Children's Hospital of Pittsburgh

Annual Progress Report: 2006 Formula Grant

Reporting Period

July 1, 2009 – June 30, 2010

Formula Grant Overview

The Children's Hospital of Pittsburgh received $732,956 in formula funds for the grant award period January 1, 2007 through December 31, 2010. Accomplishments for the reporting period are described below.

Research Project 1: Project Title and Purpose

Cell Biology of Cystic Fibrosis Transmembrane Regulator (CFTR) - CFTR is responsible for transporting chloride from the cells lining the airway, digestive tract, biliary tract, pancreatic duct and kidney tubules into the body fluids. Impairment of this function results in the lung disease, pancreatic insufficiency, liver disease, malabsorption and other clinical problems of cystic fibrosis. This project is designed to identify specific factors that determine how CFTR gets to, and stays, at the cell membrane where it needs to be for optimal functionality. The goal is to exploit knowledge of these factors for the design of novel pharmacotherapeutic agents that enhance the functionality of the mutant CFTR in patients with cystic fibrosis.

Anticipated Duration of Project

1/1/2007 - 12/31/2010

Project Overview

The objectives are to establish a program that will investigate the cellular and biochemical mechanisms by which CFTR is appropriately targeted to the site of its optimal functionality at the cell membrane. The research will provide information upon which to base novel pharmacological strategies to treat the clinical problems of cystic fibrosis.

Specific Aim 1: Identify a network of proteins that regulate CFTR endocytosis.
Specific Aim 2: Examine the specific roles of c-Cbl and Rab4 in CFTR endocytosis.
Specific Aim 3: Examine the mechanism by which sorting within the endocytic pathway determines the half-life of CFTR on the plasma membrane.

These objectives will be achieved by a combination of molecular, biochemical, functional, proteomic and morphological techniques in model cell line systems. This will include studies of protein-protein interactions, perturbations introduced by transfected specific genes and siRNA

molecules, confocal immunofluorescent imaging, immunoelectron microscopy, cell surface labeling, transepithelial conductance analysis and proteomic analysis.

Principal Investigator

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Other Participating Researchers

None

Expected Research Outcomes and Benefits

Through this basic research we will discover new methods for ameliorating the devastating clinical consequences of cystic fibrosis.

Summary of Research Completed

We have made progress in the following areas over the past year:

1) The scholar received the Recovery Act Administrative Supplement to the parent R01 award from the NIH to study the endocytic and postendocytic trafficking of CFTR.

2) The scholar published a peer reviewed article:
Ye S, Cihil K, Beer Stolz D, Pilewski JM, Stanton BA, Swiatecka-Urban A (2010) c-Cbl Facilitates Endocytosis and Lysosomal degradation of CFTR in Human Airway Epithelial Cells. Accepted for publication in JBC, June 4, 2010

3) The scholar presented her original work at an international symposium:
Ye S, Cihil KM, Stanton BA, Swiatecka-Urban A (2009) The Ubiquitin ligase, C-Cbl Facilitates CFTR Endocytosis, Ubiquitination, and Targeting for Lysosomal Degradation. Poster presentation at the 23rd North American Cystic Fibrosis Conference in Minneapolis, MN

4) The scholar submitted abstracts to the following international symposia:
a. The 24rd North American Cystic Fibrosis Conference in Baltimore, MD, October 2010:
   - Vatsyayan J, Cihil K, Swiatecka-Urban A (2010). Phosphorylation regulates the c-Cbl mediated trafficking of CFTR in human airway epithelial cells. Free Communication Session.
   - Snodgrass S, Cihil K, Madden D, Swiatecka-Urban A (2010). Dab2 mediates the TGFß-dependent downregulation of CFTR in human airway epithelial cells. Decision pending.

b. The American Society of Physiology Renal Week in Denver, CO, November 2010:

- Swiatecka-Urban A (2010) TGFß-dependent downregulation of CFTR is mediated by Dab2 in human epithelial cells. Decision pending.
- Swiatecka-Urban A (2010) Nephrin endocytosis is mediated by a tyrosine based endocytic signal. Decision pending.

5) The scholar participated in the following activities at international scientific symposia: Invited to co-chaired a symposium entitled *Modulators, Mutations & Understanding CFTR* at the 24nd North American Cystic Fibrosis Conference in Baltimore, MD, 2010

During the past year of funding we have examined the mechanisms of TGFß1-mediated downregulation of CFTR expression in the human airway epithelial cells. TGFß1 is a soluble member of the transforming growth factor ß family of cytokines. TGFß1 is a critical regulator of cell proliferation, differentiation, and migration. Furthermore, TGFß1 is an important genetic modifier in cystic fibrosis (CF). The severity of pulmonary disease in patients with CF varies substantially even among patients with identical genotypes. Genetic modifiers are an important source of this phenotypic variation. TGFβ1 gene polymorphisms are known to be modifiers of pulmonary function in CF. Our long-term objective is to elucidate the mechanisms by which TGFβ1 acts as a gene modifier through its effects on CFTR in human airway epithelial cells. Although TGFβ1 has been shown to decrease CFTR expression in nasal polyps of non-CF individuals and colonic epithelial cells, its effect on CFTR has not been studied in human airway epithelial cells. Recent work has demonstrated that the multifunctional adaptor protein Disabled-2 (Dab2) is integral to TGFβ1 signaling, although it remains unknown whether Dab2 plays a similar role in TGFβ1 signaling in human airway epithelial cells. Our previously published work demonstrated that Dab2 and CFTR co-immunoprecipitated in human airway epithelial cells. Thus, the goals of the study were: (1) to determine the effects of TGFβ1 on CFTR in human airway epithelial cells; and (2) to examine whether Dab2 mediates signal transduction from the TGFβ receptor I (TGFβ-RI) to the downstream transcription factor, Smad2. Studies were conducted in primary differentiated human bronchial epithelial cells obtained from the University of Pittsburgh Human Airway Cell Core (HBE cells; homozygous WT-CFTR) and in polarized human airway epithelial cells (CFBE41o-) stably expressing WT-CFTR or ΔF508-CFTR. Our studies demonstrate that TGFβ1 treatment induced transcriptional responses, including Smad2 phosphorylation in the HBE and CFBE41o- cells. Treatment with TGFβ1 significantly decreased CFTR expression in cell lysates and apical plasma membrane in a concentration and time-dependent manner, and treatment with TGFβ-RI inhibitors prior to stimulation with TGFβ1 prevented Smad2 phosphorylation. Endogenous Dab2 co-immunoprecipitated with members of the TGFβ1 signaling cascade and with CFTR. Moreover, Dab2 silencing reduced Smad2 phosphorylation and the nuclear translocation at baseline and after TGFβ1 treatment. These data indicate that Dab2 mediates TGFβ1 signaling in human airway epithelial cells. Our prior data have shown that silencing Dab2 increased CFTR abundance in cell lysates and in the plasma membrane and increased the CFTR mediated chloride secretion. Taken together, our data demonstrate that in human airway epithelial cells, including primary differentiated human bronchial epithelial cells, TGFβ1 downregulates CFTR expression by a mechanism that involves Dab2 mediated signal transduction to Smad2 and other downstream elements in the TGF β1 signaling cascade. We anticipate further studies will

increase our understanding of the role of TGFβ1 in CF and its role as an important genetic modifier, having relevance for further clinical implications in individuals with CF.

Research Project 2: Project Title and Purpose

Inhibition of Chloride Secretion by Cigarette Smoke - The project uses laboratory techniques to study the effect of cigarette smoke on chloride secretion in cultured human bronchial epithelial (HBE) cells. Changes in chloride secretion by airway cells, changes in airway surface liquid height, and changes in mucociliary clearance will be measured with the hypothesis that decreases in chloride secretion will result in decreased periciliary fluid height and decreased mucociliary clearance.

Duration of Project

1/1/2007 - 6/30/2007

Summary of Research Completed

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement C.U.R.E. Annual Reports on the Department's Tobacco Settlement/Act 77 web page at http://www.health.state.pa.us/cure.

Research Project 3: Project Title and Purpose

IL-23: Actions and Regulation in Pseudomonas aeruginosa Pulmonary Infection – The purpose of this project is to understand how the immune system responds to *P. aeruginosa*, a bacterium that causes severe pneumonias and lower respiratory tract infections. Understanding the immune response is key to the development of new therapies and treatments that are more effective, have fewer side effects and do not contribute to antibiotic resistance. The development of these new therapies is critical because pneumonia is among the top 10 causes of death according to the CDC National Vital Statistics Report.

Anticipated Duration of Project

1/1/2007 - 12/31/2010

Project Overview

P. aeruginosa is a Gram-negative respiratory pathogen that causes significant morbidity and mortality through actions of the pathogen and the host inflammatory response. Because the pathogen is highly adaptable, anti-pseudomonal therapies quickly become ineffective. Thus, immunomodulatory therapies could provide an effective alternative, with fewer side effects and without the development of resistance. However, their development has been impeded by an inadequate mechanistic understanding of the host's response to *P. aeruginosa*. This project focuses on one mediator of the immune response, interleukin-23 (IL-23). IL-23 is best known for stimulating the production of IL-17 by memory T cells and recruiting neutrophils to the site

of infection. In this role, IL-23 bridges the innate and adaptive immune responses. This project focuses on defining the role of IL-23 in mediating the innate response only. Specifically, it determines the role and actions of IL-23, independent of IL-17. It tests the prediction that IL-23 regulates production of IL-1α and IL-1β in the lung, independent of IL-17, and that the IL-23/IL-1 pathway elicits neutrophilic inflammation and matrix metalloproteinases (MMP) production in response to *P. aeruginosa* pulmonary infection. Through the use of recombinant cytokines, neutralizing antibodies and comparison of effects in wild type, IL-23 KO, IL-17KO and IL-23/IL-17 double KO mice the interrelationships between IL-23 and IL-1α and IL-1β will be demonstrated. Zymography will be used to quantify MMP activity.

Principal Investigator

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Other Participating Researchers

Jay K. Kolls, MD, - employed by Children's Hospital of Pittsburgh
Anuradha Ray, PhD, Joanne Flynn, PhD, - employed by University of Pittsburgh

Expected Research Outcomes and Benefits

This project will help immunology and microbiology researchers better understand how bacteria and the immune system interact to cause pneumonia and other lower respiratory infections and why these infections do not always resolve. By providing a mechanistic understanding of the immune response, this work will lay the foundations for the future development of immunomodulatory therapies to *P. aeruginosa* pulmonary infection. The development of immunotherapy is critical because, in many cases of *P. aeruginosa* infection, the *P. aeruginosa* cannot be adequately cleared from the respiratory tract and results in significant airway damage, scarring and loss of lung function. The findings of this project will have applicability to *P. aeruginosa* pulmonary infection in cystic fibrosis, HIV disease and ventilator associated pneumonia (VAP).

Summary of Research Completed

The specific mechanisms underlying the inflammation elicited during *P. aeruginosa* (PA) pulmonary infections are poorly understood. While it is clear that in patients with chronic PA infection neutrophilic inflammation is rampant, contributes to significant airway damage over time, and may not be of utility in controlling infection, the inflammatory pathways that underly this process are still not well understood. This research project seeks to elucidate these inflammatory pathways in order to identify potential targets of immunomodulatory therapy. This work has demonstrated that IL-17-mediated neutrophil recruitment is critical to inflammation in PA pulmonary infection as part of the adaptive immune response. Subsequently, this work has identified the proximal inflammatory mediators leading to IL-17 production. Specifically, we

demonstrated that IL-23 is a critical cytokine to establishing a Th17 phenotype in chronic PA pulmonary infection. Interestingly, in murine models of PA pulmonary infection, neutralizing various components of this IL-23/IL-17 inflammatory cascade does not promote worsened lung infection, nor does it promote dissemination of bacteria to the bloodstream (measured in spleen).

We have also demonstrated that IL-23 and IL-1β together mediate the production of IL-17 through the activation of γδ T cells during the acute phases of PA pulmonary infection and we have also demonstrated that IL-23 and IL-1β act synergistically to recruit neutrophils and induce proinflamamtory cytokines, chemokines and growth factors independent of IL-17.

Because IL-23 is necessary and central to these three mechanisms of neutrophilic inflammation in PA pulmonary infection, we have focused our current work on the regulation of IL-23 and its interactions with IL-1β. Through semiquantitative real time PCR, we have demonstrated that IL-23 is induced very early in PA pulmonary infection (by 1 hour) and precedes the increase in IL-1β signal (3 hours). We have also demonstrated that IL-23p19-/- mice (IL-23 deficient mice), produce significantly less IL-1β than that seen in C57Bl/6 (immune-intact control) mice. Taken together, these data demonstrate that IL-1β production exhibits IL-23-dependence.

Through semi-quantitative real time PCR performed on the lung tissue of mice administered recombinant IL-23 and IL-1β, we have also demonstrated that administration of IL-1β induces IL-1β but that the administration of IL-23 seems to have no effect on its own induction. When IL-23 and IL-1β are administered together, both IL-23 and IL-1β are induced. This positive regulation may explain the synergistic effects seen when both cytokines are administered to mice. This positive regulation may also contribute significantly to the persisting inflammation seen in chronic PA lung infections in individuals with CF and COPD. Because these individuals cannot clear the bacteria mechanically from their compromised airways, the inflammatory response may be escalated without the ability to transition to the resolution phase (e.g. conversion to a primarily macrophage infiltrate/cytokines and growth factors that promote repair). IL-23 and IL-1β could continue to promote IL-17-independent inflammation, activate γδ Tcell production of IL-17 and subsequent transition to, and augmentation of, a chronic Th17 response; all three of these mechanisms promote neutrophilic inflammation which is closely tied to airway damage and loss of lung function. Because of its inherent inflammatory actions and downstream effect of IL-17 production, IL-23 is a critical mediator of inflammation in PA pulmonary infection.

Tied to IL-17 production, we also see the induction and elaboration of IL-17F and IL-22. IL-17F is an IL-17 family member that is closely related to IL-17 (IL-17A) and also results in inflammation and neutrophil recruitment. It is produced along with IL-17 by γδ T cells and Th17 cells. IL-22 is an IL-10 family member that is associated with immune regulation and epithelial barrier integrity. It is produced by macrophages and dendritic cells but is increasingly recognized as a cytokine that is produced by γδ T cells and Th17cells as well. IL-17 (A), IL-17F and IL-22 are all produced during PA pulmonary infection and neutralization of these cytokines modifies the immune profile seen during infection. All three of these cytokines are synergistically induced by IL-23 and IL-1β.

Goals:

1. To identify and elucidate innate immune mechanisms of neutrophil recruitment during PA pulmonary infection.
2. To elucidate the regulation of critical mediators (IL-23 and IL-1β) of the innate mechanisms of neutrophil recruitment.
3. Identify critical mediators that may be potential targets for immunotherapy.

Progress:

1. We have identified three IL-23-dependent inflammatory pathways which are critical for neutrophil recruitment to the airway in PA pulmonary infection
    a. We have demonstrated the importance of IL-23 in augmenting canonical IL-1β inflammation.
    b. We have demonstrated the production of IL-17 by γδ T cells as part of the innate immune response.
    c. We have demonstrated the relevance of the Th17 response as part of the adaptive immune response
2. We have demonstrated that IL-23 and IL-1β synergistically upregulate each other, likely explaining the synergistic actions of the two cytokines.
3. IL-23 appears to augment neutrophil recruitment in excess of that necessary to contain infection; as a positive regulator of IL-1β, the synergism that it drives may result in over exuberant neutrophil recruitment as well as the polarization to an IL-17/Th17 inflammatory profile. As a result, IL-23 appears to be a linchpin of the neutrophilic inflammation that does not appear necessary for the containment of PA pulmonary infection and, therefore, a potential target of immunotherapy.

Research Project 4: Project Title and Purpose

Novel IL-17 Producing Memory Cells are Key to Vaccine-based Immunity in the Lung - The project uses laboratory techniques to study the role of a novel T-cell population, Th17 cells and how they control vaccine responses to tuberculosis (TB). This work is critical to develop a more effective vaccine against TB, which remains a global health problem.

Anticipated Duration of Project

4/1/2008 – 12/31/2010

Project Overview

Tuberculosis is a leading cause of morbidity and mortality in the world as a result of a single infectious agent. Despite the use of Bacille Calmette-Guerin (BCG, an attenuated vaccine strain) for the past 80 years, TB remains a global epidemic with one-third of the world population being infected, an annual rate of 8 million new cases and 2-2.5 million deaths. The development of a new and more effective TB vaccine remains an urgent research priority. Although several candidate vaccines are now in different phases of clinical trials, the protection afforded by these vaccines in animal models is similar to that provided by BCG. Understanding what constitutes a protective memory response to challenge with M. tuberculosis (Mtb) is required in order to

improve the design of effective vaccines against TB. Under this research project, we will determine the types of T-cells that are critical for an effective TB vaccine.

Principal Investigator

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Other Participating Researchers

None

Expected Research Outcomes and Benefits

The completion of the proposed studies will advance our understanding of TB and the types of T-cell responses that are critical for vaccine efficacy.

Summary of Research Completed

We have made progress in the following areas since the last progress report

1. The PI has received a NIH R21 grant entitled "Impact of mucosal immunization on generation of protective lung resident IL-17 producing T cells against Tuberculosis."

2. The PI has submitted and received a fundable score for an R01 grant entitled 'Role of IL-17 in protective vaccine-induced immune responses against tuberculosis."

3. The PI was invited to give the following presentations at National meetings or Seminar series

    - Feb 2010. Novel insights into the role of Th1 and Th17 pathways in intracellular pulmonary Infections. Virginia Tech Seminar Series. Invited Speaker.

    - March 21-26th 2010. Gordon Conference on Acute Lung Infections. Th1 and Th17 in Lung Immunity. Invited Speaker.

4. The following abstracts/posters from the PI's lab were presented at National meetings

    - Samantha R. Slight, Javier Rangel-Moreno, Yinyao Lin, M.Selman, Troy Randall, Shabaana A. Khader. The compositional organization of *Mycobacterium tuberculosis* granulomas is analogous to ectopic lymphoid organs. AAI Annual Meeting, Baltimore, May 2010.

- Yinyao Lin, Radha Gopal, Lokesh Guglani, Samantha Slight, Shabaana A Khader. IL-17 is critical for the generation of protective Th1 immunity against intracellular pathogens. AAI Annual Meeting, Baltimore, May 2010.

5. The following manuscripts related to this project have been published, under review or under preparation

   *Corresponding author

   - S.A.Khader*, J.Rangel-Moreno, J.J.Fountain, C.Martino, W.Reiley, J.E.Pearl, G.Winslow, D.L.Woodland, T.D.Randall and A.M. Cooper. Pulmonary expression of homeostatic chemokines is essential for protective immunity to *Mycobacterium tuberculosis* infection. J.Immunol. 2009. Dec 15;183(12):8004-14.

   - R.Gopal, Y.Lin, S.Slight, N.Nuthalapati, N.Obermajer, M.Ahmed, W.Ouyang, P. Kalinski and S.A Khader*. IL-17 is required for *Mycobacterium bovis* vaccine-induced Th1 immunity against *M.tuberculosis*. For submission to The Journal of Immunology.

   - R.Gopal, Y.Lin, S.Slight, H.Nawar, T.Cornell and S.A Khader*. Mucosal immunization confers superior protection against *Mycobacterium tuberculosis* challenge and is dependent on vaccine-induced Th17 responses. Manuscript under preparation.

   - S.R. Slight, J. Rangel-Moreno, Y.Lin, R.Gopal, B.Fallert, M.Selman, G.M. Winslow, D.L. Woodland, T.A. Reinhart, T.D. Randall and S.A. Khader*. T follicular helper cells producing Interferon gamma are critical for protection against *Mycobacterium tuberculosis* infection. Manuscript under preparation.

6. The following review articles have been published or under review.

   - S.A.Khader, Sarah Gaffen and Jay Kolls. Th17 cells at the cross roads of innate and adaptive immunity against infectious diseases at the mucosa. Mucosal Immunology. 2009. Online. 8 July 2009. doi:10.1038/mi.2009.100.

   - Y.Lin, S.Slight and S.A.Khader *. Role of IL-17 in vaccine-induced immunity. Seminars in Immunopathology. 2010 Mar; 32(1):79-90.

   - L.Guglani and S.A.Khader*. IL-17 in mucosal immunity and inflammation. Current Opinion in HIV and AIDS. In press.

   - Shabaana Khader and Radha Gopal. IL-17 in immunity against intracellular pathogens. Virulence. Submitted.

<u>Specific Aim One</u>**:** In Aim One, we proposed to characterize IL-17 producing cells and investigate the factors required for the survival and maintenance of IL-17-producing memory cells. As proposed in Aim 1, we have used flow cytometry and microarray analysis and have functionally characterized the IL-17-producing cells and compared them with naïve and IFN-γ

producing CD4 T cells. These data suggests that IL-17 producing cells express distinct gene expression patterns when compared to IFN-γ producing cells. IL-17 producing cells express mRNA for specific cytokines such as IL-17A, IL-17E, IL-21, IL-10 and IL-24 and chemokines such as CCL-20. Further, IL-17-producing cells express mRNA for chemokine receptors such as CCR-8, CXCR-4, CXCR-6, and CLR1. We have also characterized the transition of Th1 and Th17 effector to memory cells and found that one of the key differences is the low expression of IL-2R expression on Th17 memory cells when compared to Th1 memory cells. Further Th1 cells migrate in response to the chemokines CXCL-9 and CXCL-10, while Th17 cells migrate in response to CCL-20 and CCL-22. These data suggest that the Th1 and Th17 effector cells are phenotypically and functionally different from each other.

We then evaluated the ability of Th1 and Th17 rested cells to proliferate in response to antigen re-stimulation *in vitro*. Day 10 *in vitro*-rested TCR Tg Th17 and Th1 cells were re-stimulated with antigen and irradiated splenocytes, following which proliferation and activation of T cells was determined. Following antigen re-stimulation, Th17 cells but not Th1 rested memory cells rapidly proliferated as determined by loss of CFSE (Fig 1 a,b) and up-regulated CD69 expression (Fig 1c). The rapid proliferation seen in Th17 rested cells occurred even when different respiratory tract APCs were used (data not shown). These data provide compelling evidence to support our hypothesis that lung-resident Th17 memory cells are uniquely positioned in the lung to be the first responders to rapidly proliferate following antigen re-exposure.

IL-7 is a central regulator of peripheral homeostasis and is a survival factor for both naive and memory CD4+ T cells. In Aim 1.2, we had proposed to determine whether IL-7 plays a role in the establishment and persistence of the lung resident IL-17-producing cells. As a first step in that direction, we determined the expression of IL-7 expression on naïve, effector, rested effector and memory IL-17-producing cells by flow cytometry. Our data suggests that IL-17 effector cells upregulate IL-7 receptor expression as they become memory cells. This suggests that similar to Th1 and Th2 memory cells, IL-17-producing memory cells also require IL-7 for survival. However, we found that adoptive transfer of Th17 cells into IL-7 or IL-17R gene deficient mice resulted in enhanced persistence of the cells in the lymphoid and lung compartment. These studies are being written up for publication.

Specific Aim Two**:** In Aim Two, we had proposed to determine the specific location of IL-17-memory cell population in vivo and the factors that impact the establishment of IL-17-producing CD4 population in the lung. We have initiated these experiments using the OT-II TCR transgenic model. Specifically, using the OT-II transgenic adoptive transfer model, we have shown that IL-17 producing cells have the unique ability to migrate and persist in the lung (Fig 2b,c). Further, we have characterized the surface expression and phenotype and location of cells within the lung by flow cytometry, microarray analysis and thick section immunohistochemistry. These data are being prepared for publication.

Based on the preliminary data presented in the research plan, we have performed the experiments described above using an OT-II TCR transgenic mouse model to generate in vitro IL-17 and IFN-γ producing cells. We have also expanded these observations from the OT-II transgenic model to an Mtb specific TCR Transgenic mouse that recognizes a mycobacterial antigen. Specifically, Drs. David Woodland and Gary Winslow have generated an ESAT1-20 specific Tg

mouse that they have generously made available to us. Our data shows that ESAT-6 TCR Tg Th17 cells also populate the lung compartment, while Th1 ESAT-6 Tg T cells do not (Fig 2b,c). These data support our proposed hypothesis that Th17 cells populate the lung compartment preferentially (Fig 2) and can proliferate rapidly when they encounter antigen (Fig 1). This information will allow us to manipulate vaccination strategies in order to generate better protective memory in the lung against challenge with Mtb. This will influence vaccine design for tuberculosis and other pulmonary infections.

Significance

The significance of these aims is that they are designed to increase our understanding of the basic requirements for induction of long-lived effective immunity in the lung against TB and other pulmonary pathogens. This work is significant as we currently have been unable to improve upon the largely ineffective TB vaccine, BCG. Our work has identified a novel population of cells, the manipulation of which could result in a significant improvement in vaccine-induced protective response not only for TB but also for other pulmonary infections.

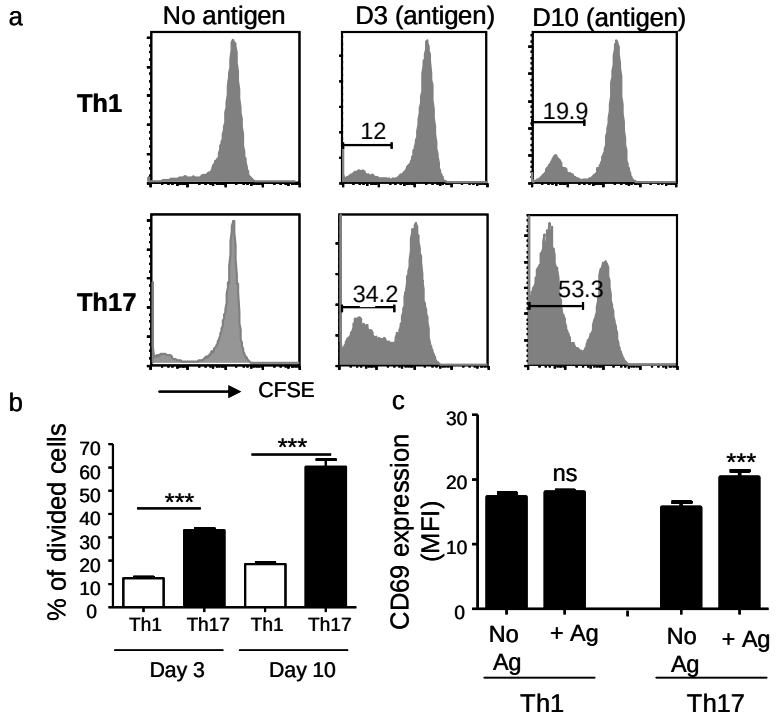

Figure 1. Th17 but not Th1 rested effector cells proliferate rapidly and upregulate activation markers following restimulation with Ag. Th1 or Th17 effectors were generated and day 10 rested-effector cells were CFSE labeled and restimulated in the presence of irradiated splenocytes (a,b) and cognate antigen. Frequency of divided cells shown as a histogram (a) or bar graphs (b). Mean fluorescence intensity for CD69 surface expression was determined on day 3 (c). (n=3-4, Student's *t*-test *** <p.0.005, ns-not significant).

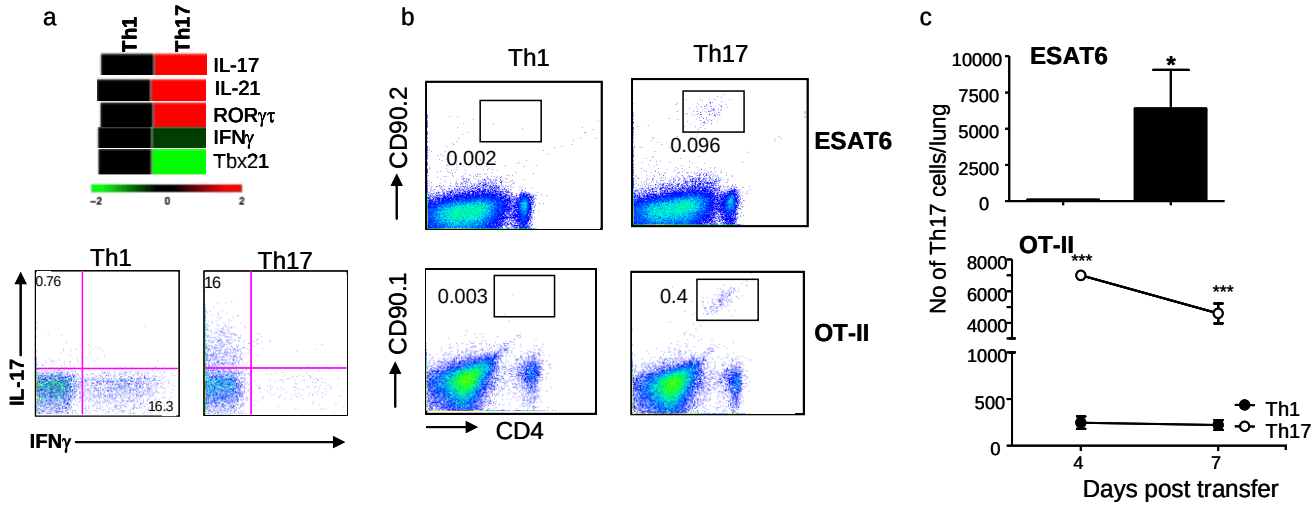

Figure 2. Th17 cells persist in the lung. Th1 or Th17 cells were generated by culturing naïve OT-II TCR Tg or ESAT-6 TCR Tg cells *in vitro* in the presence of irradiated APCs, Ag and cytokine cocktails (Th1 cells - IL-12 and anti-IL-4 antibodies; Th17 cells- IL-23, TGFβ, IL-6, anti-IL-4 and anti-IFNγ antibodies). Gene expression and cytokine profiles validate production of Th1 and Th17 cytokines (a). $2x10^6$ Th1 or Th17 cells were transferred intratracheally into B6 congenic mice and the frequency on day 4 post transfer (b) and number of Th1 or Th17 cells on day 4 (ESAT-6 Tg) or days 4 and 7 (OT-II Tg) in the lung was determined by flow cytometry (c) (n=3-4, Student's *t*-test *** <p.0.0005, * <p.0.05).