UCSF-RAP Application

SAMPLE

Grant Area: Pilots for Junior Investigators in Basic and Clinical/Translational Sciences

1. **Project Title:** **The Role of Natural Killer T cells in Sarcoidosis**

2. **Amount Request:** **$30,000**

3. **P.I. Name:** **Laura Koth, M.D.**

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4. **Abstract:**

Sarcoidosis patients have decreased numbers of NKT cells in their blood and lungs; however, whether this deficiency is due to a decrease in specific subsets of NKT cells or whether these cells have aberrant effector function is not known. We hypothesize that the numbers of CD4+ NKT cells are decreased in sarcoidosis leading to a relative increase in CD4- NKT cells which preferentially produce Th1 cytokines, a feature of sarcoidosis. Thus, we will determine the distribution of CD4+ vs. CD4- NKT cells in sarcoidosis and their cytokine responses after stimulation with CD1d ligands.

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7. **Human Subjects:**
   CHR date of approval: 03/20/2007
   CHR approval number: H5003-17407-08

8. **Animal Subjects:** Animals will not be used.

9. **Human Stem Cells:** Human stem cells will not be used.

10. **Specify if you have been funded in the past 5 years by one of the following UCSF agencies:**
    CTSI-SOS: No
    REAC: No
    CFAR: No
    Cancer Center-CIRP: No
    Academic Senate: No
    Department Startup Funds: No
    Other UCSF: No

Yes, CTSI may contact me to explore opportunities for collaboration.

11.A. SPECIFIC AIMS

Sarcoidosis is a chronic, systemic inflammatory disease of unknown cause and has no cure. It is thought to be due to an abnormal immune response to either a self or non-self antigen. Numerous clinical studies in sarcoidosis subjects over the last several decades show an excess of activated CD4+ T effector cells and a Th1 immune response with interferon-γ being the predominant cytokine (1). Despite this knowledge, little is known about the regulators of this aberrant immune response. Furthermore, current treatment approaches (e.g. systemic corticosteroids and methotrexate) are non-specific and carry significant toxicities.

Natural killer T (NKT) cells are immunoregulatory T cells that can either promote or suppress immune responses depending on local stimuli. In mice, NKT cells have been found to be *protective* in several autoimmune diseases. In humans, two independent clinical studies found that the numbers of peripheral blood and lung NKT cells were significantly lower in patients with pulmonary sarcoidosis compared to controls. Thus, NKT cells are abnormally regulated in sarcoidosis and their deficiency may contribute to pathogenesis. However, little is known about whether the NKT cell deficiency in sarcoidosis is due to a selective loss of NKT cell subsets or whether the NKT cells that are present in sarcoidosis individuals function normally.

A recent NHLBI workshop proposed that the identification of novel targets for drug development was a priority area of research in sarcoidosis (2). This proposal sets out to address this priority area by studying the role of NKT cells in the development of sarcoidosis through the use of ligand-specific stimulation to determine NKT cell subsets and effector function in sarcoidosis compared to healthy subjects.

Central Hypothesis: Sarcoidosis is associated with a deficiency of total numbers of NKT cells in the blood and lung. Furthermore, a selective deficiency of the CD4+ subset of NKT cells may skew immune responses towards Th1 responses (a characteristic of sarcoidosis). Therefore, we hypothesize that the NKT cell deficiency in sarcoidosis is due to a selective loss of the CD4+ NKT cell subset. This leads to a paucity of Th2 cytokines and a skewing of the cytokine balance in favor of Th1 cytokine-producing CD4- NKT cells which then influence conventional T cell effector function.

Aim 1: **To determine the distribution of CD4+ vs. CD4- NKT cells in the peripheral blood of subjects with sarcoidosis compared to that measured in control subjects**. We will isolate peripheral blood mononuclear cells (PBMC) from sarcoidosis patients and controls. Using multi-color flow cytometry, we will determine the fraction of NKT cells in the total T lymphocyte population, and the number of NKT cells/ml that are CD4+ vs. CD4-. Primary analyses will compare NKT cellular subsets in ~60 subjects with sarcoidosis to age-, sex- and race-matched controls (stratified by immunosuppression use if necessary).

Aim 2: **To determine NKT cell cytokine responses after stimulation with CD1d ligands in subjects with sarcoidosis compared to that measured in control subjects**. PBMCs from sarcoidosis subjects and controls will be stimulated with CD1d ligands specific for NKT cells. Using multi-color flow cytometry, we will determine which cytokines (IFN-γ, TNFα, IL-4, IL-13) are produced from CD4+ vs. CD4- NKT cells. The primary analyses will compare NKT cell cytokine responses in ~60 subjects with sarcoidosis to age-, sex- and race-matched controls (stratified by immunosuppression use if necessary). Exploratory analyses will correlate NKT cell subsets and effector function with specific clinical parameters collected at the time of blood draw (shortness of breath scale and lung function measurements) within the cohort of sarcoidosis subjects.

11.B. BACKGROUND AND SIGNIFICANCE

Importance of the problem: Sarcoidosis affects both men and women of all races and can be progressive and life-threatening. There is no cure and current therapies include immunosuppression with various agents that all cause significant toxicities. Because of the large gaps in knowledge about the inflammatory mechanisms involved with disease initiation and progression, our abilities to develop more specific treatment approaches to attenuate the inflammatory response have been hampered. Thus, the goal of this project is to determine how NKT cells in subjects with sarcoidosis function since they may contribute to pathogenesis.

Innovative nature of the proposed research: While prior studies have shown that the levels of NKT cells are abnormally regulated in sarcoidosis, studies are lacking which comprehensively examine the CD4+ and CD4- NKT cell distribution and their cytokine effector function. In the proposed study, we will use a novel method of stimulating NKT cells that involves more “physiologic” stimulation by using CD1d presentation of ligands to NKT cells. We are also equipped to analyze whether NKT cell subsets and cytokine production relate to disease severity or are affected by immunosuppression. Although it is logical to assume that immunosuppression will have some effect, little is known in this area.

Next steps and potential impact of findings: Determining the cellular subsets and cytokine profiles of NKT cells in sarcoidosis is a critical first step in determining how NKT cells may contribute to the development of sarcoidosis. Our findings would serve as necessary preliminary data for future studies to test stimulation methods and reagents that modulate the proliferation of NKT cells and their relative CD4+ to CD4- distribution. Clinical trials that stimulate NKT cells *in vitro* or *in vivo* as a way to modulate their function in the detection and eradication of tumor cells are already in progress. Thus, a better understanding of NKT cell effector function in clinical diseases is essential to move this potential "targeted" treatment strategy forward.

11.B.1 Background

NKT cells are considered "innate" immunoregulatory T cells, able to rapidly produce both T helper-1 and -2 cytokines upon stimulation with CD1d ligands.

Human NKT cells are characterized by expression of cell surface markers for both natural killer cells (e.g. CD161) and T cells (e.g. CD3). A unique aspect of NKT cells is that their activation occurs through an invariant and conserved αβT-cell receptor upon recognition of glycolipid antigens presented by CD1d--an MHC class I-like molecule expressed by antigen presenting cells including dendritic cells and B lymphocytes. This invariant αβT-cell receptor is encoded by Vα24 and JαQ gene segments whose protein products associate with the Vβ11 chain (reviewed in (3)). Thus, these cells are referred to as Vα24-invariant NKT cells or CD1d-restricted NKT cells. For purposes of this proposal, I will refer to them only as NKT cells. Although most of the information currently available about CD1d-antigen presentation to NKT cells comes from studies using the marine sponge-derived glycolipid α-galactosylceramide (α-GalCer), more recent reports have identified endogenous (isoglobotrihexosyl ceramide, iGb3)(4) and exogenous (microbially-derived) glycolipid ligands (5) for CD1d that may represent more physiological ligands for the activation of NKT cells under normal conditions. Importantly, there is evidence that different ligands induce different NKT cell cytokine responses (6). Upon stimulation of NKT cells by CD1d-presented glycolipid, these cells can rapidly (within minutes) secrete T helper 1 (Th1) and 2 (Th2) cytokines and because of this ability have been thought of as innate-like immune cells which may be critical modulators of Th1/Th2 decision of conventional CD4+ and CD8+ T cells.

Sarcoidosis is characterized by a deficiency of NKT cells in blood and bronchoalveolar lavage fluid.

The role of NKT cells in human health and disease is under intense study. In some disease models, NKT cells promote disease, while in others they are protective. For example, the mouse counterpart of NKT cell has been shown to be *protective* in Th1-biased syndromes such as type 1 diabetes and experimental autoimmune encephalomyelitis (3). Thus, a deficiency of NKT cells may be an important step in the development of these autoimmune syndromes. With regard to the human Th1-biased disease, sarcoidosis, two clinical studies in the UK and Japan found decreased total numbers of circulating and bronchoalveolar lavage NKT cells in patients with pulmonary sarcoidosis compared to controls (7, 8). However, these studies did not determine how the overall deficiency affected the distribution of CD4+ vs. CD4- NKT cells, which may determine the types of cytokines that are produced and ultimately the type of immune response that occurs *in vivo*. Nor did these studies assess the intrinsic protein effector function of NKT cells in response to more physiologic stimulation using CD1d-bound ligands. These gaps in knowledge must be addressed before novel T-cell-based therapies that modulate the function of NKT cells can be applied in sarcoidosis.

During the innate immune response to antigen, NKT cells may influence the Th1/Th2 cytokine balance and heavily contribute to the overall pattern of inflammation.

With regard to the function of NKT cells in sarcoidosis even less is known. Since NKT cells are "Th0" cells, with the capacity to rapidly produce both Th1 and Th2 cytokines and modulate the function of conventional CD4+ cells, they may have significant influence over the Th1/Th2 balance and in this way participate in the development of sarcoidal inflammation. Numerous studies of cytokine profiles in sarcoidosis have demonstrated a Th1-dominant milieu, with a predominance of IFN-γ over Th2 cytokines. Thus, if NKT cells are abnormally regulated they may promote an "imbalance" of cytokines leading to development and persistence of granulomatous inflammation. The only study to examine effector function of NKT cells in sarcoidosis was in Japanese subjects where they found mRNA for interferon-γ to be lower in NKT cells from non-remitting sarcoidosis patients than that from remitting patients (8). However, IFNγ protein was not measured and NKT cells were stimulated with 12-phorbol 13-myristate acetate (PMA) and ionomycin, which are nonspecific stimulators that may not mimic the cytokine responses induced by CD1d-presented ligands.

The subset of an NKT cell determines its effector function and an imbalance of these subsets may have consequences on immune responses.

To add a layer of complexity to the biology of NKT cells, within the last few years, investigators have identified that NKT cells are further characterized into CD4+ or CD4- subsets. In humans, CD4+ NKT cells are the predominant subset and produce both Th1 and Th2 cytokines (9). In contrast, CD4- NKT cells preferentially produce Th1 cytokines (IFNγ) and TNFα. Thus, the Th1-type inflammation characteristic of sarcoidosis may be due to a shift in NKT cell balance from CD4+ to CD4- subsets; however, no detailed studies have been done examining the cytokine profiles from CD4+ and CD4- subsets of NKT cells.

11.C PRELIMINARY STUDIES

11.C.1 Brazilian subjects with active tuberculosis (TB) infection demonstrated decreased frequencies of NKT cells.

We have recently published a study measuring NKT cells in Brazilian subjects with TB infection (10). In this study, my collaborator quantitated the number of NKT cells in PBMCs with flow cytometry using stringent criteria for NKT cells ($V\alpha24^+$, $V\beta11^+$, $6b11^+$ and CD1d dimer loaded with α-$GalCer^+$). Using these stringent criteria, we were able to measure a significant **~5-fold *decrease*** in NKT cells in PBMCs from 19 TB subjects compared to 12 controls (10).

11.C.2 Flow cytometry and NKT cell stimulation methods using CD1d ligands for measurement of NKT cell subsets and cytokine production.

With regard to assaying NKT cell effector function, over the last two years, we have developed an *in vitro* method to study the cytokine profiles of human NKT cells after stimulation with CD1d-presented ligands, as opposed to PMA and ionomycin. Our goal in using CD1d ligands is to induce a more "physiologic" stimulation of NKT cells and thus measure more meaningful cytokine profiles. The overall concept of our assay involves "loading" the CD1d receptor with CD1d ligands, such as α-GalCer. These "loaded" CD1d receptor-ligand pairs are then incubated with PBMCs. Only NKT cells within the PBMC population possess the ability to respond to ligand presented by CD1d. Figure 1 is an example of how we can detect multiple cytokines from the same population of NKT cells using multi-color flow cytometry after stimulation of PBMCs with commercially available CD1d-dimer "loaded" with α-GalCer.

Our methods have several advantages. Since NKT cells are present at relatively low numbers in normal peripheral blood, we can maximize the amount of information obtained from each subject's blood sample by using 11 color flow cytometry which we have already optimized. This method allows us 1) to detect NKT cells using 4 specific markers (minimizing the chance of detecting false positive cells); 2) to determine the numbers of NKT cells that are CD4+ and CD4-; and 3) to determine the cytokine profiles within each NKT cell.

In addition to using the CD1d ligand α-GalCer, we have also performed stimulation assays of NKT cells using different ligands, including the mycobacterial glycolipid, Phosphatidylinositol mannoside (PIM), which is a mycobacterial cell wall glycolipid that we and others (5) have found to stimulate NKT cells to produce IFNγ (data not shown). Determination of NKT cell cytokine profiles from sarcoidosis patients after stimulation with PIM would be particularly important to examine, given the resurgence of evidence suggesting the presence of mycobacterial antigens in sarcoidal biopsies (11-15).

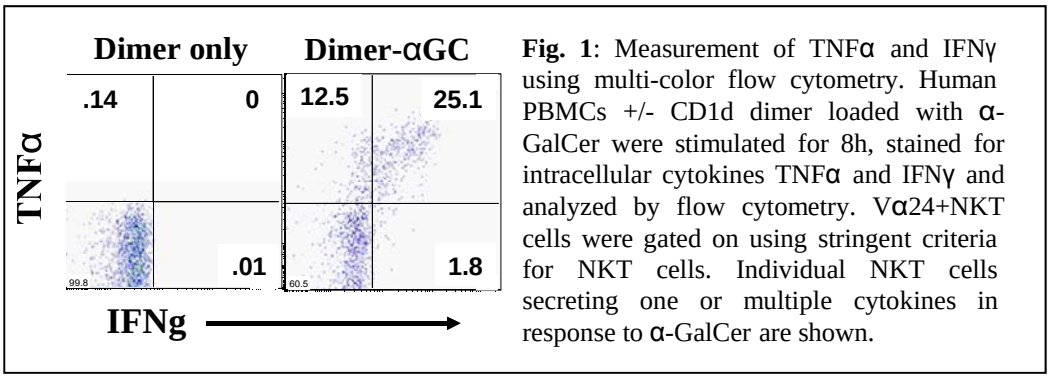

Fig. 1: Measurement of TNFα and IFNγ using multi-color flow cytometry. Human PBMCs +/- CD1d dimer loaded with α-GalCer were stimulated for 8h, stained for intracellular cytokines TNFα and IFNγ and analyzed by flow cytometry. Vα24+NKT cells were gated on using stringent criteria for NKT cells. Individual NKT cells secreting one or multiple cytokines in response to α-GalCer are shown.

11.C.3 Development of an ILD clinical database and subject recruitment

Dr. Koth has devoted significant effort since 2002 to establish a united ILD Clinic (combining clinics from SFGH and Parnassus Campus), as well as to create the ILD Clinical and Tracking Database which today contains over 800 patients with ILD with another 300+ patient data ready for entry. This research database interfaces with the UCSF ILD Clinic and contains clinical information about patients collected in standardized intake forms. We also have a protocol for blood draws with consent for research use. All subjects presenting to the ILD clinic are approached for voluntary participation in the ongoing research resource. This infrastructure will facilitate the proposed studies. Since enrollment started ~9-10 months ago, we have consented and stored blood samples from 40 subjects with sarcoidosis. Tables 1 & 2 summarize the distribution of patient characteristics.

Table 1: Blood samples obtained on sarcoidosis subjects from UCSF ILD Clinic over last 9-10 months

Total number samples	Male	Female	Caucasian	African American	Other Ethnicity
40	13	27	22	8	10

Table 2: Type of systemic immune-suppression (IS)

Total number patients on IS	Prednisone	Methotrexate	Imuran
19	12	5	2

11.D EXPERIMENTAL DESIGN AND METHODS

Rationale: NKT cells are immunoregulatory T cells that may be important modulators of the Th1/Th2 decision of conventional CD4+ T cells. CD4+ NKT cells have the ability to produce both Th1 and Th2 cytokines upon CD1d glycolipid stimulation while CD4- NKT cells preferentially produce Th1 cytokines. Two previous studies established an overall decrease in NKT cell numbers in the blood and lungs of patients with sarcoidosis. However, whether this decrease is due to a decrease in CD4+ or CD4- NKT cells is not known. Furthermore, little is known about the effector function of CD4+ and CD4- NKT cells in sarcoidosis subjects. Therefore, **Aim 1 will determine the distribution of CD4+ vs. CD4- NKT cells in sarcoidosis subjects compared to control subjects.** This will establish whether one subset is preferentially decreased or whether there is a global reduction of both subsets of NKT cells. Next, **Aim 2 will measure the effector function of CD4+ vs. CD4- NKT cells in sarcoidosis and compare it to that from control subjects.** These results will establish whether NKT cells in sarcoidosis have aberrant cytokine production and guide future studies that set out to modulate NKT cell effector function in sarcoidosis.

Overall Summary: We will collect blood and clinical data from subjects given the diagnosis of sarcoidosis and healthy controls. We will study all subjects with sarcoidosis who are or are not on immunosuppressive therapy. From each subject, we will draw ~25-30 ml of blood and 1 CBC (to determine NKT cell numbers/ml of blood). One blood draw from each subject will allow us to accomplish the studies for both Aims 1 & 2. Samples will be analyzed in a blinded fashion. Cells will be stained for markers of NKT cells, CD4 positivity and Th1 and Th2 cytokines using 11-color flow cytometry that has already been optimized for these measurements in human NKT cells. Our primary analysis will compare NKT cellular subsets (CD4+ vs. CD4-) and cytokine profiles in ~60 subjects with sarcoidosis to age-, sex- and race-matched controls (stratified by immunosuppression use if necessary).

11.D.1 Detailed Methods: Blood processing and flow cytometry analysis:

PBMCs will be isolated using standard methods. To assess NKT cell subsets (Aim 1) and cytokine responses (Aim 2), we will use multicolor flow cytometry. As described in Section 11.C.2 of preliminary data, we will use CD1d-dimer molecules loaded with CD1d ligand to measure cytokine production in NKT cells. CD1d ligands include α-galactosylceramide (α-GalCer) and Phosphatidylinositol mannoside (PIM). PBMCs will be incubated with "loaded" CD1d-dimers directly ex-vivo. After 8 hr incubation, PBMCs and supernatant will be collected and cells will be stained and analyzed by 11-color flow cytometry to determine the following:

1) the number of total lymphocytes (CD3+ cells).

2) the number of CD4+ and CD4- NKT cells (using stringent methods and markers for detection of NKT cells--$V\alpha24^+$, $V\beta11^+$, $6b11^+$ and CD1d dimer loaded with $\alpha\text{-GalCer}^+$--as described previously (10)).

3) the percentage of CD4+ and CD4- NKT cells producing Th1 (interferonγ) and Th2 (interleukin-4, 13) cytokines and TNFα.

Ligands will be tested separately, with no ligand as negative control. PBMCs from all subjects will also be stimulated with anti-CD3 and anti-CD28 to ensure the cells from each donor are viable and the FACS assays are working properly. Appropriate reagents (e.g. Brefeldin A and monensin) will be used to stop exocytosis of cytokines and allow for intracellular staining. Flow cytometry will be performed on a FACSCalibur. Data will be analyzed using FlowJo software (version 8.1.1). "Frequency minus one" stained control samples will be used to determine gate selection for all antibody panels (16). Therefore, nonspecific events will be excluded from the Va24+Vb11+ gate and will not be included in the analysis of NKT percentages. Based on our own data from tuberculosis patients who have a significant **decrease** in peripheral blood NKT cells and based on prior published data of NKT cell frequencies from sarcoidosis subjects, we know that the frequency

of NKT cells in peripheral blood may be as low as 0.01%. Thus, in our flow cytometry experiments, we routinely analyze 5-10 million lymphocytes. This generates data on at least 500-1000 NKT cells per subject. In our previous study this method was sufficient to detect a statistically significant 5-fold *decrease* in mean NKT cell frequencies in active TB (which were as low as 0.01%) vs. controls (10).

11.D.2 Detailed Methods: Data analysis:

Before performing the primary analyses, our preliminary analysis will determine how NKT cellular subsets (Aim 1) and cytokine profiles (Aim 2) differ by immunosuppression use in a cross-sectional design. The Mann-Whitney U test (or t-test if appropriate) will be used to determine whether there are significant differences in the numbers of NKT cells, the numbers of CD4+ vs. CD4- subsets, and the percentage of CD4+ NKT cells producing Th1 or Th2 cytokines between those on or not on systemic immunosuppression. If immunosuppression use does not influence the NKT cell phenotype within sarcoidosis subjects, we will analyze them as one group in the primary analysis. If, however, immunosuppression does affect NKT cell phenotype, we will stratify by immunosuppression use in our primary analysis. These initial studies will also determine whether subjects immunosuppressed with steroids (the majority) are similar to those immunosuppressed with other agents, such as methotrexate (a minority) and thus whether the "immunosuppressed" group in the primary analysis should be restricted to steroid-users.

The primary analysis will compare NKT cellular subsets (Aim 1) and cytokine profiles (Aim 2) in subjects with sarcoidosis to age (within 5 yr)-, sex- and race-matched controls. Using paired analysis (paired t-test or sign rank test as appropriate), we will determine whether there are differences in numbers of total NKT cells and whether there is a disproportionate reduction in the CD4+ NKT cell subset in sarcoidosis. If we are unable to recruit sufficient numbers of fully matched healthy controls, we will recruit controls that are comparable to our sarcoid patients in gender distribution (based on prior data from healthy controls showing that NKT cell frequency in women was slightly higher than in men (17)) and then perform unpaired analyses that control for sex, age and race (using multivariate linear regression). Exploratory secondary analyses will examine whether NKT cell numbers, cellular subsets or cytokine production in sarcoidosis correlate with pulmonary function data and dyspnea score which is collected on all patients in the clinic.

11.D.3 Detailed Methods: Power calculation:

We have previously found the percentage of PBMCs that are NKT cells to be 76% lower in TB as compared to healthy control subjects (0.048 ± 0.067% vs. 0.21 ± 0.14%, $p<0.001$). A similar decrease in NKT cell percentages was observed in a prior study of sarcoidosis (7). Assuming that the decrement in NKT cell percentages in sarcoidosis that we observe will be similar to that seen in TB infection, enrollment of 11 healthy control subjects and 11 subjects with sarcoidosis will confer 90% power to detect a difference in overall numbers of NKT cells between sarcoidosis and controls. However, our aim is to measure NKT cellular subsets and cytokine profiles between sarcoidosis and controls. We have prior data on the percentages of NKT cells that are CD4+ in a healthy control population (59 ± 21%) (17). Using these data, enrollment of 60 subjects in each group will confer 90% power to detect a 21% decline in the percentage of CD4+ NKT cells in sarcoidosis. This sample size should allow us to stratify and control for gender, if gender differences become apparent. Finally, these samples sizes will confer additional statistical power for our quantitative measures of NKT cell effector function.

11.D.4 Detailed methods: Subject enrollment and Timetable:

We will enroll patients given the diagnosis of sarcoidosis by the ILD physician at the initial clinic visit. The UCSF ILD Clinic physicians make the diagnosis of sarcoidosis based upon established criteria (18-20) (compatible clinical picture, histological demonstration of noncaseating granulomas, and exclusion of other diseases). Enrolled patient characteristics are displayed in Table 1 and 2 in section 11.C.3 and demonstrate that we enrolled 40 patients in ~9-10 months. No specific recruitment procedures were performed; all enrolled patients were referred to the UCSF ILD Clinic for diagnostic and treatment issues by their regular physicians. Based on our recruitment to date, we anticipate collecting blood from 20 sarcoidosis subjects within the first 6 months of funding (for a total of 60 sarcoidosis subjects), allowing us to perform the NKT cells assays in the 2nd 6 months of the funding period.

Our control group will consist of age- (within 5 yrs), sex- and race-matched individuals found to have a normal standardized respiratory health and medical questionnaire. Currently, we have several methods for recruiting healthy volunteers. The PI has access to a recruitment database, which contains information on 2,925 subjects who have previously participated in clinical research protocols in the Airway Clinical Research

Center at UCSF and have normal spirometry and respiratory questionnaires. These subjects are registered in an IRB-approved recruitment database and have given permission to be contacted about future studies. We will also use Dr. Nixon's CHR-approved consent and advertisement to recruit healthy volunteers for blood draws and will use newspaper advertisements as necessary for recruitment. We will recruit controls during the first 2/3 of the funding period. We will analyze matched samples in batches of 16 (8 sarcoid, 8 control) and include one healthy donor sample with a known percent of NKT cells to control for inter-assay variation.

11.D.5 Anticipated results and potential pitfalls for Aims 1 & 2: In two studies of sarcoidosis subjects vs. controls, it was demonstrated that the frequency of total NKT cells was significantly reduced in peripheral blood and bronchoalveolar lavage fluid (7, 8). However, it is not known if this reduction of NKT cell levels is due to a global reduction of both CD4+ and CD4- NKT subsets or a skewed reduction of one subset or another. Since CD4+ NKT cells have been found to produce significantly more Th2 cytokines when stimulated with CD1d ligands than that produced by CD4- NKT cells, it is critical to determine whether the homeostatic levels of these NKT subsets are altered in sarcoidosis. Based on our hypothesis, we predict that CD4+ NKT cells from sarcoidosis subjects will be decreased in peripheral blood compared to levels found in controls. This would result in a disproportionate increase in CD4- NKT cells which are biased to produce Th1 cytokines, such as IFN-γ. We speculate that IFN-γ-producing CD4- NKT cells may contribute to granulomatous inflammation in sarcoidosis. Clinical data to support this idea comes from a study of patients with Type 1 diabetes mellitus who were found to have decreased numbers of CD4+ NKT cell clones compared to controls (21).

With regard to the predicted NKT cell cytokine profile in Aim 2, we speculate that NKT cells from sarcoidosis subjects will display elevated production of Th1 cytokines based on our hypothesis of a reduction of Th2 producing-CD4+ NKT cells. However, we may instead find that NKT cells from some subjects show a Th1 bias, while others a Th2 bias. This may be informative, especially when analyzed in reference to whether the subject is on immune-suppressive therapy and in reference to clinical data. NKT cell stimulation with α-GalCer vs. PIM will also add important insight into whether different cytokine responses occur in response to stimulation with different ligands. In addition, cytokine response to PIM stimulation will be worth investigating since there is ongoing interest and evidence for mycobacterial antigens associated with sarcoidosis (12). Although not within the scope of this grant, studies that examine the functional effects of longer term stimulation of NKT cells by α-GalCer could address whether α-GalCer could be tested as a novel therapeutic for subjects with progressive sarcoidosis, since this agent has been used in Phase I studies in cancer without apparent toxicities. The studies proposed in this CTSI pilot grant would lay the framework for such future studies.

While the methods we propose to use involve a novel *in vitro* method of NKT cell stimulation to better mimic physiologic antigen presentation to NKT cells as apposed to PMA stimulation, a limitation of our study is the fact that it is an *ex vivo* stimulation. However, our stimulation protocol is directly ex vivo and minimizes the time in culture, thereby making this *in vitro* assay as physiologic as feasible and we acknowledge the inherent limitations. We do not anticipate technical difficulties with the methods as we routinely perform these assays (10, 22-26). Additionally, we will run control panels to perform compensation in our FACS experiments to eliminate spectral overlap problems inherent with multi-color FACS.

11.E How this CTSI pilot grant will lead to RO1 Funding:

The goal of this proposal is to determine whether a new class of T cells, NKT cells, could represent a new target for T cell-specific therapies in sarcoidosis. Since the cause of sarcoidosis is not known, it is critical to broaden our understanding of the key players in the immune response to be able to manipulate the immune response. The proposed studies are necessary to establish a pathogenetic role for NKT cells in sarcoidosis and set the stage for future studies that test ways to modify NKT cell function and proliferation in vitro using a variety of CD1d ligands, and test the efficacy of CD1d ligands in clinical trials. These NKT cell-specific ligands are currently being used in Phase II cancer trials and have shown minimal toxicity. Results from this project would serve as preliminary data for a recently released RO1 funding mechanism to explore novel mechanisms of immune dysregulation in sarcoidosis (http://grants.nih.gov/grants/guide/pa-files/PA-07-136.html).

Strengths of Research Plan: This application has clinical relevance since we are studying primary lymphocytes from subjects with sarcoidosis. In addition, our cell stimulation methods have been optimized and are novel and theoretically more closely mimic the type of stimulation that occurs *in vivo*. Other strengths of this application include the team of consultants and collaborators involved and the aims contain experiments that can be accomplished within the budget and timeframe outlined within.

11.F LITERATURE CITED

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9. Gumperz, J. E., S. Miyake, T. Yamamura, and M. B. Brenner. 2002. Functionally distinct subsets of CD1d-restricted natural killer T cells revealed by CD1d tetramer staining. *J Exp Med* 195(5):625-36.
10. Snyder-Cappione, J. E., D. F. Nixon, C. P. Loo, J. M. Chapman, D. A. Meiklejohn, F. F. Melo, P. R. Costa, J. K. Sandberg, D. S. Rodrigues, and E. G. Kallas. 2007. Individuals with pulmonary tuberculosis have lower levels of circulating CD1d-restricted NKT cells. *J Infect Dis* 195:in press.
11. Dubaniewicz, A., M. Dubaniewicz-Wybieralska, A. Sternau, Z. Zwolska, E. Izycka-Swieszewska, E. Augustynowicz-Kopec, J. Skokowski, M. Singh, and L. Zimnoch. 2006. Mycobacterium tuberculosis Complex and Mycobacterial Heat Shock Proteins in Lymph Node Tissue from Patients with Pulmonary Sarcoidosis. *J Clin Microbiol* 44(9):3448-51.
12. Drake, W. P., Z. Pei, D. T. Pride, R. D. Collins, T. L. Cover, and M. J. Blaser. 2002. Molecular analysis of sarcoidosis tissues for mycobacterium species DNA. *Emerg Infect Dis* 8(11):1334-41.
13. Drake, W. P., and L. S. Newman. 2006. Mycobacterial antigens may be important in sarcoidosis pathogenesis. *Curr Opin Pulm Med* 12(5):359-63.
14. Fite, E., M. T. Fernandez-Figueras, R. Prats, M. Vaquero, and J. Morera. 2006. High prevalence of Mycobacterium tuberculosis DNA in biopsies from sarcoidosis patients from Catalonia, Spain. *Respiration* 73(1):20-6.
15. Song, Z., L. Marzilli, B. M. Greenlee, E. S. Chen, R. F. Silver, F. B. Askin, A. S. Teirstein, Y. Zhang, R. J. Cotter, and D. R. Moller. 2005. Mycobacterial catalase-peroxidase is a tissue antigen and target of the adaptive immune response in systemic sarcoidosis. *J Exp Med* 201(5):755-67.
16. Roederer, M. 2001. Spectral compensation for flow cytometry: visualization artifacts, limitations, and caveats. *Cytometry* 45(3):194-205.
17. Sandberg, J. K., N. Bhardwaj, and D. F. Nixon. 2003. Dominant effector memory characteristics, capacity for dynamic adaptive expansion, and sex bias in the innate Valpha24 NKT cell compartment. *Eur J Immunol* 33(3):588-96.
18. 1999. Statement on sarcoidosis. Joint Statement of the American Thoracic Society (ATS), the European Respiratory Society (ERS) and the World Association of Sarcoidosis and Other Granulomatous Disorders (WASOG) adopted by the ATS Board of Directors and by the ERS Executive Committee, February 1999. *Am J Respir Crit Care Med* 160(2):736-55.
19. Costabel, U., and G. W. Hunninghake. 1999. ATS/ERS/WASOG statement on sarcoidosis. Sarcoidosis Statement Committee. American Thoracic Society. European Respiratory Society. World Association for Sarcoidosis and Other Granulomatous Disorders. *Eur Respir J* 14(4):735-7.

20. Hunninghake, G. W., U. Costabel, M. Ando, R. Baughman, J. F. Cordier, R. du Bois, A. Eklund, M. Kitaichi, J. Lynch, G. Rizzato, C. Rose, O. Selroos, G. Semenzato, and O. P. Sharma. 1999. ATS/ERS/WASOG statement on sarcoidosis. American Thoracic Society/European Respiratory Society/World Association of Sarcoidosis and other Granulomatous Disorders. *Sarcoidosis Vasc Diffuse Lung Dis* 16(2):149-73.
21. Kis, J., P. Engelmann, K. Farkas, G. Richman, S. Eck, J. Lolley, H. Jalahej, M. Borowiec, S. C. Kent, A. Treszl, and T. Orban. 2007. Reduced CD4+ subset and Th1 bias of the human iNKT cells in Type 1 diabetes mellitus. *J Leukoc Biol* 81(3):654-62.
22. Moll, M., J. Snyder-Cappione, G. Spotts, F. M. Hecht, J. K. Sandberg, and D. F. Nixon. 2006. Expansion of CD1d-restricted NKT cells in patients with primary HIV-1 infection treated with interleukin-2. *Blood* 107(8):3081-3.
23. Snyder-Cappione, J. E., A. A. Divekar, G. M. Maupin, X. Jin, L. M. Demeter, and T. R. Mosmann. 2006. HIV-specific cytotoxic cell frequencies measured directly ex vivo by the Lysispot assay can be higher or lower than the frequencies of IFN-gamma-secreting cells: anti-HIV cytotoxicity is not generally impaired relative to other chronic virus responses. *J Immunol* 176(4):2662-8.
24. Emu, B., E. Sinclair, D. Favre, W. J. Moretto, P. Hsue, R. Hoh, J. N. Martin, D. F. Nixon, J. M. McCune, and S. G. Deeks. 2005. Phenotypic, functional, and kinetic parameters associated with apparent T-cell control of human immunodeficiency virus replication in individuals with and without antiretroviral treatment. *J Virol* 79(22):14169-78.
25. Sandberg, J. K., C. A. Stoddart, F. Brilot, K. A. Jordan, and D. F. Nixon. 2004. Development of innate CD4+ alpha-chain variable gene segment 24 (Valpha24) natural killer T cells in the early human fetal thymus is regulated by IL-7. *Proc Natl Acad Sci U S A* 101(18):7058-63.
26. Sandberg, J. K., N. M. Fast, E. H. Palacios, G. Fennelly, J. Dobroszycki, P. Palumbo, A. Wiznia, R. M. Grant, N. Bhardwaj, M. G. Rosenberg, and D. F. Nixon. 2002. Selective loss of innate CD4(+) V alpha 24 natural killer T cells in human immunodeficiency virus infection. *J Virol* 76(15):7528-34.

Principal Investigator/Program Director (Last, First, Middle): Koth, Laura

DETAILED BUDGET FOR INITIAL BUDGET PERIOD
DIRECT COSTS ONLY

FROM | THROUGH

PERSONNEL *(Applicant organization only)*		Months Devoted to Project				DOLLAR AMOUNT REQUESTED *(omit cents)*		
NAME	ROLE ON PROJECT	Cal. Mnths	Acad. Mnths	Summer Mnths	INST.BASE SALARY	SALARY REQUESTED	FRINGE BENEFITS	TOTAL
Koth, Laura	Principal Investigator	7%						
Snyder-Cappione, Jennifer	Collaborator	2%						
Cambier, C.J.	Scientific Research Assistant	25%						
SUBTOTALS →						XXX	XXX	XXX

CONSULTANT COSTS		
EQUIPMENT *(Itemize)*		
SUPPLIES *(Itemize by category)* Flow Cytometer User Time ($720) FACS Antibodies ($3,361) Blood drawing supplies ($1,015) Tissue culture and General laboratory supplies ($600)		5,696
TRAVEL		
PATIENT CARE COSTS	INPATIENT	
	OUTPATIENT	
ALTERATIONS AND RENOVATIONS *(Itemize by category)*		
OTHER EXPENSES *(Itemize by category)* Advertisement for healthy volunteer recruitment ($700) Healthy control volunteer blood draw ($30/hr x 60 subjects =$1800)		2,500
CONSORTIUM/CONTRACTUAL COSTS	DIRECT COSTS	
SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD *(Item 7a, Face Page)*		**$** 30,000
CONSORTIUM/CONTRACTUAL COSTS	FACILITIES AND ADMINISTRATIVE COSTS	
TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD		**$** 30,000

Budget Justification

PERSONNEL

Laura L. Koth, MD, Principal Investigator (7% effort). Dr. Laura Koth is Assistant Professor of Medicine in the Divisions of Pulmonary and Critical Care Medicine and Lung Biology at the University of California, San Francisco. Dr. Koth has developed significant clinical expertise in the diagnosis and management of sarcoidosis and other interstitial lung diseases through the guidance of her clinical mentor, Dr. Talmadge E. King. She also has developed considerable experience in the collection and analysis of human biologic samples and has recently published her work on the macrophage activation state in human smokers compared to mouse models of emphysema. For this project, Dr. Koth will direct all aspects of the studies including experimental design, execution of the NKT cell assays and data analysis. Dr. Koth will also be responsible for monitoring subject recruitment, and will confirm that all subjects entered into the study meet criteria for sarcoidosis as outlined in the proposal.

Jennifer E. Snyder-Cappione, PhD, Collaborator (2% effort). Dr. Snyder-Cappione just completed the AIDS Research Program Award at the University of California, San Francisco. She is a Post-Doctoral Fellow in the laboratory of Dr. Douglas Nixon in the Gladstone Institute of Virology and Immunology. Dr. Snyder-Cappione completed her PhD in Immunology under the guidance of Dr. Tim Mosmann, who was one of the early pioneers in describing polarization of conventional T cells. Dr. Snyder-Cappione has extensive experience with in vitro methods of B and T cell stimulation and characterization, including multi-color flow cytometry and has published several influential papers in this area. She will provide ongoing expertise in the methods used to analyze NKT cell and work with Dr. Koth on the execution of the NKT cell assays.

CJ Cambier, Scientific Research Associate (25% effort). Mr. Cambier is an outstanding research associate with more than 1 year of experience as a research technician. Mr. Cambier will process blood samples from all enrolled subjects. He will also assist Dr. Koth with running the NKT cell assays. He will also maintain an inventory of samples, coded in a manner that removes protected health information using a system that we currently have in place for the ILD clinic. Mr. Cambier will also be responsible for maintaining the database on all enrolled sarcoidosis patients.

Douglas F. Nixon, MD, PhD, Consultant (effort as needed). Dr. Douglas Nixon is Professor of Medicine and Associate Director for the Division of Experimental Medicine at UCSF's San Francisco General Hospital. Dr. Nixon has over 20 years of experience studying T cell responses and was recruited to UCSF in 2001 after a comprehensive international search where he was considered one of the finest cellular immunologists in the world studying the cellular immune response to HIV. He has contributed considerable insight into cellular mechanisms of immune responses to HIV infection and his lab performs both basic and translational studies. Dr. Nixon and his lab have performed similar studies to those proposed in this application on samples from human subjects with HIV and tuberculosis, and is well-versed on the methodologies and interpretation of the data. Dr. Nixon is fully invested in this project and will serve as Dr. Koth's scientific advisor.

FRINGE BENEFITS

The fringe benefit costs are calculated using the University's established rates of 17% for academics and 22% for staff personnel.

SUPPLIES ($5,696)

(1) Flow Cytometer User Time $720

Multi-color flow cytometry will be used to detect NKT cells and determine their cytokine profiles. Use of the flow cytometer is charged at $60/hr and analysis of one sample typically requires ~0.1 hr. We have budgeted for ~60 x 2 samples.

(2) Antibodies $3,361
Multiple commercially available antibodies will be purchased to detect NKT cells (4 different antibodies) and their cytokine profiles (interleukins 4, 13 and TNFα, IFNγ). We will also use antibodies against CD3, CD4, and CD8 to distinguish T cell subsets. Antibodies will run ~$200-300/antibody and we will perform several FACS experiments per sample.
PIM will be obtained from the TB reagent facility at Colorado State University
http://www.cvmbs.colostate.edu/microbiology/tb/researchma.htm
Other FACS reagents include purified CD1d:Ig Recombinant Fusion Protein (DimerX) (~$500 for 100ug) and α-GalCer (~$200.00 for 1mg).

(3) Blood drawing supplies $1015
lavender-top tubes ($43/100 tubes x 10)
CBC tubes ($105/1000 tubes)
VACUTAINER* Blood Collection Sets ($231/200 sets)
Sterile Guaze ($185/case)
Band-aid ($64/case)

(4) Tissue Culture and General Laboratory Supplies (average of $50/mo) $600
Funds are requested for general laboratory supplies for the proposed studies. Cost of these supplies is estimated at $50/month

TRAVEL ($0)

OTHER EXPENSES ($2500)
Advertisement for healthy volunteer recruitment ($700)
Healthy control volunteer blood draw ($30/hr x 60 subjects =$1800)

BIOGRAPHICAL SKETCH

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

NAME	POSITION TITLE
Laura L. Koth, M.D.	Assistant Professor of Medicine
eRA COMMONS USER NAME LAURAKOTH	

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
University of California, Los Angeles	B.S.	06/1990	Biochemistry
Harvard Medical School	M.D.	06/1994	Medicine
Massachusetts General Hospital	Resident	1994-1997	Internal Medicine
University of California, San Francisco	Fellow	1998-2003	Pulmonary/Critical Care Medicine

A. Positions and Honors.

POSITIONS

1991 summer	Research project with Dr. Sabeeha Merchant on the regulation of chlorophyll metabolism, UCLA Department of Biochemistry, Los Angeles, CA
1991-1992	Research project and medical school thesis on parathyroid hormone gene mutations, under direction of Dr. Andrew Arnold, Massachusetts General Hospital, Boston, MA
1992	Karolinska Hospital, Dept. of Clinical Genetics, Stockholm, Sweden, worked under guidance of Dr. E. Friedman to continue my work in parathyroid biology
1991-1994	Medical Student, Harvard Medical School
1994-1997	Resident in Internal Medicine, Massachusetts General Hospital
1998-1999	Clinical Pulmonary Fellow, University of California Hospitals, San Francisco
2000-2003	Research Fellow, Lung Biology Center and Cardiovascular Research Institute, University of California, San Francisco
2003-present	Assistant Professor of Medicine, Lung Biology Center, University of California, San Francisco
2003-2004	Director, Interstitial Lung Disease Clinic, San Francisco General Hospital
2004-present	Co-Director, Interstitial Lung Disease Clinic, University of California, San Francisco
2006-present	Preceptor, Internal Medicine Residency Training Program Subspecialty ILD Clinic Experience

HONORS AND AWARDS

Anatomy and Physiology Student of the Year, 1985
American Chemical Society, Chemistry Student of the Year, 1987
Recipient of the Southern California Edison Co. Career Development Award, 1987
Recipient of the UCLA Regional Alumni Scholarship, 1987
UCLA Alumni Association Achievement Award, 1988 and 1989
Dunn Award, UCLA Biochemistry Student of the Year, 1990
First Place, National Basic Science Research Award, Third Annual Respiratory Disease Young Investigator's Forum, 2006

B. Selected peer-reviewed publications (in chronological order).

Original Research

1. J.H. Lee, N. Kaminski, G. Dolganov, G. Grunig, **L. Koth**, C. Solomon, D.J. Erle, D. Sheppard. Interleukin-13 induces dramatically different transcriptional programs in three human airway cell types. *Am J Respir Cell Mol Biol*, 2001 Oct; 25(4):474-85.

2. D.A. Kuperman, X.Z. Huang, **L. Koth**, Z. Zhu, J.A. Elias, D. Sheppard, D.J. Erle. Direct effects of interleukin-13 on epithelial cells cause airway hyperreactivity and mucus overproduction, two central features of asthma. *Nat Med*, 2002 Aug; 8(8): 885-9.

3. M.B. Gotway, J.A. Golden, M. Warnock, **L.L. Koth**, R. Webb, G.P.Reddy, J.R. Balmes. Hard metal interstitial lung disease: high-resolution computed tomography appearance. *J Thorac Imaging*. 2002 Oct;17(4):314-8.

4. A. Barczak, M.W. Rodriquez, K. Hanspers, **L.L. Koth**, Y.C. Tai, B.M. Bolstad, T.P. Speed, D.J. Erle. Spotted long oligonucleotide arrays for human gene expression analysis. *Genome Res.* 2003 Jul;13(7):1775-85.

5. **L.L. Koth**, M.W. Rodriguez, X.L. Berstein, S. Chan, X. Huang, I.F. Charo, B.J. Rollins, D.J. Erle. Aspergillus antigen induces robust Th2 cytokine production, inflammation, airway hyperreactivity and fibrosis in the absence of MCP-1 or CCR2. *Respir Res*, 2004 Sep; 15;5(1):12.

6. Marras TK, Wallace RJ Jr, **Koth LL**, Stulbarg MS, Cowl CT, Daley CL. Hypersensitivity pneumonitis reaction to Mycobacterium avium in household water. *Chest*. 2005 Feb;127(2):664-71.

7. **L. L. Koth**, P.G. Woodruff, Y.H. Yang, M.W. Rodriguez, S. Favoreto, G.M. Dolganov, A.C. Paquet, D.J. Erle. A distinctive alveolar macrophage activation state induced by cigarette smoking. *Am J Res Crit Car Med*, 2005 Dec 1;172(11):1383-92. Epub 2005 Sep 15.

8. BW Kinder, HR Collard, **L Koth**, DI Daikh, PJ Wolters, B Elicker, KD Jones, TE King Jr. Idiopathic NSIP: Lung Manifestation of Undifferentiated Connective Tissue Disease? *Am J Respir Crit Care Med*. 2007 Jun 7; [Epub ahead of print].

9. **LL Koth**, BT Alex, S Hawgood, MA Nead, D Sheppard, DJ Erle, DG Morris. Integrin {beta}6 Mediates Phospholipid and Collectin Homeostasis by Activation of Latent TGF{beta}1. *Am J Respir Cell Mol Biol*. 2007 Jul 19; [Epub ahead of print].

Abstracts

1. D. Kuperman, **L. Koth**, J. Mandac, J. Elias, Z. Zhu, D.J Erle. The role of IL-13 and STAT6 in a murine model of asthma. *Am J Respir Crit Care Med*, 2001 April; 163 (5) A437.

2. D. J. Erle, **L. Koth**, O. Abramson, M. Rodriguez, A. Barczak. Use of spotted oligonucleotide arrays for large-scale analysis of mammalian gene expression. *Chest*, 2002 Mar; 121(3 Suppl):80S.

3. **L. Koth**, M. Rodriguez, X.Z. Huang, D.J. Erle. Effects of MCP-1 deletion on lung inflammation, airway hyperresponsiveness (AHR) and fibrosis. *Am J Respir Crit Care Med*, 2002 April; 165 (8) A448.

4. **L.L. Koth**, D.G. Morris, Y. Wang, D. Sheppard, S. Hawgood, D.J. Erle. The integrin subunit β6 (*Itgb6*) is required for normal mouse lung surfactant homeostasis. *Am J Respir Crit Care Med*, 2003 April; 167(7) A288.

Chapters:

1. **L. Koth**, D. Sheppard. Integrins and pulmonary fibrosis. In: Lung Biology in Health and Disease, ed. Lenfant C: Volume 158: Idiopathic pulmonary fibrosis, ed Lynch JP. New York: Marcel Dekker, 2004.

2. **L. Koth**, King TE Jr., Heldmann M., Berney S.N., Berney S.M. Pulmonary vasculitis In: Bone's Atlas of Pulmonary. Philadelphia: W.B. Saunders Co., 2005; pp: 201-210.

3. J. Alexander and **L. Koth**. Smoking-related interstitial lung diseases. Pulmonary and Critical Care Update Online (PCCU) [serial online]. 2005; vol 19, lesson 20. Available at: www.chestnet.org/education/online/pccu/vol18/lessons09_10/lesson10.php

C. Research Support.

Ongoing Research Support

K08 HL072915 Koth (PI) 05/01/2003-04/30/2008
NIH/NHLBI
Mcp-1 and TGFB in Macrophage Activation and Emphysema

This KO8 award is a Career Development Award from the NIH to support the applicant's research related and career development activities. The specific aims of the grant are to determine the contribution of Mcp-1 and TGFβ in the development of macrophage activation and subsequent emphysema in a mouse model.

16RT-0072 Koth (PI) 07/01/2007-06/30/2010
UC Tobacco-Related Disease Research Program
Macrophage DAP12 pathway in emphysema

The major goal of this project is to study a novel signaling pathway which our data suggest may be an important contributor of smoking-induced macrophage activation and the development of emphysema.

OTHER SUPPORT

Laura Koth

Active

K08 HL072915 Koth (PI) 05/01/2003-04/30/2008 75%
NIH/NHLBI $xxx
Mcp-1 and TGFB in Macrophage Activation and Emphysema

This K08 award is a Career Development Award from the NIH to support the applicant's research related and career development activities. The specific aims of the grant are to determine the contribution of Mcp-1 and TGFβ in the development of macrophage activation and subsequent emphysema in a mouse model.

16RT-0072 Koth (PI) 07/01/2007-06/30/2010 Yr 1: 25%; Yr 2 &3: 50%
UC Tobacco-Related Disease Research Program $xxx
Macrophage DAP12 pathway in emphysema

The major goal of this project is to study a novel signaling pathway which our data suggest may be an important contributor of smoking-induced macrophage activation and the development of emphysema.

Pending

(Koth) 07/01/08-06/30/2010 7%
American Lung Association-Dalsemer Research Grant $xxx
Effector Function of Natural Killer T (NKT) Cells in Sarcoidosis

The major goal of this project is to determine NKT cell function in sarcoid individuals longitudinally and the effect of immunosuppressants on NKT cell function.

(Koth) 07/01/08-06/30/2010 12%
NIH/R03 $xxx
Effector Function of Natural Killer T (NKT) Cells in Sarcoidosis

The major goal of this project is to determine how NKT cell effector function relates to disease severity in sarcoidosis subjects and the effect of immune-suppression on NKT cell subsets and cytokine responses in a longitudinal study.

Overlap

Dr. Koth will adjust her effort accordingly should a grant be funded before completion of her K08 award on 4/30/08.

BIOGRAPHICAL SKETCH

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

NAME	POSITION TITLE
Douglas F. Nixon	Professor of Medicine (in Residence)
eRA COMMONS USER NAME dnixon	

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
University College London, University of London, England	B.Sc. (Hons)	1981	Immunology
Westminster Medical School, London, England	M.B., B.S.	1984	Medicine and Surgery
University of Oxford, England	M.A.	1991	Immunology
University of Oxford, England	D. Phil.	1992	Immunology

A. Positions and Honors.

Positions and Employment

1989-1993	Clinical Lecturer, Medicine, University of Oxford, U.K.
1993-1995	Director, Immunotherapy, United Biomedical, Inc., New York
1996-1997	Visiting Scientist, Aaron Diamond AIDS Research Center, New York
1997-2000	Staff Investigator, Aaron Diamond AIDS Research Center, New York
1997-2000	Assistant Professor, The Rockefeller University, New York
2000-present	Associate Investigator, Gladstone Institute of Virology and Immunology, San Francisco
2001-2006	Associate Professor of Medicine (in residence), University of California, San Francisco
2006-present	Professor of Medicine (in residence), University of California, San Francisco

Other Experience and Professional Memberships

1981	B.Sc., Immunology, University College London, First Class Honors
1988	Vaccine Research Trust, U.K., First Prize (joint)
1989-1992	Junior Research Fellowship, Merton College, University of Oxford, U.K.
1989-1993	Special Fellowship, Merton College, University of Oxford, U.K.
2000-2005	Elizabeth Glaser Pediatric AIDS Foundation Scientist
2003-present	Appointed to Editorial Board, Journal of Virology
2003-present	Member of American Society for Clinical Investigation (ASCI)
2003-present	Member, NIH study section, AARR-2 (now AIP)
2003-present	Member, IAVI Vaccine Science Committee
2005-present	Contributing Editor, Journal of Clinical Investigation

B. Selected peer-reviewed publications (OUT OF MORE THAN 100).

1. Nixon DF, Townsend ARM, Elvin JG, Rizza CR, Gallwey J and McMichael AJ. HIV-1 gag-specific cytotoxic T lymphocytes defined with recombinant vaccinia virus and synthetic peptides. *Nature,* 1988 336:484-487.
2. Phillips RE, Rowland-Jones S, Nixon DF, Gotch FM, Edwards JP, Ogunlesi AO, Elvin JG, Rothbard J, Bangham CRM, Rizza CR and McMichael AJ. Human immunodeficiency virus genetic variation that can escape cytotoxic T cell recognition. *Nature,* 1991 354:453-459.
3. Ogg GS, Jin X, Bonhoeffer S, Dunbar PR, Nowak MA, Monard S, Segal JP, Cao Y, Rowland-Jones S, Cerundolo V, Hurley A, Markowitz M, Ho DD, Nixon DF and McMichael AJ. Quantitation of HIV-1-specific cytotoxic T lymphocytes and plasma load of viral RNA. *Science,* 1998 279:2103-2106.
4. Ortiz GM*, Nixon DF*, Trkola A, Binley J, Jin X, Bonhoeffer S, Kuebler PJ, Donahoe SM, Demoitie MA, Kakimoto WM, Ketas T, Clas B, Zhang L, Cao Y, Hurley A, Moore JP, Ho DD and Markowitz M. HIV-1-specific immune responses in subjects who temporarily contain virus replication after discontinuation of

highly active antiretroviral therapy. *Journal of Clinical Investigation,* 1999 104:R13-R18. *Shared primary authorship.

5. Rose, NF, Marx PA, Luckay A, Nixon DF, Moretto WJ, Donahoe SM, Montefiori D, Roberts A, Buonocore L and Rose JK. An effective AIDS vaccine based on live attenuated vesicular stomatitis virus recombinants. *Cell,* 2001 106:539-549.
6. Ortiz GM, Wellons M, Brancato J, Vo HTT, Zinn RL, Clarkson DE, Miralles GD, Van Loon K, Bonhoeffer S, Montefiori D, Bartlett JA and Nixon DF. Structured antiretroviral therapy interruption in chronically HIV-1-infected subjects. *Proc. Natl. Acad. Sci. USA*, 2001 98:13288-13293.
7. Sandberg JK, Fast NM, Palacios EH, Fennelly G, Dobroszycki J, Palumbo P, Wiznia A, Grant RM, Bhardwaj N, Rosenberg MG and Nixon DF. Selective loss of innate $CD4^+$ V alpha 24 NKT cells in HIV infection *J. Virology,* 2002 76:7528-7534.
8. Aandahl EM, Moretto WJ, Haslett PA, Vang T, Bryn T, Tasken K and Nixon DF. Inhibition of antigen-specific T cell proliferation and cytokine production by protein kinase A type I. *J. Immunology,* 2002 169:802-808.
9. Shacklett BL, Means RE, Larsson M, Wilkens DT, Beadle TJ, Merritt MJ, Bhardwaj N, Palumbo PE, Skurnick JH, Louria DB and Nixon DF. Dendritic cell amplification of HIV-1 specific $CD8^+$ T-cell responses in exposed, seronegative heterosexual women. *AIDS Research and Human Retroviruses,* 2002 18:805-815.
10. Jennes W, Kestens L, Nixon DF and Shacklett BL. Enhanced ELISPOT detection of antigen-specific T cell responses from cryopreserved specimens with addition of both IL-7 and IL-15—the Amplispot assay*. J. Immunology Methods,* 2002 270:99-108.
11. Aandahl EM, Sandberg JK, Beckerman KP, Moretto WJ, Tasken K and Nixon DF. CD7 Is a differentiation marker that identifies multiple CD8 T-cell effector Subsets *J. Immunol*ogy, 2003 170:2349-2355.
12. Sandberg JK, Fast NM, Jordan KA, Furlan SN, Barbour J, Fennelly G, Dobroszycki J, Spiegel HML, Wiznia A, Rosenberg MG, and Nixon DF. $CD4^+$ T cell and age requirements for effective, antigen-driven $CD8^+$ T cell responses in pediatric HIV-infection. *J. Immunology,* 2003 170:4403-4410.
13. Shacklett BL, Cox CA, Sandberg JK, Stollman NH, Jacobson MA and Nixon, DF. Trafficking of HIV-specific CD8+ T cells to gut-associated lymphoid tissue (GALT) during chronic infection. *J. Virology,* 2003 77:5621-5631.
14. Karlsson AC, Deeks SG, Barbour JD, Heiken BD, Younger SR, Hoh R, Lane M, Sällberg M, Ortiz GM, Demarest JM, Liegler T, Grant RM, Martin JN and Nixon DF. Dual pressure from antiretroviral drug and cell mediated immune response on the HIV-1 protease gene. *J. Virology,* 2003 77:6743-6752.
15. Aandahl EM, Michaelsson J, Moretto WJ, Hecht FM, Nixon DF. Human CD4+ CD25+ regulatory T cells control T cell responses to HIV and CMV antigens. *J. Virology,* 2004 78(5):2454-2459.
16. Sandberg JK, Stoddart CA, Brilot F, Jordan KA, and Nixon DF. Development of innate CD4+ Vα24 NKT cells in the early human fetal thymus is regulated by IL-7. *Proc. Natl. Acad. Sci. USA,* 2004 101(18):7058-7063*.*
17. Shacklett BL, Cox CA, Quigley MF, Kreis C, Stollman NH, Jacobson MA, Andersson J, Sandberg JK, Nixon DF. Abundant expression of granzyme A, but not perforin, in granules of CD8+ T Cells in GALT: Implications for immune control of HIV-1 infection. *J. Immunology,* 2004 173:641-648.
18. Schweighardt B, Roy AM, Meiklejohn DA, Grace EJ 2^{nd}, Moretto WJ, Heymann JJ, Nixon DF. R5 human immunodeficiency virus type 1 (HIV-1) replicates more efficiently in primary CD4+ T-cell cultures than X4 HIV-1. *J. Virology,* 2004 17:9164-9173.
19. Legrand FA, Abadi J, Jordan KA, Davenport MA, Deeks SG, Fennelly GJ, Wiznia AA, Nixon DF, Rosenberg MG. Partial treatment interruption of protease inhibitors augment HIV-specific immune response in vertically infected pediatric patients. *AIDS*, 2005 15:1575-1585.
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23. Jordan KA, Furlan SN, Gonzalez VD, Karlsson AC, Quigley MF, Deeks SG, Rosenberg MG, Nixon DF, Sandberg JK. CD8 T cell effector maturation in HIV-1 infected children. *Virology,* 2006 347(1):117-126.
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25. Legrand FA, Nixon DF, Loo CP, Ono E, Chapman JM, Miyamoto M, Diaz RS, Santos AM, Succi RC, Abadi J, Rosenberg MG, de Moraes-Pinto MI, Kallas EG. Strong HIV-1 specific T cell responses in HIV-1 exposed uninfected infants and neonates revealed after regulatory T cell removal. *PLoS ONE*. 2006 Dec 20; 1:e102.
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27. Carr WH, Rosen DB, Arase H, Nixon DF, Michaelsson J, Lanier LL. Cutting edge: KIR3DS1, a gene implicated in resistance to progression to AIDS, encodes a DAP12-associated receptor expressed on NK cells that triggers NK cell activation. *J Immunol.* 2007 Jan 15; 178(2):647-51.
28. Snyder-Cappione JE, Nixon DF*, Loo CP, Chapman JM, Meiklejohn DA, Melo FF, Costa PR, Sandberg JK, Rodrigues DS, Kallas EG. Individuals with Pulmonary Tuberculosis have lower levels of circulating CD1d-restricted NKT cells. *Journal of Infectious Diseases*, in press. *Corresponding author.
29. Sequential Broadening of CTL Responses in Early HIV-1 Infection is Associated With Viral Escape. Karlsson AC, Iversen AKN, Chapman JM, de Oliviera T, Spotts G, McMichael AJ, Davenport MP, Hecht FM^, and Nixon DF^. *PLoSONE*, in press. ^ Shared senior authorship.

C. Research Support.

Ongoing Research Support

R01 AI060379 Nixon (PI) 04/01/05 - 03/31/10
NIH/HIAID
Cell Mediated Immunity in HIV Infected Children
The major goal of this project is to 1) determine the relationship between age and development of cellular immune responses in HIV infected children, 2) determine the effect of race on HIV specific immune responses in Pediatric populations, and 3) determine the effect of partial, intermittent or continued viral suppression, and of interruption in protease inhibitor therapy, on HIV specific immune responses and T cell activation. Role: PI

R01 A1068498 Nixon (PI) 07/01/06 - 06/30/08
NIH/NIAID
T Regulatory Cells in HIV Infection
The overall aim of this study is to determine the phenotype, function, and role of CD4+CD25+ regulatory T cells (Treg cells) in HIV infected and uninfected humans. Role: PI

R01 AI062333 Grant (PI) 09/01/04 - 06/30/10
NIH
Chemoprophylaxis and HIV Host Interactions
The major goal of this project is to 1) test the hypothesis that anti-HIV chemoprophylaxis may have durable benefits (or risks) that extend beyond the period treatment, including attenuation of the course of infection among those who become infected despite chemoprophylaxis, 2) prolongation of the period of viral exposure prior to seroconversion, 3) and induction of cell-mediated antiviral immune responses among those who remain seronegative despite viral exposure, and 4) we also assess the rate of chemoprophylaxis may be lower if protective immunity is inducted, or higher if chemoprophylaxis suppresses viral infection below the antigenic threshold for antibody responses without providing durable containment. Role: Co-Inv

PO1 AI064520 Brodsky (PI) 09/16/05 - 02/28/10
Human Natural Killer Cell Biology: Proj 5 NK cells and
HIV infection
The major goals of this project are to 1) determine the relationship between the frequency, number, function, and phenotype of NK cells and plasma viral load early in HIV infection, and during structured treatment interruption, and 2) determine the effect of race on HIV specific immune responses in Pediatric populations,

and 3) to determine the effect of early versus late initiation of HAART, and the effect of interleukin-2 (IL-2) therapy on NK cell frequency, number, and function in HIV-infected patients. Role: Co-Inv

R21 AI065241 Nixon (PI) 07/01/06 - 06/30/11
NIH/NIAID
Viral Tropism and Immune Control in HIV
The major goals of this project are 1) to determine the role of the infected cell phenotype on the susceptibility to CD8+ T cells control, and 2) to investigate the effect of CD8+ T cells on the growth of X4 and R5 in CD4+ T lymphocytes in vitro. Role: PI

Nixon (PI) 01/01/07 - 12/31/07
Pfizer
Immunity to HERV and LINE-1
The major goal of this project is to determine the impact of immunity to HERV and LINE-1. Role: PI

R37 AI052731 Nixon (PI) 05/01/02 - 06/30/12
NIH/NIAID
Innate NKT Cells in HIV Infection
The major goal of this project is to determine the effects of HIV infection on the natural killer T (NKT) cell compartment. Role: PI

COMPLETED RESEARCH SUPPORT

U01 Cooperative Agreement Levy (PI) 07/01/02 - 06/30/07
NIH/NIAID
Acute Infection and Early Disease Research Program
The major goal of this project was to investigate the pathogenesis of acute and early HIV infection.
Role: Co-Inv

R01 AI52745 Deeks (PI) 09/01/02 - 02/28/06
NIH/NIAID
Immunologic Control of Drug-Resistant HIV
The major goals of this project were to examine TREC, Ki67, and CD4 proliferation and their relationship to CFC and virologic outcomes. Role: Co-Inv

R21 AI060407 Nixon (PI) 03/01/04 - 02/28/06
NIH/NIAID
T Regulatory Cells in HIV Infection
The goal of this proposal was to generate data to determine the importance of T_{REG} cells in HIV infection.
Role: PI

OTHER SUPPORT

Douglas Nixon

Active

P01 AI064520 Brodsky (PI) 09/16/2005–02/28/2010 15%
UCSF/NIH/NIAID $xxx
Human Natural Killer Cell Biology

The major goals of this project are 1) to focus on the relationship between the frequency, number and function of NK cells early in HIV infection and the level of plasma viremia post seroconveresion, the temporal kinetics of the NK cell response in relationship to plasma HIV viral load in early HIV infection, and the effect of HIV viremia on NK cell receptor expression and 2) to determine how the time of initiation of treatment with highly active antiretroviral drug therapy (HAART) and how interleukin-2 therapy impact the frequency and function of NK cells in HIV-infected subjects enrolled in the “Options Project”.

R01 AI060379 Nixon (PI) 04/15/2005–12/31/2009 15%
NIH/NIAID $xxx
Cell Mediated Immunity in HIV Infected Children

The major goals of this project are 1) to determine the relationship between age and development of cellular immune responses in HIV infected children, 2) to determine the effect of race on HIV specific immune responses in pediatric populations, and 3) to determine the effect of partial, intermittent or continued viral suppression, and of interruption in protease inhibitor therapy, on HIV specific immune responses and T cell activation.

R01 AI068498 Nixon (PI) 07/1/2006–06/30/2011 15%
NIH/NIAID $xxx
T Regulatory Cells in HIV Infection

The major goals of this project are: 1) to determine a functional phenotype of human Treg cells in healthy HIV uninfected individuals, 2) to determine the impact of removal of Treg cells on the avidity, breadth, phenotype and function of HIV specific responding T cells, and also ascertain whether HIV specific Treg cells can be induced during HIV infection, and 3) to determine the frequency and function of Treg cells in primary HIV infection.

R01 AI062333 Grant (PI) 09/1/2004–01/31/2010 10%
NIH/NIAID $xxx *(salary support only)*
Chemoprophylaxis and HIV Host Interactions

The major goal of this project is: 1) to test the hypothesis that anti-HIV chemoprophylaxis may have durable benefits (or risks) that extend beyond the period of treatment, including attenuation of the course of infection among those who become infected despite chemoprophylaxis, 2) prolongation of the period of viral exposure prior to seroconversion, 3) and induction of cell-mediated antiviral immune responses among those who remain seronegative despite viral exposure, and 4) we will also assess the rate of chemoprophylaxis may be lower if protective immunity is inducted, or higher if chemoprophylaxis suppresses viral infection below the antigenic threshold for antibody responses without providing durable containment.

R21 AI065241 Nixon (PI) 07/1/2006–06/30/2008 15%
NIH $xxx
Viral Tropism and Immune Control in HIV

The major goals of this project are 1) to determine the role of the infected cell phenotype on the susceptibility to CD8+ T cells control, and 2) to investigate the effect of CD8+ T cells on the growth of X4 and R5 in CD4+ T lymphocytes in vitro.

Nixon (PI) 01/01/2007–12/31/2007 10%
Pfizer $xxx
Immunity to HERV and LINE-1

The major goal of this project is to determine the correlation between magnitude, phenotype or breadth of HERV specific response in relation to HIV-1 viral load.

R01 AI52731 Nixon (PI) 07/01/2007–06/30/2012 15%
NIH/NIAID $xxx
Innate NKT Cells in HIV Infection

The major goal of this project is to determine the effects of HIV infection on the natural killer T (NKT) cell compartment.

Pending

PO1 Hecht (PI) 04/01/2007–03/31/2008 20%
NIH $xxx
Biology of HIV Transmission

The major goal of this project is to study immunologic factors in the transmission and subsequent reversion of cytotoxic T-lymphocyte escape mutations in HIV when transmitted to a new host.

R01 Nixon (PI) 07/01/2007–06/30/2012 15%
NIH/NIAID $xxx
Human Endogenous Retroelements in HIV Immunopathogenesis

The major goal of this project is to understand the consequence of HERE antigen production and presentation in HIV-1 infection, and to determine whether the immune response generated to these elements can eliminate HIV-1 infected cells.

R21 AN2983922 Nixon (co-PI) 12/01/2007–11/30/2009 10%
NIH/NIAID $xxx
NK cell and airway epithelial responses to influenza

The major goals of this project are to better understand the pathogenesis of the airway epithelial and innate immune cell interactions following influenza virus infection and vaccination in humans.

Overlap
Dr. Nixon will adjust his effort accordingly as grants are funded.

BIOGRAPHICAL SKETCH

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

NAME	POSITION TITLE
Jennifer E. Snyder-Cappione	Postdoctoral Scholar
eRA COMMONS USER NAME jsnyder-cappione	

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
Brandeis University, Waltham, MA	B.S.	1996	Biology
Wadsworth Center for Labs and Research, Albany, NY	Lab Technician	1996-1998	Immunotoxicology
University of Rochester Medical Center, Rochester, NY	Ph. D.	1998-2004	Microbiology and Immunology
Gladstone Institute of Virology and Immunology, UCSF, San Francisco, CA	Postdoctoral Fellow	2004-present	Viral Immunology

A. Positions and Honors.

Universitywide AIDS Research Program (UARP) Award Recipient (#F05-GI-209): September 2005- September 2007

Scholarship Recipient and Selected Speaker: 3rd IAS conference on HIV Pathogenesis and Treatment, Rio de Janeiro, Brazil, July 24-27, 2005

Pre-doctoral Trainee: HIV-1 Replication and Pathogenesis Training Grant #T32 AI49815
University of Rochester Medical Center, 2001-2004

University of Rochester Graduate Student Society Travel Award, 2000

B. Selected peer-reviewed publications (in chronological order).

Snyder J.E., Filipov N.M., Parsons P.J., Lawrence D.A. The efficiency of maternal transfer of lead and its influence on plasma IgE and splenic cellularity of mice. *Toxicological Sciences* 2000 Sept;57(1): 87-94.

Kuzin II, **Snyder J.E.**, Ugine GD, Wu D, Lee S, Bushnell T Jr, Insel RA, Young FM, Bottaro A. Tetracyclines inhibit activated B cell function. *Int Immunol*. 2001 Jul;13(7):921-31.

Snyder J.E., Bowers W.J., Livingstone A.M., Lee F.E., Federoff H.J., Mosmann T.R. Measuring the frequency of mouse and human cytotoxic T cells by the Lysispot assay: independent regulation of cytokine secretion and short-term killing. *Nature Medicine* 2003 Feb;9(2):231-5.

Snyder, J.E. and Mosmann, T.R. How to "spot" a real killer. *Trends Immunol.* 2003 May;24(5):231.

Lee FE, Walsh EE, Falsey AR, Liu N, Liu D, Divekar A, **Snyder-Cappione J.E.**, Mosmann TR. The balance between influenza- and RSV-specific CD4 T cells secreting IL-10 or IFNgamma in young and healthy-elderly subjects. *Mechanisms of Ageing and Development*; 2005 Nov;126(11):1223-9.

Moll M, **Snyder-Cappione J**, Spotts G, Hecht FM, Sandberg JK, Nixon DF. Expansion of CD1d-restricted NKT cells in patients with primary HIV-1 infection treated with interleukin-2. *Blood*; 2006 Apr 15;107(8):3081-3.

Snyder-Cappione J.E., Divekar A.A., Maupin G.M., Jin, X, Demeter L.M., Mosmann T.R. HIV-Specific Cytotoxic Cell Frequencies Measured Directly Ex Vivo by the Lysispot Assay Can Be Higher or Lower Than the

Frequencies of IFN-{gamma}-Secreting Cells: Anti-HIV Cytotoxicity Is Not Generally Impaired Relative to Other Chronic Virus Responses. *Journal of Immunology*; 2006 Feb 15;176(4):2662-8.

Snyder-Cappione J.E., Nixon D.F., Loo C.P., Chapman J.M., Meiklejohn, D.A., Melo, F.F., Costa P.R., Sandberg J.K., Rodrigues D.S., and Kallas E.G. Individuals with Pulmonary Tuberculosis have lower levels of circulating CD1d-restricted NKT cells. *Journal of Infectious Diseases*; 2007 May 195(9):1361-4

OTHER SUPPORT

Jennifer E. Snyder-Cappione

Active

R01 AI52731 (Nixon, PI)	07/01/2007-06/30/2012	100%
NIH/NIAID	$xxx	
Innate NKT Cells in HIV Infection		

The major goal of this project is to determine the effects of HIV infection on the natural killer T (NKT) cell compartment.

Overlap

Dr. Snyder-Cappione will adjust her effort accordingly as grants are funded.

University of California
San Francisco (UCSF)

DEPARTMENT OF MEDICINE

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SAN FRANCISCO GENERAL HOSPITAL
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Biomedical Research

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Hal F. Yee, Jr., M.D., Ph.D.
Interim Chief of Medical Service, San Francisco General Hospital

November 12, 2007

Re: Laura Koth

Dear RAP Committee Members,

This letter is to support Laura Koth's application to study natural killer T cells in sarcoidosis. I am aware of her project and am very pleased that she has joined forces with Dr. Douglas Nixon to study the immunology of sarcoidosis. She will be taking advantage of the Interstitial Lung Disease Clinic's clinical infrastructure and database, which she played a large role in developing.

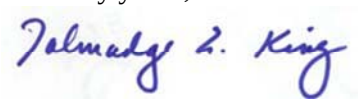
Her project represents a completely independent area of research than that performed by her K08 mentor, Dr. David Erle.

The Department of Medicine is committed to Dr. Koth and will ensure the availability of lab space and other resources for the proposed research.

Dr. Koth is an outstanding young researcher who is committed to a career in academic Pulmonary Medicine. One of the primary goals of the Department of Medicine, and the Division of Pulmonary and Critical Care Medicine, is to make the best possible resources available to promising candidates, such as Dr. Koth, and to help them to become successful, independent researchers. She has a very sound research project, an outstanding environment, experienced and committed mentors and the drive to succeed.

I give her my strongest support and I wish her luck in her project.

Sincerely yours,



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November 28, 2007

Laura L. Koth, MD
Lung Biology Center
University of California, San Francisco
Rock Hall, Mission Bay Campus
1550 4th Street.
San Francisco, CA 94158

RE: RAP application for LAURA KOTH

Dear Laura,

I am very enthusiastic to support your grant application to the UCSF CTSI SOS grant program and serve as your scientific consultant for your experiments addressing the role of NKT cells in sarcoidosis. As we discussed in our last meeting, it will be very interesting to examine the NKT cell subsets from sarcoid patients and how CD1d antigens activate these NKT cells. I will provide ongoing expertise in experimental study design, interpretation of the results and help you troubleshoot any unforeseen issues. In addition, Dr. Jennifer Snyder-Cappione, your collaborator and my post-doctoral fellow, also has extensive experience performing multi-color FACS and *in vitro* stimulation assays and we are both fully committed to your project.

I have complete confidence that the findings obtained from this project will advance the field of sarcoidosis immunobiology and will serve as a critical next step in defining how the NKT cell population may contribute to sarcoidal inflammation. In fact, I cannot think of a reason why the studies you proposed should not be pursued in light of the current thinking about sarcoidosis disease pathogenesis. You have organized the Interstitial Lung Disease Clinic to a point that you can now take advantage of the clinical database and blood drawing system that you helped establish. I think your project is exciting and novel and I look forward to working together.

Sincerely,

Douglas F. Nixon, M.D., Ph.D.,
Professor of Medicine, University of California, San Francisco
Associate Chief, Division of Experimental Medicine, UCSF

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E-mail: douglas.nixon@ucsf.edu