function mutant RTK Torso triggers ectopic Dpp and STAT signaling. ***Genetics*** 2003;164: 247-258. PMID: 12750336.

3. I then worked on the JAK/STAT pathway focusing on the function of STAT in heterochromatic gene silencing. We demonstrated a non-canonical mode of JAK/STAT signaling. We found the unphosphorylated STAT (U-STAT) promoted heterochromatin formation to silence gene transcription. As a result, U-STAT functions as a tumor suppressor, not only to counteract the transcriptional activity of pSTAT, but also to silence multiple gene transcription. This has been studied both in *Drosophila* model and human cancer cells *in vitro* and *in vivo.* Currently, I am working on further characterizing the non-canonical function of STAT3 in NSCLC.

a. **Jinghong Li**, Fan Xia, and Willis X. Li. Coactivation of STAT and Ras is required for germ cell proliferation and invasive migration in *Drosophila*. ***Dev. Cell*** 2003;5:787-798. PMID: 14602078.
b. **Jinghong Li**, Wenjun Li, Healani C. Calhoun, Fan Xia, Fen-Biao Gao and Willis X. Li. Patterns and functions of STAT activation during *Drosophila* embryogenesis. ***Mech. Dev.*** 2003;120(12):1455-1468. PMID: 14654218.
c. Song Shi, Healeni C. Calhoun, Fan Xia, **Jinghong Li**, Long Le and Willis X. Li. JAK signaling globally counteracts heterochromatic gene silencing. ***Nature Genetics*** 2006;38:1071-6. PMID: 16892059.
d. Amy Tsurumi, Fan Xia, **Jinghong Li**, Kimberly Larson, Russell LaFrance, Willis X. Li. STAT Is an Essential Activator of the Zygotic Genome in the Early *Drosophila* Embryo. ***PLoS Genet.*** 2011;7(5):e1002086. PMID: 21637778.
e. Xiaoyu Hu, Pranabananda Dutta, Amy Tsurumi, **Jinghong Li**, Jingtong Wang, Hartmut Land, Willis X. Li. Unphosphorylated STAT5A stabilizes heterochromatin and suppresses tumor growth. ***Proc Natl Acad Sci U S A.*** 2013; 110(25):10213-8. PMID: 23733954.
f. Pranabananda Dutta, Nafiseh Sabri, **Jinghong Li**, and Willis Li. Role of STAT3 in lung cancer. ***JAKSTAT.*** 2014; 3: e999503.

4. It has been recognized that many pulmonary diseases manifestation are acute and chronic inflammation. Immune cells such as T cells play important roles in regulating immune homeostasis including cells proliferation, growth and cytokine secretion. We investigated the regulation and function of one of the important checkpoint protein, cytotoxic T lymphocyte antigen 4 (CTLA4) in T cells and in mouse asthma and acute lung injury (ARDS) models. We are currently working on the interaction between CTLA4 and PD-1/PD-L1 signaling by using T cells, lung epithelial cells and mouse models for asthma and lung cancer.

a. **Jinghong Li**, Ko-Wei Lin, Fiona Murray, Takeshi Nakajima, Yandong Zhao, David L. Perkins, and Patricia W. Finn. Regulation of cytotoxic T lymphocyte antigen 4 by cAMP. ***Am J Respir Cell Mol Biol.*** 2013; 48(1):63-70. Epub 2012 Sep 28. **Featured in "*Red Alert":* Highlighted Papers by Junior Investigators.** PMID: 23024062.

b. Ko-Wei Lin, **Jinghong Li**, and Patricia W. Finn. Emerging pathways in asthma: Innate and adaptive interactions. ***Biochim Biophys Acta.*** 2011;1810:1052-8. PMID: 21596099.
c. Takeshi Nakajima, Ko-Wei Lin, **Jinghong Li**, Halvor S. McGee, Jennifer M. Kwan, David L. Perkins, and Patricia W. Finn. T Cells and Lung Injury: Impact of Rapamycin. ***Am J Respir Cell Mol Biol.*** 2014; 51(2):294-9.
d. **Jinghong Li**. Environmental fine particular matter and airway epithelium cell stress. ***Current Pulmonol Rep***. 2015; 4:111-6.
e. **Jinghong Li**, Willis X. Li, Chunxue Bai, Yuanlin Song. Particulate matter-induced epigenetic changes and lung cancer. ***Clin Respir J.*** 2015 Sep 25 [Epub ahead of print]

Complete List of Published Work in MyBibliography:
http://www.ncbi.nlm.nih.gov/sites/myncbi/1hQdxHKqQQrQj/bibliograpahy/48008356/public/?sort=date&direction=ascending

D. Research Support

Recent Completed Research Support
American Thoracic Society (ATS) Excellent Service Award 2015
Cytotoxic T lymphocyte antigen 4 (CTLA4) expression profile in non-small cell lung cancer
Role: PI. 3/15-2/16

T32HL098062 Training in respiratory biology: innovate, integrate, and translate.
Role: Trainee. 07/11 – 06/13

BIOGRAPHICAL SKETCH

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

NAME: Finn, Patricia W.

eRA COMMONS USER NAME (credential, e.g., agency login): PWFINN

POSITION TITLE: Earl M. Bane Professor and Chair, Department of Medicine

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
State University of New York, Buffalo, NY	BA	05/1976	Anthropology
Albert Einstein College of Medicine, NY	MD	05/1980	Medicine

A. Personal Statement

My prior research has been primarily focused on the investigation of immune mediated diseases. Specifically, my laboratory analyzes mechanisms of immune activation and the development of inflammatory disorders (e.g., asthma, transplantation, lung injury, and sarcoidosis), as well as the interface between innate and adaptive immunity. I focus on translational investigations and molecular biological studies in murine models (in vitro and in vivo) as well as human clinical studies.

As additional methods to identify key regulatory immune pathways, I am also furthering the analysis of inflammatory models incorporating bioinformatic approaches. Recent advances in the fields of personalized medicine, omics biology, and analysis of big data have facilitated recasting my investigation to the effects of the metagenome on immunity and disease.

I am past president of the American Thoracic Society, the largest professional international society addressing pulmonary, critical care and sleep disorders. I have a history of NIH funding, have served as PI on T32 HL098062 at University of California, San Diego (UCSD), and am currently PI of T32 HL82547 training grant at University of Illinois at Chicago (UIC). I have served as permanent member and chair of the NIH LCMI study section as well as other NIH study sections and workshops. I am currently chair of the NHLBI Heart, Lung Blood Program Project Review Committee.

I moved from UCSD to become chair of the Department of Medicine at UIC. Relocation to UIC has afforded me the opportunity to work at an institution with multiple colleges of health science and personnel and resources to analyze large datasets. In this proposal, we will focus on integration of large data with clinical outcomes, focusing on early life virome and immune interactions related to the onset of later allergic disease.

B. Positions and Honors

Positions and Employment

1980-1983 Intern & Resident in Medicine, Montefiore Hospital and Albert Einstein College of Medicine, NY
1983-1986 Fellow in Pulmonary Medicine, Massachusetts General Hospital
1986-1988 Research Fellow, Department of Cancer Biology, Harvard University School of Public Health
1988-1991 Instructor in Immunology, Department of Cancer Biology, Harvard School of Public Health
1990-1991 Instructor in Medicine, Harvard Medical School and Associate Physician, Brigham & Women's Hospital and Beth Israel Hospital
1991-2000 Assistant Professor of Medicine, Harvard Medical School and Associate Physician, Brigham & Women's Hospital
2000-2005 Associate Professor of Medicine, Harvard Medical School and Associate Physician, Brigham & Women's Hospital
2005-2011 Kenneth M. Moser Professor of Medicine, University of California, San Diego, School of Medicine

2005-2011 Division Director, Pulmonary and Critical Care Medicine, University of California, San Diego, School of Medicine
2012- Earl M. Bane Professor and Chair, Department of Medicine, University of Illinois at Chicago

Honors (Selected)

1983 Distinguished Medical Teaching Award, Albert Einstein College of Medicine
1997 Career Investigator, American Lung Association
1999 Lynn M. Reid Scholar in Medicine Award, Harvard Medical School
1999 Nomination, Excellence in Teaching Award, Harvard Medical School
2002-2004 Chair Elect and Chair, Program Committee, Allergy, Immunology, and Inflammation Assembly, American Thoracic Society
2007- American Association of Physicians (AAP)
2007-2009 Chair Elect and Chair, Allergy, Immunology, and Inflammation Assembly, American Thoracic Society
2009 Elizabeth Rich Award, American Thoracic Society
2010-2011 Secretary-Treasurer, American Thoracic Society
2011-2012 Vice President, American Thoracic Society
2012-2013 President Elect, American Thoracic Society
2013-2014 President, American Thoracic Society
2014-2015 Immediate Past President, American Thoracic Society

Editorial Boards and Study Sections

1995- Reviewer, *Journal of Immunology, American Journal of Respiratory, Cell and Molecular Biology, Journal of Clinical Investigation*, *Clinical and Experimental Allergy, American Journal of Respiratory and Critical Care Medicine, New England Journal of Medicine*
2000-2009 Associate Editor, Immunology Section, *Respiratory Research*
1998-2002 American Lung Association, Research Fellowship Review Committee
1998, 2002 Ad hoc Reviewer, Lung Biology and Pathology Study Section, NIH
2002 Immunology Editor, *Encyclopedia Respiratory Medicine,* Harcourt, Elsevier Science
2002-2005 American Heart Association Review Committee
2003-2008 Permanent Member, Lung Cellular Molecular Immunology Study Section, NIH
2003-2008 Editorial Board, *American Journal of Respiratory Cell and Molecular Biology*
2006 NIH/NIEHS, ONES Review Committee
2006 NIH/NHLBI, Strategic Planning Committees, Co-Chair and Chair
2007-2008 Chair, Lung Cellular Molecular Immunology Study Section, NIH
2008 Chair, NIH/NHLBI Working Group, "Immuno-genetics of Chronic Lung Disease"
2008-2012 Section Editor, *Journal of Immunology*
2008-2013 Associate Editor, *American Journal of Respiratory Cell and Molecular Biology*
2013-2017 Permanent Member, NHLBI Program Project Review Committee
2015-2017 Chair, NHLBI Program Project Review Committee

C. Contribution to Science

1. Early life immune responses

Our previous studies have investigated immune responses in early life following mothers and infants in birth cohorts. Findings indicate that distinct fetal and perinatal exposures and black race/ethnicity may be associated with increased cord blood lymphoproliferative responses. NF-kappaB activity is differentially activated in cord blood and associated with a distinct cytokine pattern. We have also examined the relationship between cytokine secretions by cord blood mononuclear cells and lower acute respiratory illness in a birth cohort. Increased IL-10 and Foxp3 induction in cord blood mononuclear cells of non-atopic compared to atopic mothers, may indicate an increased capacity to respond to microbial stimuli.

1. Schroeter CS, Schaub B, Gold DR, Contreras PJ, Manrique O, Gillman MW, Weiss S, Palmer LJ, Perkins DL, **Finn PW**. Nuclear factor kappa B activation in human cord blood mononuclear cells. Pediatric Research. 2004;56:212-218. PMCID: PMC1488728
2. Willwerth BM, Schaub B, Tantisira KG, Gold DR, Palmer LJ, Litonjua AA, Perkins DL, Schroeter C, Gibbons FK, Gillman MW, Weiss ST, **Finn PW**. Prenatal, perinatal, and heritable influences on cord blood immune responses. Ann Allergy Asthma Immunol. 2006;96:445-53. PMCID: PMC1562525

3. Schaub B, Campo M, He H, Perkins D, Gillman MW, Gold DR, Weiss S, Lieberman E, **Finn PW**. Neonatal immune responses to TLR2 stimulation: Influence of maternal atopy on Foxp3 and IL-10 expression. Respir Res. 2006;7:40. PMCID: PMC1435749
4. Ly NP, Rifas-Shiman SL, Litonjua AA, Tzianabos AO, Schaub B, Ruiz-Perez B, Tantisira KG, **Finn PW**, Gillman MW, Weiss ST, Gold DR. Cord blood cytokines and acute lower respiratory illnesses in the first year of life. Pediatrics. 2007;119,171-8. PMCID: PMC1994927

2. Metagenome analysis

Our previous studies have documented changes in the metagenome induced by the stressors of kidney transplantation including prophylactic antibiotics and immunosuppressive drugs, taking into account differences in ethnicity. With my collaborator, Dr. David Perkins, we are expanding our analysis of the metagenome to other clinical disorders. These studies have the potential to elucidate local or systemic biomarkers of disease, which may contribute to personalized therapeutics.

1. Rani A, Ranjan R, McGee HS, Andropolis KE, Panchal DV, Brennan DC, **Finn PW**, Perkins DL. Urinary microbiome of kidney transplant patients reveals shift to pathogenic bacteria with enhanced antibiotic resistance potential. (submitted 2016).
2. Rani A, Ranjan R, McGee H, Brennan DC, **Finn PW**, Perkins DL. The urinary virome in kidney transplant recipients. (submitted 2016).
3. Kwan J, Hajjiri Z, Perkins DL, **Finn PW**. Ethnicity Associations with Renal Graft Adverse Outcomes. (submitted 2016).

3. Cytotoxic T lymphocyte antigen 4 (CTLA4) mediates decreased pulmonary inflammation

We analyzed cytotoxic lymphocyte antigen 4 (CTLA4), which we have shown to be a negative regulator of pulmonary inflammation. Pulmonary lipopolysaccharide (LPS) administration promotes CD4(+) T cells and T cell pathways involving CTLA4 contribute to acute lung injury (ALI). We demonstrate that rapamycin decreases cAMP accumulation and CTLA4 expression in ALI, suggesting that cAMP may negatively regulate pulmonary inflammatory responses in vivo and in vitro by altering CTLA4 expression. Rapamycin also significantly decreases inflammatory parameters and Foxp3, CTLA4, and CD69 in CD4(+) T cells. Rapamycin administration before or after the onset of lung injury, as well as systemically or by pulmonary routes, ameliorates inflammation in ALI. We have also demonstrated that Treg peripheral homeostasis can be specifically modulated in vivo to promote Treg expansion and tolerance by increasing conjugation between Tregs and dendritic cells (DCs).

1. Nakajima T, Suarez CJ, Lin KW, Jen KY, Schnitzer JS, Makani SS, Parker NJ, Perkins DL, **Finn PW**. T cell pathways involving CTLA4 contribute to a model of acute lung injury. J of Immunol. 2010;184:5835-5841. PMCID: PMC3068917
2. Li J, Lin KW, Murray F, Nakajima T, Zhao Y, Perkins DL, **Finn PW**. Regulation of Cytotoxic T Lymphocyte Antigen 4 by cAMP. Am J Respir Cell Mol Biol. 2012 Sep 28. PMCID: PMC3547085
3. Nakajima T, Lin KW, Li J, McGee HS, Kwan JM, Perkins DL, **Finn PW**. T Cells and Lung Injury: Impact of Rapamycin. Am J Respir Cell Mol Biol. 2014 Aug;51(2):294-9. PMCID: PMC4148036
4. Camirand G, Wang Y, Lu Y, Wan YY, Lin Y, Deng S, Guz G, Perkins DL, Finn PW, Farber DL, Flavell RA, Shlomchik WD, Lakkis FG, Rudd CE, Rothstein DM. CD45 ligation expands Tregs by promoting interactions with DCs. J Clin Invest. 2014 Oct;124(10):4603-13. PMCID: PMC4191025

4. Surfactant protein D (SP-D) decreases allergic responses

Pulmonary surfactant protein D (SP-D), a member of the collectin family, is an innate immune molecule critical for defense that can also modulate adaptive immune responses. Our findings support a model in which surfactant apoproteins D is important to both innate immunity and adaptive immune responses to allergens. SP-D may be critical for the modulation of early stages of allergic inflammation in vivo. Our results indicate that SP-D decreases allergen responses, an effect that may be mediated by increase of CTLA4 in T cells.

1. Haley KJ, Ciota A, Contreras JP, Liou, HC, Boothby MR, Perkins DL, **Finn PW**. Alterations in the collectins surfactant A and surfactant D in an adaptive allergic immune response. Am J Physiology. 2002;282:L573-84.
2. Schaub B, Westlake R, He H, Arestides R, Hawgood S, Poulain FR, Perkins DL, **Finn PW**. Surfactant protein D influences the development of allergic immune responses. Clin Exp Allergy. 2004;34:1819-26.

3. Lin KW, Jen KY, Suarez CJ, Crouch E, Perkins DL, **Finn PW**. Surfactant protein D mediated decrease of allergen-induced inflammation is dependent upon CTLA4. J of Immunol. 2010;184:6343 -6349. PMCID: PMC2905687

5. Analysis of gene networks

With my collaborator, Dr. David Perkins, our laboratory group has analyzed hubs in biological interaction networks as a method of understanding, predicting, and possibly treating disease. We have used self-organizing maps to demonstrate a crucial role for NF-kappaB in acute allograft rejection, identify different molecular mechanisms of rejection by distinct NF-kappaB family members, and identify a small subset of inducible genes whose inhibition is linked to graft acceptance. In our model of allergic asthma, our analysis suggests that a combination of differential gene expression plus topological characteristics of the interaction network provides enhanced understanding.

1. **Finn PW**, He H, Ma C, Mueller T, Stone JR, Liou HC, Boothby MR, Perkins DL. Molecular profiling of the role of the NF-kappaB family of transcription factors during alloimmunity. J Leukoc Biol. 2002 Nov;72(5):1054-62. PMID:12429729
2. Lu X, Jain VV, **Finn PW**, Perkins DL. Hubs in gene network exhibit low changes in expression in allergic response in experimental asthma. Mol Syst Biol. 2007; 3:98.
3. Jain VV, Perkins DL, **Finn PW**. Costimulation and allergic responses: Immune and bioinformatic analyses. Pharmacol Ther. 2008; 117:385-92.
4. Kadota P, Hajjiri Z, **Finn PW**, Perkins DL. Precision Medicine Applied to T Cell Mediated Rejection. Frontiers in Immunology. 2015 Nov 6;6:536. PMCID: PMC4635852

D. Research Support

Ongoing Research Support

5T32HL082547-08 Role: PI 05/01/2012 – 04/30/2017
NIH/NHLBI
Pulmonary and Critical Care Post-Doctoral Research Training Program
The major goal of this institutional training grant is to train the next generation of investigators focusing on pulmonary and critical care medicine research.

Pending Research Support

1R01DK112320-01 (MPI: Perkins, Finn) 12/01/2016 – 11/30/2021
NIH/NIDDK
The Virome in Kidney Transplantation
The major goal of this project is to determine the viruses that impair the kidney allograft and analyze how the body's immune response is influenced by viruses.

Completed Research Support

5R01AI053878 Role: PI 04/01/2009 – 03/31/2014
NIH/NIAID NCE: 04/01/2014 – 03/31/2015
Pulmonary Interactions with Innate Immunity
The major goal of this project is to determine the mechanisms of modulation of adaptive responses, focusing on molecular and biochemical characterizations of innate immune responses.

Role: PI 07/01/2012 – 06/30/2014
Grifols, Inc NCE: 07/01/2014 – 06/30/2015
Transplantation: Alpha-1 Antitrypsin Deficiency and Microbial Community
The major goal of this project is to analyze the microbiome in the lung and analyze the effects of specific drugs in changes in the microbiome following transplantation.

1R01AI075317-01A2 Role: PI 08/01/2009 – 07/31/2012
NIH/NIAID
Transplantation: Alloimmune Networks
The major goal of this project is to understand the molecular interactions that regulate the immune response during graft rejection.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Gen-Sheng Feng	Professor
eRA COMMONS USER NAME (credential, e.g., agency login) GSFENG	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Zhejiang University, Hangzhou, Zhejiang	B.Sc.	01/82	Biology
2nd Medical University of Army, Shanghai	M.Sc.	12/84	Immunology
Indiana University Bloomington, Indiana	Ph.D.	08/90	Molecular Biology
The Hospital for Sick Children, Toronto	Postdoctoral	08/91	Molecular Biology
Research Institute, Mt Sinai Hospital, Toronto	Postdoctoral	04/94	Signal Transduction

A. Personal Statement

Our research program aims at understanding the dynamic interplay between signaling pathways in different cell types under physiological or pathological conditions. This work was initiated during my postdoctoral studies, by cloning of mouse interferon-induced and dsRNA-dependent protein kinase (PKR) and identification of its dsRNA-binding motifs in Bryan Williams lab, and discovery of an SH2 domain-containing tyrosine phosphatase Shp2 (originally called Syp) in Tony Pawson lab. The current lab focuses are on deciphering signal cross-talks in liver cancer and leukemia. In recent experiments, we and others have uncovered new and "paradoxical" anti-oncogenic roles for many oncoproteins in liver cancer. Elucidating the mechanisms of the dual roles of the classical oncoproteins in the liver may lead to a paradigm shift in understanding the initiation and development of liver cancer that is now the 2nd leading cause of cancer death worldwide. Our goal is to provide novel diagnostic and therapeutic strategies for liver cancer patients. Meanwhile, we are very interested in dissecting the antagonistic and cooperative roles of Pten- and Shp2-regulated signals in leukemia and anemia, which were just unlocked in our lab most recently. Together, these studies will provide better understanding of cell signaling mechanisms in carcinogenesis.

1. Feng GS, Hui CC, Pawson T. 1993. SH2-containing phosphotyrosine phosphatase as a target of protein-tyrosine kinases. ***Science*** 259: 1607-11. PMID: 8096088
2. Bard-Chapeau EA, Li S, Ding J, Zhang SS, Zhu HH, Princen F, Fang DD, Han T, Bailly-Maitre B, Poli V, Varki NM, Wang H, Feng GS. 2011. Ptpn11/Shp2 acts as a tumor suppressor in hepatocellular carcinogenesis. ***Cancer Cell*** 19: 629-39 (*Highlighted in Hepatology 55:322-4, 2012*). PMID: 21575863 PMCID: PMC3098128
3. Feng GS. 2012. Conflicting roles of molecules in hepatocarcinogenesis: paradigm or paradox. ***Cancer Cell*** 21: 150-4. PMID: 22340589 PMCID: PMC3285429
4. Li S, Hsu DD, Li B, Luo X, Alderson N, Qiao L, Ma L, Zhu HH, He Z, Suino-Powell K, Ji K, Li J, Shao J, Xu HE, Li T, Feng GS. 2014. Cytoplasmic Tyrosine Phosphatase Shp2 Coordinates Hepatic Regulation of Bile Acid and FGF15/19 Signaling to Repress Bile Acid Synthesis. ***Cell Metab*** 20: 320-32. *(This article was recommended by Faculty 1000, Previewed by Perino and Schoonjans, Another Shp on the horizon for bile acids, Cell Metab 20, 203-204, 2014).* PMID: 24981838 PMCID: PMC4365973
5. Bard-Chapeau EA, Hevener AL, Long S, Zhang EE, Olefsky JM, Feng GS. 2005. Deletion of Gab1 in the liver leads to enhanced glucose tolerance and improved hepatic insulin action. ***Nat Med*** 11: 567-71. PMID: 15821749

B. Positions and Honors

Positions and Employment

1994-1999 Assistant Professor, Dept. of Biochemistry, Indiana University School of Medicine

1999-1999 Associate Professor, Dept. of Biochemistry, Indiana University School of Medicine
2000-2005 Associate Professor, Burnham Institute for Medical Research, La Jolla, CA
2005-2009 Professor, Burnham Institute for Medical Research, La Jolla, CA
2009- Professor (with tenure), Department of Pathology, School of Medicine, and Molecular Biology Section, Division of Biological Sciences, University of California San Diego, La Jolla, CA

Selected Honors

1988-90 Indiana University Floyd Fellowship, Indiana University Bloomington, Indiana
1990 Outstanding Associate Instructor Award, Howard Hughes Medical Institute & Biology Department, Indiana University Bloomington
1990-1993 Post-doctoral Fellowship of Medical Research Council of Canada
1995-1997 Career Development Award of American Diabetes Association
1995 Carrie E. Wolff Award of American Heart Association-Indiana Affiliate, Inc. for the highest grant-in-aid merit score
1995-2000 NIH First Award
2001 Distinguished Lecturer, Cleveland Clinic Foundation, Cleveland, Ohio
2015 Elected Fellow, American Association for the Advancement of Science

Public Service

2003-2007 Member, Cell Signaling and Dynamics, CSD (formerly CDF-3) study section, NIH
2006-2011 Editorial Board, *Journal of Biological Chemistry (JBC)*
2006- Editorial Board: *Molecular and Cellular Biology (MCB)*
2015- Editorial Board: *Journal of Hepatology*
1998 American Heart Association, Mid-America Res. Consortium Committee 5B
1999 American Heart Great America Research Consortium Study Group
1994 Ad hoc Reviewer for International Human Frontier Science Program
1996 - Ad hoc reviewer for The U.S. Veterans Affairs Medical Research System.
1998 University Grants committee of Hongkong
2000 Ad hoc reviewer for American Cancer Society, Tumor Biochem. and Endocrinol. Committee
2001-04 Ad hoc member for NIH Hem1, Hem2, CDF3, CDF5, Dev2 study sections
2001 The Wellcome Trust, 2001
2002 Ad hoc reviewer for Canadian Institutes of Health Research
2002 Ad hoc reviewer for Ohio Cancer Research Associates
2004 National Cancer Institute of Canada, 2004
2003-06 American Heart Association-Western Consortium, 2003-2006
2007-08 Ad hoc reviewer, Integrative Physiology of Obesity and Diabetes (IPOD) study section, NIH
2009-10 Ad hoc reviewer, MIST study section, NIH, 2009, 2010
2009-11 Ad hoc member for NIH Special Emphasis Panels (SEP)
2013 Ad hoc member, Hepatobiliary Pathophysiology (HBPP) study section
2013-14 Ad hoc Member, Molecular and Cellular Hematology (MCH) study section
2014 Ad hoc Member, Tumor Cell Biology (TCB) study section
2014-18 Member, Hepatobiliary Pathophysiology (HBPP) study section

C. Contributions to Science:

Identification and characterization of new signaling molecules. In 1990's, my lab identified several important signaling molecules. For example, a new adaptor molecule that shares the same SH3-SH2-SH3 architecture as Grb2, and we named this protein Grap (Grb2-related adaptor protein). Grap, predominantly expressed in lymphocytes, acts to negatively regulate TCR signaling, IL-2 production and lymphocyte proliferation, in contrast to a positive role of Grb2 in cell proliferation. We also identified Gab2, an adaptor molecule that is structurally similar to Gab1 and contains a PH domain, SH3- and SH2-binding motifs. In characterizing Gab2, we further identified GC-GAP, a Rho GTPase-activating protein that binds both Gab1 and Gab2. In further studies, we found a specific role of Gab2 in promoting mammary tumor metastasis.

1. Feng GS, Ouyang YB, Hu DP, Shi ZQ, Gentz R, Ni J. 1996. Grap is a novel SH3-SH2-SH3 adaptor protein that couples tyrosine kinases to the Ras pathway. ***J Biol Chem*** 271: 12129-32. PMID: 8647802

2. Shen R, Ouyang YB, Qu CK, Alonso A, Sperzel L, Mustelin T, Kaplan MH, Feng GS. 2002. Grap negatively regulates T-cell receptor-elicited lymphocyte proliferation and interleukin-2 induction. ***Mol Cell Biol*** 22: 3230-6. PMID: 11971956 PMCID: PMC133801
3. Zhao C, Yu DH, Shen R, Feng GS. 1999. Gab2, a new pleckstrin homology domain-containing adapter protein, acts to uncouple signaling from ERK kinase to Elk-1. ***J Biol Chem*** 274: 19649-54. PMID: 10391903
4. Zhao C, Ma H, Bossy-Wetzel E, Lipton SA, Zhang Z, Feng GS. 2003. GC-GAP, a Rho family GTPase-activating protein that interacts with signaling adapters Gab1 and Gab2. ***J Biol Chem*** 278: 34641-53. PMID 12819203
5. Ke Y, Wu D, Princen F, Nguyen T, Pang Y, Lesperance J, Muller WJ, Oshima RG, Feng GS. 2007. Role of Gab2 in mammary tumorigenesis and metastasis. ***Oncogene*** 26: 4951-60. PMID: 17310989

Positive and negative control of intracellular signaling pathways. In the past two decades, we have extensively investigated regulation of intracellular signaling events using Shp2 as an entry point. We have determined that Shp2 modulates signal strength of multiple pathways in positive or negative manner. For example, Shp2 acts to suppress interferon-stimulated Jak-Stat pathway, in contrast to its positive role in Erk activation. However, Shp2 acts to promote IL-6 induction by IL-1 and TNFα through modulation of the NF-κB pathway. Interestingly, we demonstrated that Shp2 also regulates fibroblast cell migration and focal adhesion by counteracting FAK activity, an interesting finding independently confirmed by other groups. This in vitro data in cell culture was substantiated by experiments showing a role of Shp2 in orchestrating granule cell migration during cerebellar development. All of these experimental data from our lab have contributed to better understanding of cell signaling events in various cell types in health and alternations in diseases.

1. You M, Yu DH, Feng GS. 1999. Shp-2 tyrosine phosphatase functions as a negative regulator in the interferon-stimulated Jak/STAT pathway. ***Mol Cell Biol*** 19: 2416-24. PMID: 10022928 PMCID: PMC84034
2. You M, Flick LM, Yu D, Feng GS. 2001. Modulation of the Nuclear Factor kappaB Pathway by Shp-2 Tyrosine Phosphatase in Mediating the Induction of Interleukin (IL)-6 by IL-1 or Tumor Necrosis Factor. ***J Exp Med*** 193: 101-10. PMCID: 2195877
3. Yu DH, Qu CK, Henegariu O, Lu X, Feng GS. 1998. Protein tyrosine phosphatase Shp-2 regulates cell spreading, migration and focal adhesion. ***J Biol Chem*** 273: 21125-31. PMID: 9694867
4. Hagihara K, Zhang EE, Ke YH, Liu G, Liu JJ, Rao Y, Feng GS. 2009. Shp2 acts downstream of SDF-1alpha/CXCR4 in guiding granule cell migration during cerebellar development. ***Dev Biol*** 334: 276-84. PMID: 19635473 PMCID: PMC2744846

Elucidating new mechanisms of liver tumorigenesis. Our current focus is on understanding pathways that control cell proliferation. By biochemical and genetic analyses, we demonstrated a positive role of Shp2 in enhancing Ras-Mek-Erk signaling, contributing to establishment of a dogma that a phosphatase enhances signaling from RTKs-to-Erk. Consistently, we have found that formation of a Gab1/Shp2 complex is necessary to promote hepatocyte proliferation following liver damage. However, we were surprised to observe that ablating Shp2 in hepatocytes resulted in enhanced liver tumorigenesis, identifying a new tumor suppressor role of Shp2 in the liver. Consistently, other groups detected similar anti-oncogenic effects of many classical pro-oncogenic molecules. We believe that elucidating the dual roles of these molecules will lead to a new paradigm in understanding mechanisms of liver carcinogenesis and will catalyze design of new pharmaceuticals for treatment of liver cancer patients. Along this line, we have elucidated a role of Shp2 in coordinating bile acid and FGF15/19 signaling for the control of hepatic bile acid biosynthesis.

1. Shi ZQ, Yu DH, Park M, Marshall M, Feng GS. 2000. Molecular mechanism for the Shp-2 tyrosine phosphatase function in promoting growth factor stimulation of Erk activity. ***Mol Cell Biol*** 20: 1526-36. PMID: 10669730 PMCID: PMC85329
2. Qu CK, Yu WM, Azzarelli B, Feng GS. 1999. Genetic evidence that shp-2 tyrosine phosphatase is a signal enhancer of the epidermal growth factor receptor in mammals. ***Proc Natl Acad Sci USA*** 96: 8528-33. PMID: 10411909 PMCID: PMC17550
3. Bard-Chapeau EA, Yuan J, Droin N, Long S, Zhang EE, Nguyen TV, Feng GS. 2006. Concerted functions of Gab1 and Shp2 in liver regeneration and hepatoprotection. ***Mol Cell Biol*** 26: 4664-74. PMID: 16738330 PMCID: PMC1489129

Metabolic signaling and metabolic disorders. Identification of leptin greatly facilitated understanding the physiology of energy metabolism and obesity. We found that Shp2 binds the leptin receptor to amplify leptin signal in the hypothalamus (16). We further demonstrated that Shp2 couples leptin and estrogen signals by association with ERα (17). Detection of the Shp2/ERα complex in connection with leptin signaling may help explain: *a)* why estrogen has leptin-like effect in the hypothalamus; *b)* why postmenopausal women have tendency to become over-weight or obese; and *c)* why obesity promotes breast cancer development. We also found that deletion of Shp2 in adipocytes leads to severe lipodistrophy (18). Together, these data provide new mechanistic insights into obesity and adipogenesis. Insulin deficiency and Insulin resistance are the two key diagnoses for type 1 and 2 diabetes, respectively. By creating gene KO animal model, we have uncovered a new mechanism for coordinated regulation of multiple signaling events in control of insulin biosynthesis in pancreatic beta cells (19). In dissecting hepatic response to insulin, we have also demonstrated that Gab1, structurally similar to IRS1 and IRS2, acts to negatively regulate insulin signaling in hepatocytes (20).

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Regulation of hematopoiesis and stem cell differentiation. Elucidating the molecular mechanisms that control stem cells' self-renewal *versus* differentiation is at the heart of stem cell biology. We have addressed this issue in embryonic, hematopoietic and neural stem cells (ESCs, HSCs and NSCs). Our group uncovered an essential role for Shp2 in development of all blood cell lineages, in particular Shp2 is positively required for hematopoietic stem/progenitor cell fate commitment and differentiation during embryonic hematopoiesis. We further demonstrated an essential role of Shp2 in maintenance of the hematopoietic stem cell pool in adult mammals. Of note, Shp2 acts downstream of Kit receptor to control expression of the *Kit* gene, thus forming a Kit-Shp2-*Kit* signaling circuit. In addition, we found that Shp2 operates in thymocytes to promote both pre-TCR and TCR signaling during T lymphopoiesis. A similar signaling mechanism is conserved between HSCs and NSCs. Elucidating the positive role of Shp2 in hematopoiesis facilitated characterization of a leukemogenic effect of Shp2/Ptpn11 in Noonan syndrome patients and also in JMML with somatic Ptpn11 mutations. Together, these data allowed for identification of Ptpn11 as the 1st proto-oncogene that codes for a tyrosine phosphatase. We provided data showing that mouse ES cells homozygous for Shp2 deletion are defective in differentiation in vitro, and we elucidated a mechanism of LIF-Shp2-Stat3 signaling pathway in control of ES cell self-renewal. Our experimental results further suggest that the role of Shp2 in promoting ES cell differentiation is conserved in mouse and human ES cells.

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Complete List of Published Work in MyBibliography:

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D. Research Support

Active:

R01CA176012 (PI: FENG) 03/01/14 - 02/28/19 2.40 calendar
NIH (NCI)
Molecular and cellular communications in liver tumorigenesis
The primary goal of this project is to determine how hepatocytes and Kupffer cells communicate in control of hepatocyte proliferation and cancer development, and to elucidate how Stat3 mediates the intrahepatic cell-cell interactions.

R01CA188506 (PI: FENG) 07/01/15 - 06/30/20 2.40 calendar
NIH (NCI)
Dissection of Pten-regulated signals in hepatopathogenesis
The goal of this project is to determine the molecular mechanisms of Pten-regulated signaling pathways in liver diseases and cancer development.

R01HL129763 (PI: FENG) 09/01/15 - 03/31/19 2.40 calendar
NIH (NHLBI)
Shp2 and Pten in leukemia and anemia
The objective of this project is to elucidate the antagonistic effects of Pten and Shp2 in myelopoiesis and leukemogenesis, while cooperative roles in erythropoiesis.

Completed:

R01 HL096125 (PI: FENG) 05/01/99 – 06/31/14 2.40 calendar
Tyrosine dephosphorylation and blood cell development
The major goal of this project is to specifically determine roles of Shp2 in hematopoietic stem/progenitor cell commitment and differentiation using inducible gene targeting mouse models.

R01 DK075916 (PI: FENG) 12/01/09 - 11/30/13 2.40 calendar
Coordinated regulation of signaling events for insulin biosynthesis and secretion
The long-term goal of this project is to determine how multiple pathways are coordinated in control of insulin synthesis and secretion in pancreatic beta-cells.

RESEARCH PLANS

A. STATEMENT OF PROBLEM AND SPECIFIC AIMS

STATEMENT OF PROBLEM

Asthma is an inflammatory disorder characterized by airway inflammation and airway hyperresponsiveness (AHR). The incidence of asthma is increasing worldwide in the past decades. In Los Angeles/Southern California, the incidence of asthma is relatively high, partially due to the local weather condition and air pollution status. The Santa Ana winds are strong, extremely dry down-slope winds that originate from inland and blow to Southern California. The winds bring dust, pollen, fungal spores and occur mostly in autumn and winter, the seasons with increased respiratory disease patients. It has long believed that Santa Ana winds can aggravate respiratory illness especially in patients who have pre-exist respiratory diseases such as asthma with increased emergency department visits and increased hospitalization (1). In addition, the air pollution in Los Angeles area has been suspected for decades to cause respiratory symptoms with recent evidence that decreases in ambient pollution levels were associated with statistically significant decreases in bronchitic symptoms in children (2).

Asthma is a disease with marked heterogeneity. Asthma can be subdivided into several phenotypes on the basis of clinical, physiological and inflammatory markers (3). For example, asthma with elevated eosinophils; asthma with allergic phenotype manifested by Th2 cytokines secretion; asthma with non-allergic phenotype manifested by the involvement of innate immune response (4). These phenotypes are often mixed. Approximately 5% of patients have severe refractory asthma that responds poorly to all the available treatment including high dose inhaled or systemic glucocorticoid treatment.

It was generally believed that most patients with asthma have an immune-mediated component mediated by adaptive immune response with T cell activation and the type 2 T helper (Th2) cytokines secretion. Therefore, inhibition of the Th2 cytokines was the focus of most current therapeutic approaches. Pharmaceutical approaches targeting IgE, IL-4, IL-5 and IL-13 by antibodies were extensively studied and tested in clinical trials. However, with the exception of the anti-IgE monoclonal antibody, other biological therapies have not yield significant clinical impact in the treatment of asthma (5-7). On the other hand, some Th1 cytokines, such as interferon-γ (IFN-γ), was increased in asthma patients and murine model of allergic asthma (8, 9). Only limited studies were done to address IFN-γ, as a therapeutic target for asthma (10).

Complete T cell activation requires two signals from antigen presenting cells (APCs): an antigen specific signal mediated by the T cell receptor (TCR), and a costimulatory signal mediated by CD28 and CD80/CD86 (11) **(Figure 1)**. CD28 family members include CD28, cytotoxic T lymphocyte antigen 4 (CTLA4), and programmed death-1 (PD-1) (12, 13). CD28 binds to CD80/CD86 and positively mediates the costimulatory signal leads to complete T cell activation. CTLA4 binds to CD80/CD86 and blocks the costimulatory signal, leads to inhibition of T cell activation. PD-1 is responsible for the T-cell "exhaustion" and prevents autoimmune disease (14). CTLA4 or PD-1 knockout mice display lymphoproliferation in lymph nodes and spleen, consistent with their roles of negative regulators of T cell activation (15-17).

We previously reported that CTLA4-Ig, a fusion protein known to inhibit the engagement of CD28 and CD80/CD86, achieved therapeutic effects in murine asthma model, but not effective enough in severe asthma models (18, 19). The role of PD-1 in asthma is controversial. PD-1 ligand 1 (PD-L1) knockout mice have reduced airway hyperresponsiveness (AHR), but not the PD-L2 knockout mice (20). PD-L1 expression is upregulated by Th1 cytokines, predominantly by IFN-γ (21). PD-L1 and PD-L2 are widely expressed including bronchial epithelial cells. The roles of bronchial epithelial cells in the pathogenesis of asthma were re-defined. The bronchial epithelial cells release TSLP, IL-25 and IL-33 in response to various stimuli or cellular damage. These cytokines result in airway remodeling and pathological

changes resemble asthma (22).

Taken together, CD28 contributes to T cell activation and PD-L1 contributes to increased AHR, whereas CTLA4 contributes to T cell inhibition and PD-L2 contributes reduced AHR. We speculate that IFN-γ, the best-known stimulator of PD-L1 expression, may contribute to the induction of PD-L1 in bronchial epithelial cells. The upregulation of PD-L1 in bronchial epithelial cells promotes severe inflammation result in airway remodeling and AHR.

We reasoned that in order to treat the severe refractory asthma, we will need to address not only the T cells activation and Th2 cytokines, we need to address the bronchial epithelial cells, especially in the patients with severe inflammation and airway remodeling. We need to better understand the (1) What is the function of Th1 cytokines, such as IFN-γ, in asthma; (2) the interaction between T cells and bronchial epithelial cells in asthma; (3) the bronchial epithelial cells as the target of asthma treatment. We focus on CTLA4, which we have done extensive work in the past, both in T cells and animal asthma models. We propose that other that targeting CTLA4 in T cells and PD-1/PD-L1 in bronchial epithelial cells, will provide us with greater opportunities to improve clinical practice by identifying new molecular therapeutic targets for intervention.

We **hypothesize** CTLA4-Ig inhibits T cell activation, Th2 cytokines production and more importantly, IFN-γ production, which is the major stimulator of PD-L1 expression. As a result, the PD-L1 levels in T cells and bronchial epithelial cells will be decreased. We **hypothesize** that the combination of CTLA4-Ig and anti-PD-L1 antibody will have synergistic therapeutic effects in allergic asthma model. CTLA4-Ig inhibits the T cell activation and IFN-γ production. Meanwhile, anti-PD-L1 will further block the function of PD-L1 in bronchial epithelial cells to reduce AHR.

SPECIFIC AIMS

Aim 1. The effects of CTLA4-Ig on IFN-γ and PD-L1 expression in spleen T cells and bronchial epithelial cells.

CTLA4-Ig is a fusion protein that prevents the engagement of CD28 and CD80/CD86 and thus inhibits T cell activation. It has been shown that CTLA4-Ig can inhibit IFN-γ production in some disease models (23, 24). IFN-γ is known to be the major stimulator of PD-L1 expression (21, 25). We will examine the effects of CTLA4-Ig on IFN-γ and PD-L1 expression in mouse primary spleen CD4+ cells and human bronchial epithelial cells, BEAS-2B. We will determine if CTLA4-Ig can inhibit IFN-γ and PD-L1 expression. Non-specific IgG and anti-CTLA4 antibody will be used as controls.

Aim 2. Determine the effects of combination of CTLA4-Ig and anti-PD-L1 antibody on OVA-induced mouse allergic asthma model *in vivo.*

By using OVA-induced mouse asthma models, we will examine the effects of CTLA4-Ig, anti-PD-L1 antibody, and in combination of the two, in mouse allergic asthma model. We will examine the AHR, inflammation parameters and cytokines in bronchoalveolar lavage (BAL), serum IgE to evaluate the therapeutic effects.

B. BACKGROUND AND SIGNIFICANCE

BACKGROUND

Allergic asthma is an inflammatory disorder characterized by increased antigen-dependent immune responses. Asthma has become an epidemic affecting 300 million people in the world. The chronic inflammation in asthma is due to the persistence of Th2 lymphocytes and Th2 cytokines produced by both the structural cells of the lung as well as the infiltrating lymphocytes, eosinophils, and mast cells. New therapeutic approaches and strategies are needed for asthmatic patients.

The pathogenesis of asthma was previously described as an imbalance in Th1/Th2 T cell subset function. Th cells were divided into two subsets on the basis of their cytokine secretion profiles. Specifically, Th1 cells produce IFN-γ and TNF-α, whereas Th2 cells produce IL-4, IL-5, and IL-13 (26). Immune mediated diseases and animal models were previously characterized as primarily a Th1 (e.g., autoimmunity) or Th2 (e.g., asthma and allergy) predominant state. However, Th1 cytokines (e.g. IFN-γ) may also play a crucial role in allergic airway inflammation (27). IFN-γ is detected in human allergic responses and murine allergic models (8, 9). Thus, a strict Th1 and Th2 paradigm was found to be insufficient to explain allergic asthmatic phenotypes. Antibodies against several of the Th2 cytokines were tested in clinical trials without success. Only limited studies were done to address Th1 cytokines, such as IFN-γ, as a therapeutic target for asthma (10).

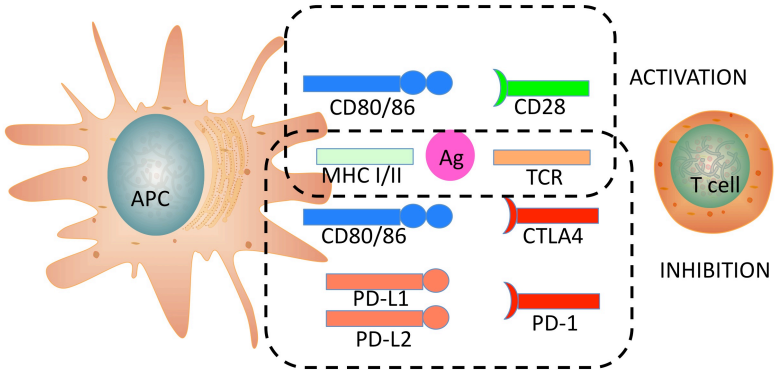

Figure 1. Model of immune checkpoint molecules and T cell function. Inhibitory T cell co-receptors such as CTLA4 and PD-1 cause T cell anergy and apoptosis.

Ag = antigen
APC = antigen-presenting cell
CTLA4 = cytotoxic T lymphocyte antigen 4
PD-1= Programmed death 1
PD-L1= Programmed death ligand1
PD-L2= Programmed death ligand2
MHC I/II = major histocompatibility complex class-I and class-II
TCR = T cell receptor

Complete T cell activation requires two signals: an antigen specific signal mediated by T cell receptor (TCR), and a costimulatory signal mediated by the engagement of CD28 on T cell surface with CD80/CD86. CTLA4 is expressed in T cells at low levels and is rapidly upregulated after T cell activation (28, 29). CTLA4 is structurally similar to CD28 and can competitively bind to CD80/CD86 with much higher affinity. The binding of CTLA4 and CD80/CD86 therefore blocks the costimulatory signal mediated by CD28 and inhibits the T cell activation (30). CTLA4 expression is regulated mainly at the transcriptional level by T cell activation (28). In CTLA4 knockout mouse, the mouse exhibits lymphoproliferation in lymph nodes and spleen, indicating that CTLA4 is a negative regulatory T cell activition (15, 16).

PD-1 is another member in the CD28 family. Similar to CTLA4, PD-1 knockout mouse showed moderate splenomegaly and increased cellularity of lymphoid and myeloid cells (17). PD-1 has been shown to negatively regulate TCR signaling and to decrease T cell proliferation and cytokine production (31). The mechanism of how PD-1 negatively regulates T cell activation is unclear. In contrast to the well-defined role of CTLA4 in asthma, the role of PD-1 is somewhat controversial. PD-1 has two ligands, PD-1 ligand 1 (PD-L1) and PD-1 ligand 2 (PD-L2). PD-L1 and PD-L2 expression can be rapidly upregulated on various cells and tissues (32, 33). PD-L1 expression is upregulated by Th1 cytokines, predominantly by IFN-γ (21). PD-L2 is upregulated by Th2 cytokines, predominantly IL-4 (21). PD-L1 and PD-L2 have opposite functions in mouse asthma model. PD-L1 contributes to increased AHR, this is evident by respiratory infection-induced PD-L1 promotes severe inflammation and development of severe allergic airway disease (34). PD-L1 knockout mice have reduced airway hyperresponsiveness

(AHR) (20). In contrast, PD-L2 knockout mice developed higher AHR (20). PD-L1/PD-L2 double knockout mice neutralize the effects (20). These data indicate that the PD-L1/PD-L2 ratio is important to the development of allergic asthma. Interestingly, PD-L1 expression is upregulated by Th1 cytokines, predominantly by IFN-γ (21), the Th1 cytokines found to be increased in asthma but not well studied as a therapeutic target in asthma (10).

The roles of CTLA4 in allergic asthma models were extensively studied. CTLA4 can bind to CD80/CD86 with greater affinity than to CD28 to block the costimulatory function of CD28. In the mouse allergic asthma model, mice lacking CD80 or CD86 or both did not develop pulmonary inflammation and AHR, indicates the important role of the costimulatory signal in the pathogenesis of allergic asthma (35, 36).

We previously reported that blockade of the CD28/CD80/CD86 costimulatory pathway dramatically inhibits pulmonary inflammation and AHR in a murine model of allergic asthma (37, 38). We then focused on CTLA4-Ig (FDA approved drug for renal transplant), a fusion protein known to inhibit the engagement of CD28 and CD80/CD86. CTLA4-Ig again showed therapeutic effects in murine asthma model, but not effective enough in severe asthma models (18, 19). With the recent development of checkpoint proteins blockade in cancer immunotherapy, the interactions between CTLA4 and PD-1/PD-L1 are better understood. In melanoma, the upregulation of PD-L1 by CTLA4 blockade therapy is considered as possible reason for the uneffectiveness of CTLA4 blockade therapy (39). Combined CTLA4 and PD-1/PD-L1 blockade therapy showed better efficacy than single blockade (40).

Recently, the interaction between CTLA4 and PD-1 are under investigation in cancer immunotherapy. Combined CTLA4 and PD-1/PD-L1 blockade therapy showed better efficacy than single blockade. We ask whether there are interactions between CTLA4 and PD-1/PD-L1 in asthma and whether IFN-γ mediates the interactions. More importantly, we ask whether CTLA4-Ig can inhibit IFN-γ production, which is the main stimulator of PD-L1 expression and whether additional anti-PD-L1 antibody will inhibit the PD-L1 in bronchial epithelial cells.

We therefore ask whether there are interactions between CTLA4 and PD-1/PD-L1 in asthma and whether IFN-γ mediates the interactions. Moreover, we ask whether CTLA4-Ig can inhibit IFN-γ production, which is the main stimulator of PD-L1 expression and whether additional anti-PD-L1 antibody will inhibit the PD-L1 in bronchial epithelial cells. We expect that targeting CTLA4 in T cells and PD-1/PD-L1 in bronchial epithelial cells simultaneously will provide us with more effective therapeutics in severe asthma, like the synergistic effects observed in cancer treatment.

SIGNIFICANCE

The idea that CTLA4 and PD-1/PD-L1 interactions play roles in asthma is innovative. The idea that CTLA4-Ig may not only inhibit T cells activation, but also inhibit IFN-γ production, leading to decreased PD-L1 expression in T cells and lung epithelial cells is innovative.

The limited effectiveness of CTLA4-Ig in treatment of mouse asthma model may be explained by the still abundance of PD-L1 expression in T cells and lung epithelial cells. Based on the roles of PD-L1 (but not PD-L2) in the pathogenesis of asthma, we may need to further inhibit the PD-L1 expression and/or function to achieve a more effective treatment.

This provides us with the innovative idea that combination of CTLA4-Ig and anti-PD-L1 antibody may have synergistic therapeutic effects in allergic asthma model. CTLA4-Ig inhibits the T cell activation and IFN-γ production. Meanwhile, anti-PD-L1 may further block the function of PD-L1 in peripheral tissues to reduce the manifestations of asthma.

Both drugs in our proposal are FDA approved drugs. CTLA4-Ig was approved for renal transplant patients. Anti-PD-L1 was approved in May 2016 for cancer patients. They both have a relative low

profile of side effects. Although pneumonitis was described in patients treated with PD-1 blocking agents, overall safety profiles are good and well tolerated (41). The treatment for the pneumonitis is glucocorticoids, for which most asthma patient would be on already. We hope that by knowing the safety profiles of the drugs well, our mice research will be able to translate to human studies soon.

C. WORK ACCOMPLISHED AND PRELIMINARY STUDIES

1. Expression of CTLA4 and PD-L1 in bronchial epithelial cells

We examined the protein expression and the cell immunostaining of CTLA4, PD-1, and PD-L1 in multiple bronchial epithelial cell lines. In normal bronchial epithelial cells, BEAS-2B, there is no expression of CTLA4 or PD-1 (data not shown). CTLA4 is expressed at various levels in lung cancer bronchial epithelial cell lines. CTLA4 is expressed at relatively high levels in A549, H460, H1975, and HCC827 cells. CTLA4 is expressed at relatively low levels in H661 cells and is not detectable at H1650 cells **(Figure 2A)**. The cellular localization of CTLA4 is in the cytoplasm, the high concentration of the protein is found to surround the nuclear membrane **(Figure 2B)**.

PD-1 expression is undetectable in the cell lines we examined (data not shown). PD-L1 is expressed at various levels in bronchial epithelial cell lines. **(Figure 2A)**. The cellular localization of PD-L1 is in the cytoplasm **(Figure 2B)**.

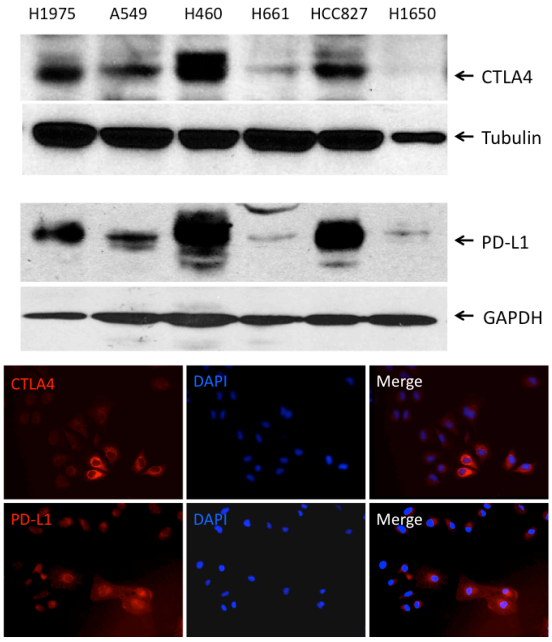

Figure 2. CTLA4 and PD-L1 expression in NSCLC cells
A.CTLA4 is expressed at various levels. PD-1 expression is undetectable (data not shown). PD-L1 is expressed in all NSCLC cell lines.
B.CTLA4 and PD-L1 are localized in the cytoplasm of NSCLC cells.

2. IFN-γ or anti-CTLA4 treatment upregulates PDL1 expression in bronchial epithelial cells

IFN-γ or anti-CTLA4 antibody treatment can increase PD-L1 expression **(Figure 3A)**. IFN-γ (100ng/mL) was used as a positive control. The upregulation of PD-L1 expression is observed in the cell lines with higher CTLA4 expression, such as in A549 and H460, but not the in the cells with low or no CTLA4 expression, such as H661 and H1650 **(Figure 3A)**. This is

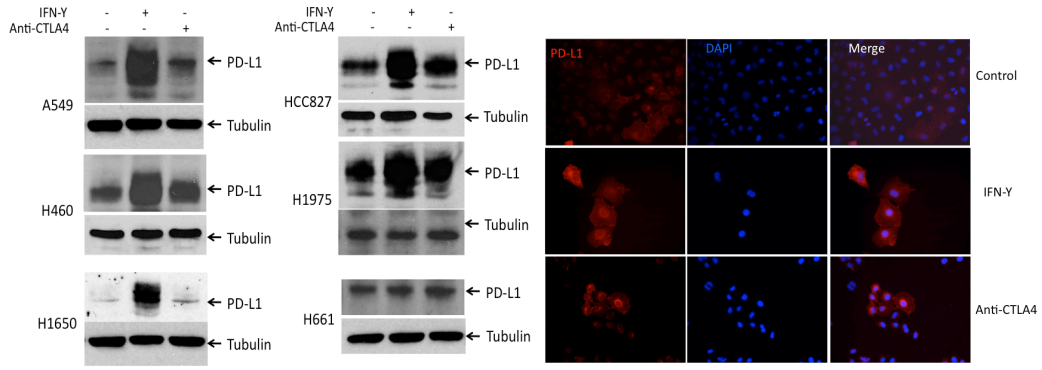

Figure 3. Effects of IFN-γ and anti-CTLA4 antibody treatment on PD-L1 expression in NSCLC cells
A.The indicated cells were treated with IFN-γ (100ng/mL) or anti-CTLA4 antibody (10 μg/ml) for 24 hours. Anti-CTLA4-induced PD-L1 expression is observed in the cell lines with higher intrinsic CTLA4 levels, such as in A549 and H460, but not the in the cells with low or no intrinsic CTLA4 expression, such as H661 and H1650.
B.IFN-γ or Anti-CTLA4 antibody treatment increases cellular PD-L1 (red) in A549 cells.

also demonstrated by cell immunostaining of PD-L1 expression **(Figure 3B),** anti-CTLA4 antibody treatment can increase PD-L1 expression.

3. The *in vivo* effects of CTLA4-Ig in mouse allergic asthma model
In mouse allergic asthma model, we tested the effectiveness of CTLA4-Ig, a fusion protein known to block the CD28 and CD80/CD86 engagement, thus inhibit T cell activation. CTLA4-Ig inhibits airway hyperresponsiveness and inflammatory infiltration in the mouse allergic asthma model (18) **(Figure 4)**.

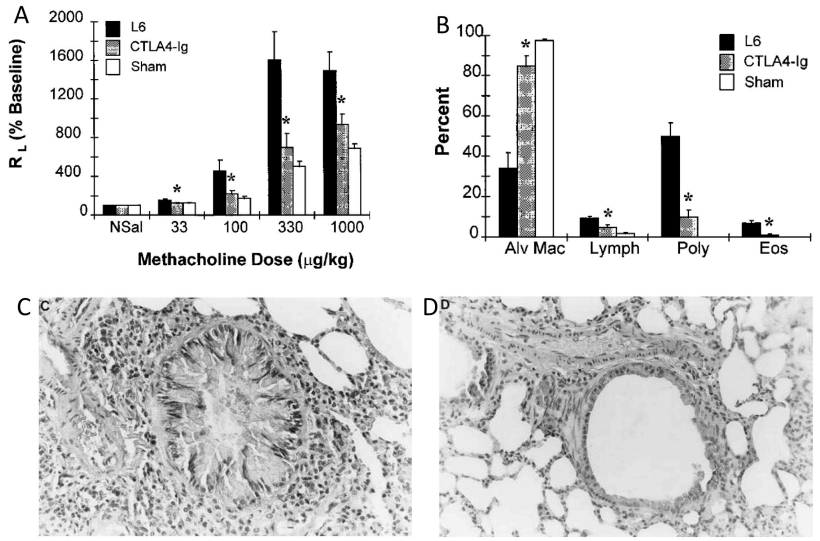

Figure 4. Effect of CTLA4-Ig on OVA induced mouse asthma model
Groups: (1) L6, control antibody in OVA model; (2) CTLA4-Ig in OVA model, and (3) Sham, no OVA, PBS model.
A. Airway resistance in response to methacholine.
B. Relative proportions of inflammatory cells in BAL fluid.
C. Peribronchial and perivascular inflammation in L6-treated mice.
D. Lung sections from CTLA4-Ig-treated mice, demonstrating significantly less of inflammatory cells.

Summary of preliminary data:
1. We found that CTLA4 is expressed not only in T cells, but some lung epithelial cell lines.
2. We demonstrated that IFN-γ or anti-CTLA4 can upregulate PD-L1 expression in bronchial epithelial cells. Anti-CTLA4 antibody can increase PD-L1 expression in lung epithelial cell lines with higher CTLA4 expression, but not the ones with low or no CTLA4 expression. IFN-γ can even increase PD-L1 expression in lung epithelial cell lines with low CTLA4 expression.
4. CTLA4-Ig, a fusion protein known to block the CD28 and CD80/CD86 engagement, has favorable therapeutic effects in mouse allergic asthma model.

D. RESEARCH DESIGN AND METHODS

We will investigate whether the combination of CTLA4-Ig and anti-PD-L1 antibody will have synergistic therapeutic effects in allergic asthma model. CTLA4-Ig inhibits the T cell activation and IFN-γ production. Meanwhile, anti-PD-L1 will further block the function of PD-L1 in bronchial epithelial cells to reduce AHR.

Aim 1. The effects of CTLA4-Ig on IFN-γ and PD-L1 expression in spleen T cells and bronchial epithelial cells.

A. Rationale:

It was reported that CD4+ T cells from peripheral blood and tumor tissues from anti-CTLA4 treatment patients had markedly increased production of IFN-γ (42). IFN-γ is known to mediate the induction of PD-L1 expression. It was reported that PD-L1 expression was upregulated in melanoma cells after receiving anti-CTLA4 antibody treatment (39). Therefore, we will investigate whether CTLA4-Ig inhibits T cell activation, Th2 cytokines production and more importantly, IFN-γ production, which is the major stimulator of PD-L1 expression. If we were able to observe the decrease of PD-L1 mRNA and protein, we will perform the PD-L1 promoter reporter assay to identify

B. Experimental Protocol:

1. Isolation of mouse primary spleen CD4+ cells

To obtain mouse primary spleen CD4+ cells, single-cell suspensions will be prepared from spleen. CD4+ T cells will be isolated using negative selection by magnetic-activated cell sorting system (Miltenyi Biotec Inc., Auburn, CA).

2. Cells proliferation

We will examine the effects of CTLA4-Ig on IFN-γ and PD-L1 expression in mouse primary spleen CD4+ cells and normal human bronchial epithelial cells, BEAS-2B. We will treat the cells with 1) control; 2) non-specific IgG (control, 50 μg/ml); 3) CTLA4-Ig (50 μg/ml); 4) anti-CTLA4 (50 μg/ml); and 5) CTLA4-Ig+anti-CTLA4. T cell activation parameters including T cell proliferation after stimulation with mitogen Concavalin A (ConA) will be determined by BrdU incorporation.

The CD4+ T cells collected as above will be grown in RPMI 1640 medium with 10% heat-inactivated fetal bovine serum. Proliferation (2×10^5 cells per well) will be determined using a colorimetric immunoassay, based on the measurement of BrdU incorporation during DNA synthesis. CD4+ T cells will be stimulated with ConA (1 mg/mL) for 48 hours. 48 hrs after stimulation, cells were pulsed with BrdU solution and denatured with FixDenat solution, then incubated with mouse anti-BrdU mAbs conjugated to peroxidase. The absorbance will be measured at 370 nm using an ELISA plate reader.

The bronchial epithelial cells will be grown in RPMI 1640 medium with 10% heat-inactivated fetal bovine serum. Proliferation will be determined by BrdU incorporation.

3. ELISA Cytokines

Both of the CD4+ T cells and bronchial epithelial cells culture supernatant will be collected and tested for IFN-γ, IL-4, IL-5, IL-6, IL-13 by ELISA.

4. Real time PCR of PD-L1 mRNA levels and Western blots of PD-L1 protein

Total RNA will be isolated with TRI reagent (Sigma-Aldrich). Isolated RNA is reverse-transcribed with SuperScript II RNase reverse transcriptase (Invitrogen, Carlsbad, CA). Specific primer pairs for GAPDH and PD-L1 will be used. Direct detection of the PCR product was monitored by measuring the increase in fluorescence caused by the binding of SYBR Green to dsDNA (Applied Biosystems) per well during 40 cycles. The cycle threshold (C_t) method was used to assess changes in mRNA expression: fold change = $2^{-\Delta\Delta Ct}$. The lysate containing equal amounts of protein (20 μl) will be loaded on a 10% SDS/PAGE. Anti-PD-L1 antibodies will be used to detect the protein levels by Western blotting.

5. Identify the promoter region mediates the suppression of PD-L1 by CTLA4-Ig

We will use reporter assays to identify the promoter region responsible for the suppression of PD-L1 expression by CTLA4-Ig. We will transfect the cells with the full-length (1.8 Kb) PD-L1-luc reporter (pGL3) gene (Gift from Inhak Choi, MD, PhD) (43). In order to narrow down and identify the promoter region mediates the suppression of PD-L1 expression, the full-length (1.8 Kb) promoter will be used as a template to generate truncated constructs, as previously described (44). Transient transfections will be performed using FuGENE 6 (Roche). After 12 hours of transfection, cells will be treated with 1) non-specific IgG (control, 50 μg/ml); 2) IFN-γ (positive control, 100ng/mL) 3) CTLA4-Ig (50 μg/ml); for 24 hours. PD-L1 reporter assay will be done by measuring the luciferase activity (Promega).

C. Interpretation, potential pitfalls, and alternative approaches:

CTLA4-Ig has been originally developed based on the concept that specific binding to CD80/CD86 molecules to interrupt CD28 mediated signaling to T cells and thus can propagate the emergence of anergy (45). As a potent inhibitor of T cell costimulation signal, CTLA4-Ig is a FDA approved immunosuppressant for renal transplant patients. It can suppress inflammation even if administered when disease is established. A CD28-independent mechanism by which CTLA4-Ig inhibits activated T cells was also identified recently (46). CTLA4-Ig can turn off already activated effector T cells by TGF-ß–dependent pathway (46). It was shown to decrease the manifestation of allergic asthma models by multiple groups including our group (18, 19).

We expect CTLA4-Ig will inhibit cell proliferation and decrease Th2 cytokine secretion in T cells. We expect CTLA4-Ig will also inhibit the IFN-γ secretion, which is not a Th2 cytokine, but frequently increased in asthma patients (8), as well as murine model of allergic asthma (9). We expect anti-CTLA4 antibody will do the opposite. Anti-CTLA4 antibody will increase cell proliferation, Th2 cytokines, and IFN-γ.

We expect CTLA4-Ig will decrease PD-L1 mRNA and protein expression in both T cells and bronchial epithelial cells. We expect anti-CTLA4 antibody will do the opposite, will increase PD-L1 mRNA and protein expression, as we observed in our preliminary data in bronchial epithelial cells. We expect the suppression of *of PD-L1 by CTLA4-Ig is mediated by* IFN-γ at the transcriptional level. If we deleted the IFN-γ responsive region, interferon regulatory factor-1 (IRF-1), at the PD-L1 promoter (43), the suppression would be eliminated.

Aim 2. Determine the effects of combination of CTLA4-Ig and anti-PD-L1 antibody on OVA-induced mouse allergic asthma model *in vivo.*

By using OVA-induced mouse asthma models, we will examine the effects of CTLA4-Ig, anti-PD-L1 antibody, and in combination of the two, in mouse allergic asthma model. We will examine the AHR, inflammation parameters and cytokines in bronchoalveolar lavage (BAL), serum IgE to evaluate the therapeutic effects.

A. Rationale:

The two ligands of PD-1, PD-L1 and PD-L2, have opposite functions in mouse asthma model. PD-L1 knockout mice have reduced AHR (20). PD-L2 knockout mice developed higher AHR (20). These data indicate that the PD-L1/PD-L2 ratio is important to the development of allergic asthma. Because PD-L1 knockout mice have reduced AHR (20), we reasoned that if we block the PD-L1 function by anti-PD-L1 antibody, we will achieve the goal to reduce AHR and inflammation in an allergic asthma model.

B. Experimental Protocol:

1. Mouse model of allergen (ovalbumin, OVA) induced pulmonary inflammation

We have previously characterized a mouse model of allergen (ovalbumin, OVA) induced pulmonary inflammation in wild types strain (BALB/c, or C57BL/6) (18, 38, 47). We will have five groups 1) control; 2) non-specific IgG (control, 50 μg/ml); 3) CTLA4-Ig (50 μg/ml); 4) anti-PD-L1 (50 μg/ml); and 5) CTLA4-Ig+anti-PD-L1 **(Table 1)**. Six- to eight-week-old mice will be sensitized via intraperitoneal injection with 10 μg of chicken OVA and 1 mg of Al(OH)2 (alum) in 0.2 ml of PBS on Day 1, followed by a boosting injection on Day 7 with the identical reagents. Control mice will receive 1 mg of alum in 0.2 ml of PBS without OVA. On Days 15–20, mice will receive aerosolized challenges with 6% OVA or PBS, respectively, for 20 min/day via an ultrasonic nebulizer. Different groups will be sacrificed at Day 0, 15, 21.

2. Administration of CTLA4-Ig and PD-L1 antibody

The timing of administration of CTLA4-Ig and PD-L1 antibody is selected to represent intervention prior to sensitization (Day -2) or during challenge (Day +15). Antibody of 200 μg in 100 μl of PBS will be given by intraperitoneal injection. Experimental groups are as described **(Table 1)**. We will analyze allergic manifestations as described below at the following time points. In addition to the harvest on the last day of the protocol (21 day protocol), we will analyze additional time points relevant to sensitization or challenge (days 0, 15) to monitor changes in cytokines or inflammatory cells that may be manifested early in the protocol.

3. Detection of airway resistance, BAL cell counts, cytokines, histology and immunohistochemistry

Baseline airway resistance and methacholine challenge test will be performed on a mechanical ventilator (FlexiVent, SCIREQ; Quebec, Canada). Allergic parameters include BAL (bronchoalveolar

lavage) eosinophilia and cytokines (IL-4, IL-5, IL-6, IL-13, IFN-γ) and serum IgE levels will be measured. Lung tissue will be examined for histology and immunohistochemistry of CTLA4, PD-1, and PD-L1.

BAL eosinophils: BAL cells will be pelleted and the supernatant stored at -80°C. Cells are resuspended in RPMI and slides for differential cell counts are prepared with Cytospin and fixed and stained with Diff-Quik (Dade Behring). For each sample an investigator blinded to the treatment groups will perform two counts of 100 cells. BAL Cytokines: We will analyze cytokines in the BAL as indicative of a local response to the in vivo treatment. We will focus on the measurement of cytokines reflective of a Th1, Th2 responses. We will analyze IFN-γ, IL-4, IL-5, IL-6, IL-13 by ELISA. IgE analysis: Blood will be withdrawn by cardiac puncture and centrifuged at 13,000 rpm for 20 minutes to recover serum. Total serum IgE levels will be determined by ELISA.

Histology and Immunohistochemistry: Lung tissue will be stained using hematoxylin and eosin and Periodic acid–Schiff (PAS) to compare the histology changes. Immunohistochemistry of CTLA4, PD-1, PD-L1 will be done.

C. Interpretation, potential pitfalls, and alternative approaches:

To confirm the role of the inducible PD-L1 expression *in vivo*, the mice will be treated with CTLA4-Ig, anti-PD-L1 antibody, or both on Day -2 and Day 15 in OVA-induced mouse allergic asthma model. Mice will be sacrificed at Days 0, 14, or 21. Day 0 is selected to examine the cytokine milieu after CTLA4-Ig and/or anti-PD-L1 antibody treatment, and before allergen sensitization. Day 15 will provide information prior to the first aerosol challenge. Day 21 is the last day of protocol.

We expect that IFN-γ will be induced in BAL and PD-L1 will be increased in lung tissue in the mouse allergic asthma model. We expect that CTLA4-Ig will inhibit the IFN-γ, and Th2 cytokines secretion. We expect the combination of CTLA4-Ig and anti-PD-L1 antibody will have synergistic therapeutic effects in allergic asthma model.

We have previous data (Figure 4) to show that CTLA4-Ig is effective in inhibiting AHR and inflammatory infiltration in the mouse allergic asthma model. We expect that we will achieve synergistic therapeutic effects by the combination of CTLA4-Ig and anti-PD-L1 antibody. We have the group with anti-PD-L1 antibody only, so we will able to compare and analyze the difference.

Group	BAL eosinophils	BAL IFN-γ	AHR	PD-L1
Control	-	-	-	
non-specific IgG	-	-	-	
CTLA4-Ig	Decrease	Decrease	Decrease	Decrease
anti-PD-L1	Decrease	-	Decrease	-
CTLA4-Ig + anti-PD-L1	Decrease	Decrease	Decrease	Decrease

Table 1. The effects of CTLA4-Ig and anti-PD-L1 antibody on OVA-induced mouse asthma model *in vivo*

Five groups of male C57BL/6 mice will be analyzed.
Mice will be administered via in-traperitoneal injection with CTLA4-Ig (200 μg), anti-PD-L1 (200 μg) or a control IgG monoclonal antibody on Day -2, 15.

Statistics

Data will be reported as means ± SD. Data from *in vitro* assays will be analyzed by a *t*-test for comparison of two groups or analysis of variance for more than two means. For analysis of multiple conditions across multiple groups a two-way ANOVA will be performed. Sample size for animal experiments has been proposed based on a power analysis using an anticipated treatment effect of 50% fall in airway resistance or a 50% fall in total cell counts anticipating a $p \leq 0.05$. Data will be analyzed by a two-way analysis of variance or repeated measures ANOVA as indicated.

As outlined in the Experimental protocol section, we will analyze mice in a well characterized and previously published mouse model of allergic (ovalbumin, OVA) pulmonary inflammation. We will use wild type C57BL/6 mice. The mice used will be: males, 6-8 weeks of age. We anticipate that each experimental condition will require approximately 6 mice per group (repeated 3 times) to obtain statistical significance. Total number will be 90 mice.

Time line

	Year 1	Year 2
Aim 1	→	
Aim 2		→
Manuscript preparation		By 18 months
R01 Grant Proposal (PI)		By 18-24 months

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Type of Expense	Period 1	Period 2
Start Date	15-Dec-2016	14-Dec-2017
End Date	15-Dec-2017	14-Dec-2018
Personnel Direct Costs		
Salaries & Wages	$10,125	$10,429
Benefits	$4,668	$4,943
SubTotal: Personnel Costs	$14,793	$15,372
Non-Personnel Direct Costs		
Consultant Costs		
Equipment		
Supplies	$23,727	$23,147
Travel	$1,000	$1,000
Patient Care In-Patient		
Patient Care Out-Patient		
Alterations and Renovations		
Other Expenses	NGN: $481	NGN: $481
Consortium & Contractual Direct		
Sub Total: Non-Personnel Costs		
TOTAL DIRECT COSTS	$40,000	$40,000
Indirect Costs		
INDIRECT COSTS	ONLY Allowed for the PHA Proof-of-Concept grants	
TOTAL COSTS	$40,000	$40,000

BUDGET JUSTIFICATION

PERSONNEL:

Jinghong Li, MD, PhD (PI), (50% effort, no salary), Assistant Professor of Medicine, is an experienced physician-scientist in the fields of inflammatory lung diseases. She will be responsible for administration and scientific progress of this grant, overall direction of the project. She will design the experiments, coordinate with Collaborators. She will apply her Molecular Biology expertise to the analysis of gene regulations both *in vitro* and *in vivo*. She will analyze the data and write the papers.

Patricia W. Finn, MD (Collaborator), (5% effort, no salary), Professor and Chair, Department of Medicine, University of Illinois at Chicago. Dr. Finn is an expert in CTLA4 and T cell activation in pulmonary inflammatory disorders. She will collaborate with Dr. Li on the proposed project, with her expertise in T cell activation and pulmonary inflammatory disorders animal models including allergic asthma models.

Gen-Sheng Feng, PhD (Collaborator), (5% effort, no salary), Professor of Pathology and Biology, UCSD. Dr. Feng is an expert in CTLA4 and SH2 domain-containing tyrosine phosphatase (Shp2) pathway. CTLA4 was found to bind to Shp2, and they both play important roles in T cell activation. Dr. Feng has been working on Shp2 airway knockout mouse in OVA induced asthma model to analyze Shp2 function in asthma. He will be responsible to help with the related assays required for the proposal and provide the Shp2 knockout mouse if needed.

TBN, Research Assistant (part time), (25% effort, with salary support), will be responsible to assist the PI in performing experiments. We have two new graduates from UCSD (Biology major, graduated in June 2016) working at lab. They both have experience in molecular biology experiments. If funded, will hire one of them as part time research assistant.

Fringe benefits are calculated at rates currently in effect for the University of California.

SUPPLIES:
General lab supplies are calculated based on past experience in carrying out research in these fields.
First year:
Molecular biological reagents: $9,000; disposable plastic ware and pipettes: $4,000; antibodies: $5,000; animals: $2000; tissue culture media: $3,727 are included.
Total: $23,727
Second year:
Molecular biological reagents: $9,000; disposable plastic ware and pipettes: $4,000; antibodies: $5,000; animals: $3000; tissue culture media: $2,147 are included.
Total: $23,147

TRAVEL:
Travel cost of $1,000 per year for the PI to attend scientific meeting to present the data from the project is included.

NGN:
UCSD Next Generation Network (NGN) communication charge of $481 per year is included.

Applicant's Name **Jinghong Li**

FACILITIES:

LABORATORY:
The laboratory research will be done in Medicine Building 4 on the UCSD campus. The laboratory includes ~1,000 square feet available for research use. Communal space includes 700 square feet of laboratory space, 200 square feet for additional equipment space and a 100 square feet cell culture room.

On the UCSD campus, there are Microscopy Core Facility, Flow Cytometry Facility, Transgenic Mouse Facility, Histology & Immunohistochemistry Facility, Biomedical Genomics Core Facility (BIOGEM), Human Stem Cell Core Facility and many others. All of these cores have consultants available for project specific and technical guidance.

CLINICAL:
Not applicable.

ANIMAL:
Animals are maintained in vivarium rooms, one of which is a total barrier facility all located in the laboratory building. Mice are maintained in vented racks with constant temperature and humidity. Separate rooms are available for animal surgery, storage of feed and bedding, and cage washing. Access to the facility is very limited to specific laboratory personnel. The University of California, San Diego is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International (AAALAC), and holds an approved NIH Assurance and USDA License. There are 148,000 square feet of animal facilities at 27 locations. Support includes quarantine rooms, sterile operating rooms, post surgical recovery rooms, radiology and diagnostic laboratory services. There is a farm facility that provides indoor and outdoor housing for farm animals and other species. Veterinary care is provided on a 24-hour basis, including weekends and holidays, by a staff comprised of veterinarians and animal health technicians.

COMPUTER:
More than 10 PCs and Macs running various versions of Windows and Mac OS are available throughout lab space and offices.

OFFICE:
Office space and/or desk space is available for all personnel, including investigators and administrators.

OTHER:
There are excellent secretarial supports in the Division of Pulmonary Critical Care Medicine, as well as the Department of Medicine for all the investigators and staff.

MAJOR EQUIPMENT:
(List the most important items already available to this project, noting the location and pertinent capabilities of each.)

Chemical fume hood
Laminar flow hoods for cell culture
Liquid N2 storage tank
37 degree/CO2 incubator
Precision 37 degree incubator
Brunswick Scientific incubator shaker
4 degree Refrigerators
-20 degree Freezers
-80 degree Freezer
Spectrophotometer
PTC-100 programmable thermal controller
ABI 7000 Real-Time PCR System
Microscopes
Zeiss Axio Observer A1 Inverted microscope
Digital video camera
Laboratory cell counter
Precision water baths
Eppendorf centrifuges
Refrigerated centrifuge
Centrifuge, Heraeus Biofuge Pico
Biorad electroporator
Electrophoresis power supplies
Induction Chamber

Additional Shared Equipments:

Applied Biosystems StepOne Real-time PCR System (VA)
BD FACSAria (Cancer Center)
Sonicator (Cellular Molecular Medicine)
Olympus Confocal Microscope with FRAP capacity (Microscope Facility, UCSD)
L Max ll 384 Illuminometer (Cellular Molecular Medicine)
Illumina HiSeq2000 sequencing systems (IGM, UCSD)
Agilent 2100 Bioanalyzer (IGM, UCSD)
NanoDrop ND-1000 (IGM, UCSD)
Genotyping Arrays, Methylation Arrays (IGM, UCSD)
Affymetrix Microarrays / Nimblegen Arrays (VA/VMRF Microarray & NGS Core)
Luminex 100 (Cancer Center)
Histology Immunohistochemistry core (Cancer Center)

Atul Malhotra, M.D.
Chief, Pulmonary and Critical Care Medicine
Director of Sleep Medicine
Kenneth M. Moser Professor of Medicine
President, American Thoracic Society (2015-2016)
amalhotra@ucsd.edu

September 5, 2016

RE: Jinghong Li, M.D., Ph.D.

Dear Members of ATS Foundation Research Program Review Committee:

It is a pleasure to write the letter to support Dr. Jinghong Li's application for ATS Foundation/Breathe California of Los Angeles Research Grant. I have known Dr. Li for over three years since I became the Division Chief of Pulmonary, Critical Care and Sleep Medicine at University of California San Diego in 2013.

Dr. Li brought with her considerable experience in molecular biology. She had been investigating several signal transduction pathways, in particular TGF-β signaling pathway, by using mouse and Drosophila models. She has multiple publications in some of the top journals including Nature Cell Biology, Nature Genetics, and Journal of Biological Chemistry. She identified eIF4A (eukaryotic translation initiation factor 4A) as a novel negative regulator of the TGF-β signaling pathway and her paper was elected for the "News and Views" by the editor of Nature Cell Biology.

During her Pulmonary Critical Care fellowship, she joined the laboratory of Dr. Patricia Finn to work on the immunology of lung inflammatory diseases, particularly in asthma and acute lung injury. She developed a project to study the regulation of an important immune molecule, cytotoxic T lymphocyte antigen 4 (CTLA4), in both in vitro and in vivo animal models. Her first author research paper was featured in "Red Alert": Highlighted Papers by Junior Investigators at ATS Journal American Journal of Respiratory Cell and Molecular Biology.

Dr. Li has managed to stay active in research in spite of her busy clinical schedule. In 2014, she was one of the three ATS Young Investigators to attend The 54th Annual Meeting of The Japanese Respiratory Society. Her presentation was very well received. She continues to demonstrate a high degree of responsibility and capacity for scientific investigation, as evident by the current proposal: Targeting CTLA4 and PD-1/PD-L1 for treatment of allergic asthma. This is an extension of her fellowship project.

She has laid out a logical, exciting, and doable proposal that is important to advance our understanding of asthma, leading to potential therapeutic intervention.

In my opinion, Dr. Li is one of the few individuals among Pulmonary and Critical Care Medicine junior faculty that has extensive background in both scientific and clinical fields. The span of her research is broad, from mouse disease models, molecular biology, molecular immunology, and human disease clinical research. She has great potential for contribution to medical research as a physician scientist. I have no doubt that her strong commitment and dedication make her an outstanding candidate for the ATS Foundation/Breathe California of Los Angeles Research Grant.

Sincerely,

Atul Malhotra, MD
Chief, Pulmonary, Critical Care and Sleep Medicine
Director of Sleep Medicine
Kenneth M. Moser Professor of Medicine

UC San Diego
SCHOOL OF MEDICINE

Wolfgang H. Dillmann, M.D.
Helen M. Ranney Distinguished Professor
Chair, Department of Medicine
wdillmann@ucsd.edu

September 13, 2016

Department Head's Letter

To Whom It May Concern:

The Department of Medicine and Division of Pulmonary and Critical Care Medicine are fully committed to supporting Jinghong Li, M.D., Ph.D. to meet all of the requirements of the **ATS Foundation/Breathe California of Los Angeles Research Grant** as detailed below.

Dr. Jinghong Li has been appointed as a full time Assistant Professor as of October 1, 2013. She will devote at least 50% of effort to the research project proposed. The remainder of her effort (less than 50%) will be devoted to clinical care with the Pulmonary and Critical Care Division of the Department of Medicine and to teaching fellows, residents, and medical students.

Dr. Jinghong Li has been provided office space and administrative support within the Division of Pulmonary and Critical Care Medicine. Dr. Jinghong Li will conduct the proposed research at her collaborator, Dr. Willis Li's laboratory, located at the School of Medicine Building 4. Approximately 1000 sq ft of fully equipped space as detailed in the facilities and equipments statement is fully available to Dr. Jinghong Li, as well as additional resources on the campus including the Core Microscopy facility, and Core Molecular Biology/Genetics/Genomics facilities of UCSD.

Dr. Jinghong Li has already published first-author papers on the data related to the current proposal, she will be expected to write two senior-authored original research manuscripts to be published in high impact journals. Based on the data obtained from current proposal, she is expected to continue her grant applications for independent research funding (e.g., NIH R01) within the next two years.

Please feel free to contact my office with any questions.

Sincerely,

W. Dillmann

Wolfgang H. Dillmann, M.D

THE UNIVERSITY OF ILLINOIS AT CHICAGO
UIC

September 8, 2016

Jinghong Li, MD, PhD
Assistant Professor of Medicine
Department of Medicine
University of California, San Diego

Dear Jinghong,

I am writing to express my willingness to serve as a collaborator on your proposal “Targeting CTLA4 and PD-1/PD-L1 for treatment of allergic asthma”. Your data shows that two of the most investigated immune checkpoint proteins, CTLA4 and PD-1, interact and regulate each other. This is important finding that may explain why blockade of a single checkpoint protein is not as effective as previously thought. The availability of multiple antibodies from rapid growing cancer immunotherapy research may provide therapeutic interventions targeting CTLA4 and PD-1 in pulmonary inflammatory diseases such as asthma.

My expertise is in the area of immune mediated pulmonary disorders, including asthma, acute lung injury, and transplantation. I have a history of NIH funding, including serving as PI on the T32 HL82547-05 training grant at UIC.

I enjoyed mentoring and collaborating with you over the past six years, when I was director of the Division of Pulmonary and Critical Care Medicine at the University of California, San Diego. I have since moved to the University of Illinois at Chicago to assume the position of chair of the Department of Medicine. Our recent publication entitled “Regulation of cytotoxic T lymphocyte antigen 4 by cAMP” in the American Journal of Respiratory Cell and Molecular Biology was selected for the “Red Alert” section of the journal, which highlights important contributions from junior investigators.

I look forward to our continued collaboration. I am very confident that our collaboration will be highly productive.

Sincerely,

Patricia W. Finn

Patricia W. Finn, MD
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September 8, 2016

Jinghong Li, M.D., Ph.D.
Department of Medicine
UC San Diego

Re: Consultation/Collaboration

Dear Jinghong,

This letter is to confirm that I am more than happy to provide collaboration, technical support, and advice regarding your research project investigating the "Targeting CTLA4 and PD-1/PD-L1 for treatment of allergic asthma".

My research interest has been in molecular mechanisms of T cell function and cell signaling involved in cancer, metabolic disorders, and stem cells. We have expertise in CTLA4 and SH2 domain-containing tyrosine phosphatase (Shp2) pathway. CTLA4 was found to bind to Shp2, and they both play important roles in T cell activation. My group has developed a wide range of reagents and protocols, which should be helpful for your proposed research project. In particular, we have generated Shp2 knockout mouse and examined the Shp2 function in OVA induced mouse asthma model. I look forward to our collaboration.

Sincerely,

Gen-Sheng Feng, Ph.D.
Professor of Pathology and Biology

Please scan and upload this document with signatures to your application on Foundant.

APPLICANT ASSURANCE: I certify that the statements herein are true, complete and accurate to the best of my knowledge. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. I agree to accept responsibility for the scientific conduct of the project and to provide the required progress reports if a grant is awarded as a result of this application.

SIGNATURE OF APPLICANT *(In ink. "Per" signature not acceptable)*:

DATE___8/23/16_____

SIGNING OFFICIAL ASSURANCE: I certify that the statements herein are true, complete and accurate to the best of my knowledge. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties.

SIGNATURE OF SIGNING OFFICIAL *(In ink. "Per" signature not acceptable.)*:

12 Sept. 2016

DATE_____