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TITLE:
IL-15–Mediated IL-6Ra Effector Memory CD8+ T Cells in Dysfunctional Lung Responses During Cigarette Smoke Exposure and Influenza Viral Infections

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RECIPIENT: Yale University School of Medicine

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Our proposal focuses on the interface of IL-15 and IL-6Rα effector memory CD8+ T cells as a significant contributor in the development of pathological hyperinflammatory and fibrotic responses in the smokers and COPD subjects during influenza infection. The goal of the studies is to characterize the IL-15 regulatory pathways in the lung that are induced by CS and influenza virus and the mechanisms by which IL-15 mediates induction of these pathways that contribute to the AECOPD and fibrotic phenotype of COPD and CS-related lung diseases. We will test the hypothesis this using a series of mouse and human studies. In Aim 1, we propose to determine the importance of IL-15 in regulating macrophage and fibroblast function and their contribution to lung inflammation, injury and fibrotic responses during CS exposure + Flu infection mouse model. In Aim 2, we will determine the molecular and cellular mechanisms by which IL-15 modulates IL-6Rα^{high} EM CD8+ T cells and its contribution to lung injury and fibrotic responses during CS + Flu. Lastly in Aim 3, we will study the clinical implication of a novel IL-6Rα^{high} EM CD8+ T cell subset with the capacity to produce type II cytokines smokers and in patients with COPD.

15. SUBJECT TERMS

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1. **INTRODUCTION:** Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

Using the mouse modeling system and human samples from patients, we have identified important immune pathways that may contribute to the increased pathology of viral infections in smokers and patients with COPD. We will explore the interface of IL-15 (thus macrophages, which are major secretors) and IL-6Rα^{high} EM CD8+ T cells as a significant contributor in the development of pathological hyperinflammatory and fibrotic responses in the smokers and COPD subjects during influenza infection. ***The overall goal of the studies is to characterize the IL-15 regulatory pathways in the lung that are induced by cigarette smoke (CS) and influenza (Flu) virus and the mechanisms by which IL-15 mediates induction of IL-6Rα^{high} EM CD8+ T cells and their contribution to the AECOPD and fibrotic phenotype of COPD and CS-related lung diseases.***

2. **KEYWORDS:** Provide a brief list of keywords (limit to 20 words).

Lungs, COPD, viral infections, influenza, cigarette smoke, pneumonia, interleukin-15, CD8 T cells, inflammation, fibrosis

3. **ACCOMPLISHMENTS:** The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.

What were the major goals of the project?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Major Goals/Milestones:

1. Yale IACUC and ACURO Approval: 100% completed
2. Macrophage subpopulation: 50% completed
3. Macrophage and fibroblast interaction: 30% completed
4. Alveolar macrophage transfer studies:
5. IL15 genetically modified mouse studies: 30% completed
6. Co-culture of macrophage and T cell studies: 20% completed
7. Macrophage depleting studies: 20% completed
8. Blocking antibody studies: 0% completed
9. CD8 T cell sorting and transfer studies: 0% completed
10. Ex vivo culture studies with fibroblasts: 30% completed
11. Yale IRB & HRPO Approval: 100% completed
12. Recruitment of subjects for airway and blood samples: 10%
13. Collection of peripheral mononuclear cells from subjects: 20%
14. Measurement of circulating IL-15 in plasma: 20%
15. Collection of clinical subject information: 20%
16. Single cell sequencing: 20%
17. CyTOF analyses: 0%
18. In vitro co-culture of human macrophages and fibroblasts: 20%
19. Evaluation of EM CD8 T cells: 20%

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

This reporting period allowed analyses of macrophage subpopulations in mice exposed to cigarette smoke and or influenza virus, isolation of specific macrophage and CD8 T cells, measurements of cytokines including IL-15, ex vivo fibroblasts studies, measurements of cytokines in existing repository, as well as the start of recruitment of patients infected with respiratory viruses with varying smoking history and COPD. Due to COVID-19, non-COVID-19 research work could not be done until this summer. In the meantime, we were able to process mouse and human samples previously obtained for analyses.

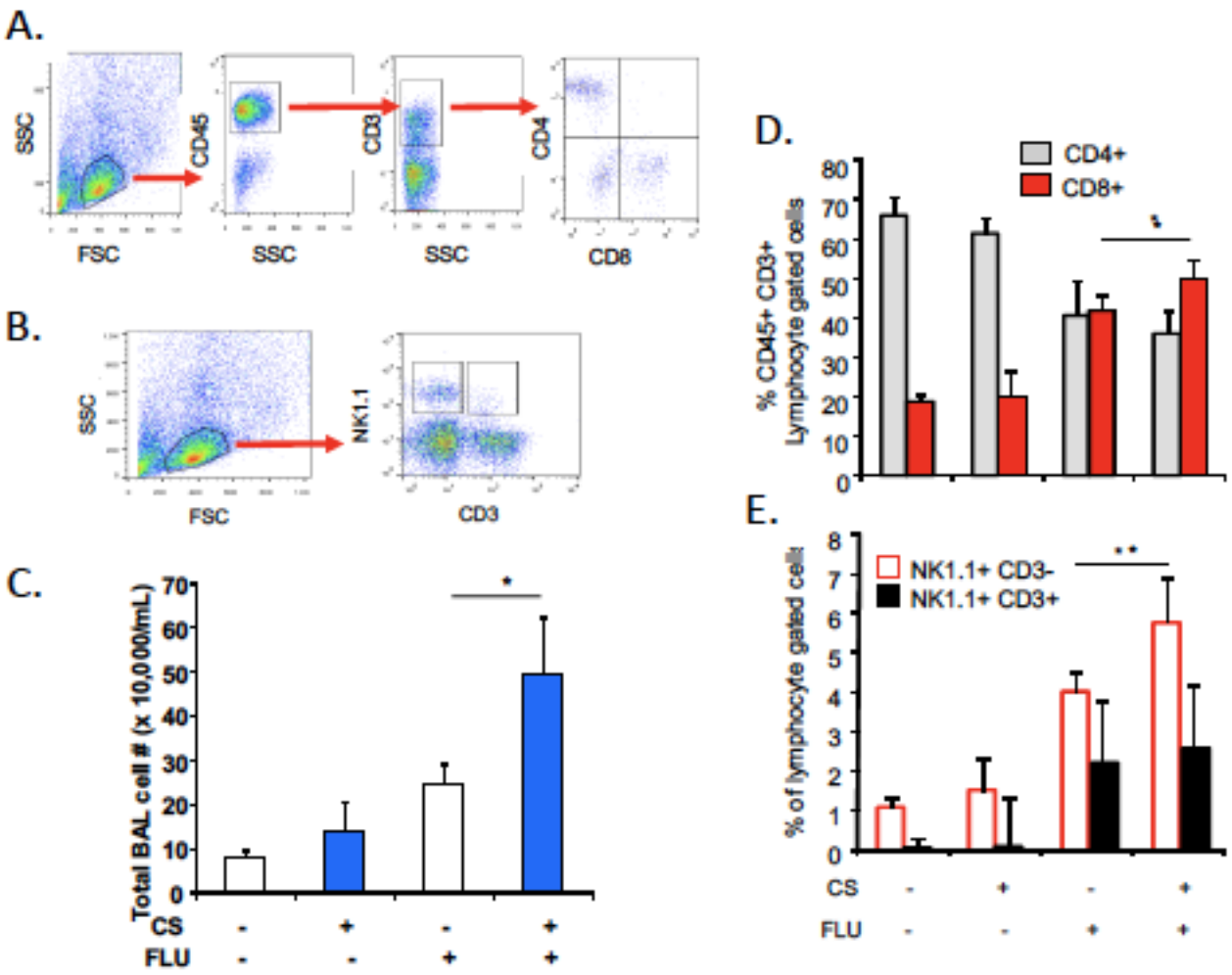

Figure 1. The effect of cigarette smoke (CS) and influenza virus on CD8 T cells. The mice were exposed to CS or room air for 1 months (2 cigarettes a day for 5 days / week) and were infected with 0.05 LD50 A/PR8/34 H1N1 influenza virus for 7 days. 5-8 mice are in each treatment groups. (A) FACS gating strategy to identify CD3+CD8+ T cells and CD3+CD4+ T cells and (B) for CD3-NK1.1+ (NK) cells. (C) Total bronchoalveolar cell numbers for each of the treatment group. (D) Percent CD45+CD3+ of lymphocyte gated cells that are CD4+ or CD8+ in the different mouse groups as noted. (E) Percent of lymphocyte gated cells based on FSC and SSC that are NK1.1+CD3- or NK1.1+ CD3+ cells in the lung.

Analysis/Interpretation: Various lung lymphocyte population were measured using flow cytometry of single cell suspension of lungs harvested from mice exposed to room air or CS followed by influenza viral infection or vehicle control. The findings show that mice exposed to either CS or influenza alone resulted in increased lung inflammation but significantly more with the mice were expose to both CS and influenza. Dual exposure resulted in increased percentages of CD3+CD8+ T cells in the lung while maintaining the same percentages of CD3+CD4+ T cells. Interestingly, the dual exposure resulted in increased NK cells as determined by NK1.1+CD3- staining compared to NKT cells (NK1.1+CD3+).

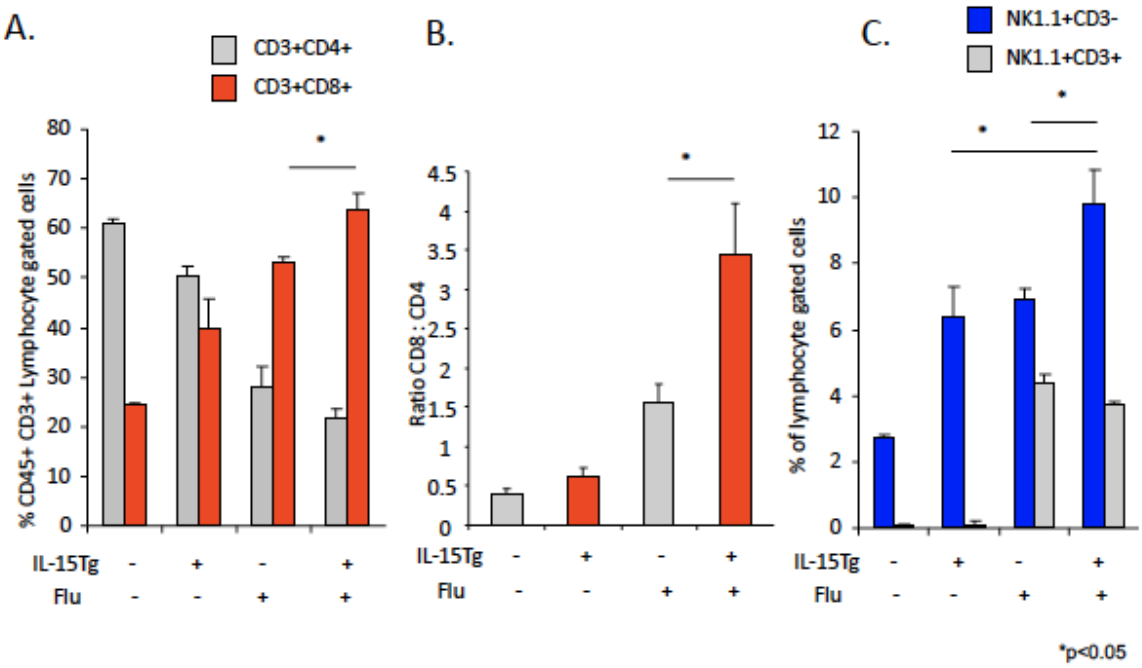

Figure 2. The effect of overexpression of interleukin-15 in the lung. C57BL6 mice genetically modified to overexpress IL-15 using the CC10 promoter compared to non-transgenic C57BL6 mice. Mice are treated with doxycycline to allow for the expression of the transgene provided into the drinking water of the mice for 2-4 weeks. These mice were then infected with 0.05 LD50 A/PR8/34 H1N1 influenza virus for 7 days. 5-7 mice are in each treatment groups. (A) Flow cytometry results showing the percentage of CD45+CD3+ lymphocytes in the various treatment group. (B) Ratio of CD8 to CD4 cells in IL-15Tg mice and control mice that were infected with or without influenza. (C). Flow cytometry results showing the percentage of NK1.1+CD3- and NK1.1+CD3+ cells in the different treatment groups.

Analysis/Interpretation: Lung lymphocyte population were measured using flow cytometry of single cell suspension of lungs harvested from mice overexpressing interleukin-15 in the lungs compared to controls followed by influenza viral infection or vehicle control. The findings show that mice that overexpress IL-15 in the lung have higher percentages of CD8+ T cells with increased CD8 to CD4 ratio in the lungs suggesting the potential role of CD8 T cells in response to IL-15 overexpression and influenza virus infection. Moreover, the overexpression of IL-15 in the lung resulted in increased percentages of NK cells.

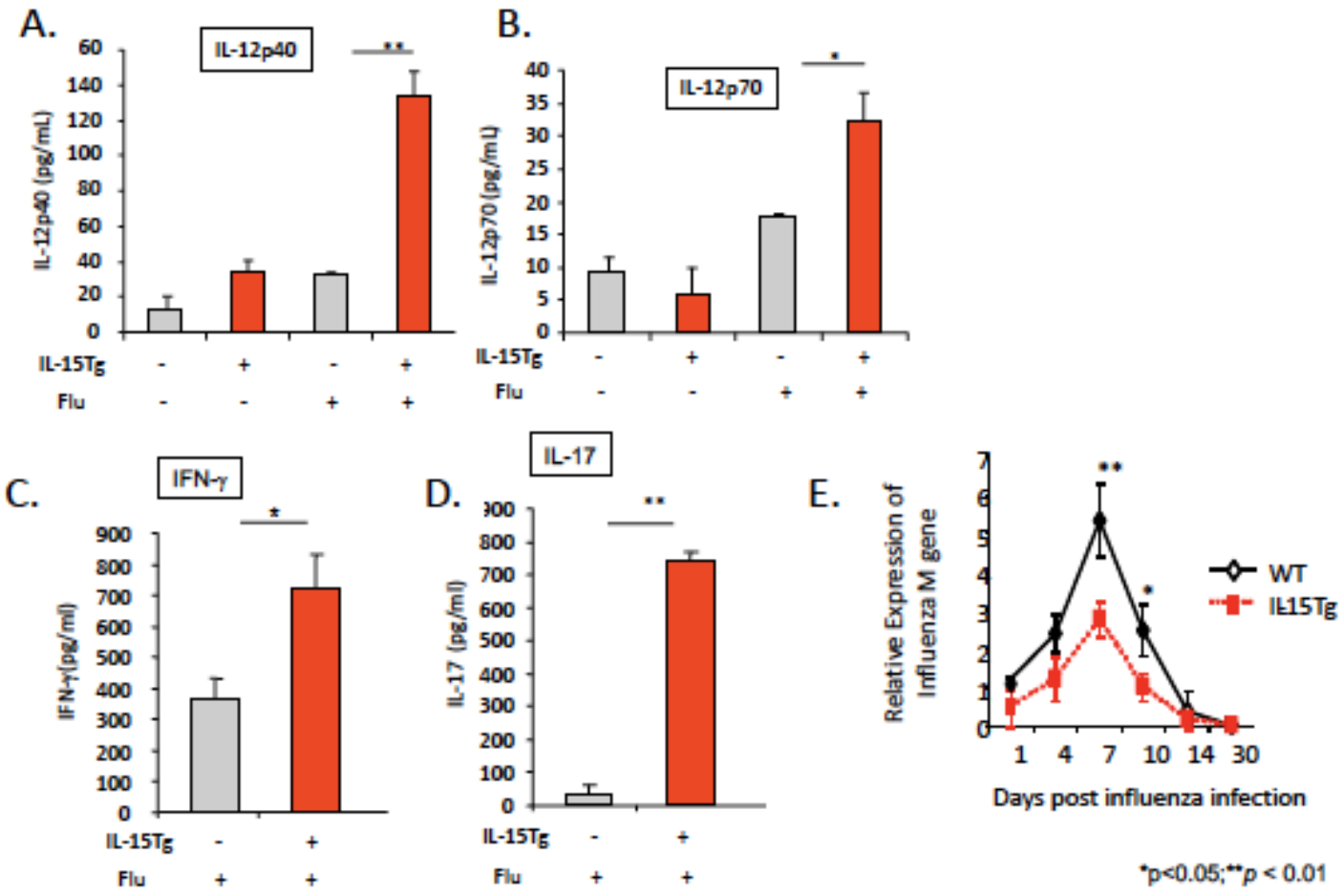

Figure 3. Overexpression of lung interleukin-15 results in increased cytokine and decreased viral load during influenza infection. C57BL6 mice genetically modified to overexpress IL-15 using the CC10 promoter compared to non-transgenic C57BL6 mice. Mice are treated with doxycycline to allow for the expression of the transgene provided into the drinking water of the mice for 2-4 weeks. These mice were then infected with 0.05 LD50 A/PR8/34 H1N1 influenza virus for 7 days. 5-7 mice are in each treatment groups. Bronchoalveolar lavage fluid and lung tissues are harvested at day 7 after infection for cytokine measures and viral load determination. (A-D) BAL fluid levels of IL-12p40, IL-12p70, IFNg and IL-17 from the various treatment group as shown. (E) Relative expression of influenza M gene as measurement of influenza viral in WT and interleukin-15 transgenic mice at various days post influenza infection.

Analysis/Interpretation: Bronchoalveolar lavage fluids are isolated from the lungs of WT and IL-15 transgenic mice infected with influenza virus. Mice infected with influenza virus showed increased IL-12 compared to non-infected controls. In addition, IL15-Tg mice had significantly increased levels of both IFN-12p40 and IL-12p70 in the BAL compared to littermate non-Tg controls. Both IFN-g and IL-17 are elevated in IL15Tg mice infected with influenza highlight the role of IL-15 in mediating a T helper 1 and T helper 17 responses in the lung during viral infections. The overexpression of IL-15 resulted in decreased viral load suggesting the impact of IL-15 expression in viral control and clearance. This was associated with the IL-15 Tg mice with increased cytokine in the lung during influenza viral infections.

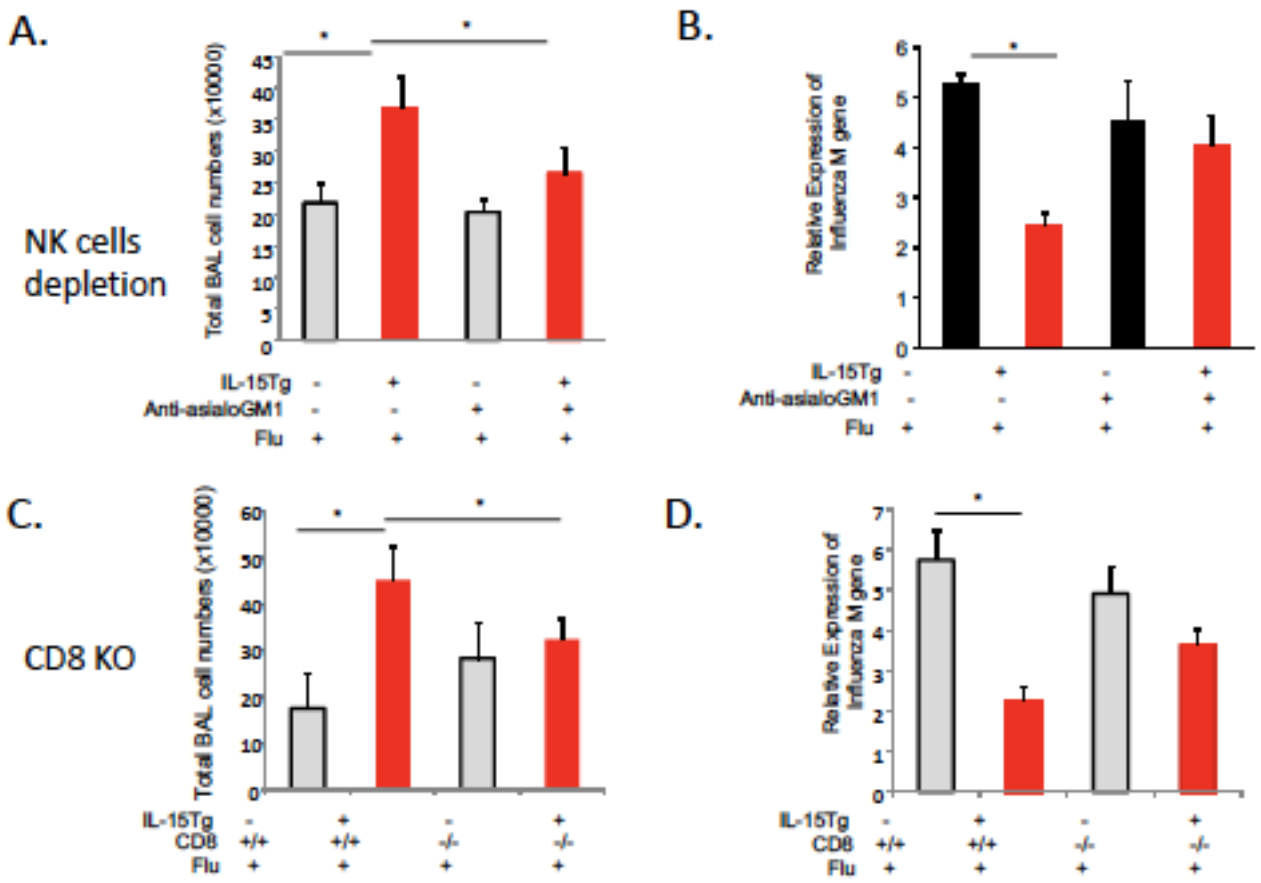

Figure 4. Role of CD8 and or NK cells in the IL15 Tg mice during influenza virus infection. C57BL6 mice genetically modified to overexpress IL-15 using the CC10 promoter compared to non-transgenic C57BL6 mice. Mice are treated with or without doxycycline to allow for the expression of the transgene provided into the drinking water of the mice for 2-4 weeks. These mice were then infected with 0.05 LD50 A/PR8/34 H1N1 influenza virus for 7 days. 5-7 mice are in each treatment groups. IL15 transgenic mice and non-Transgenic mice treated with anti-asialoGM1 antibody before influenza virus infection. Results show (A) total BAL cell numbers at day 7 after influenza infection and (B) relative expression of influenza M gene from lung lysates harvested from the various transgenic and non-transgenic mouse treated with or without the anti-asialoGM1 antibodies to deplete NK cells. Results show (C) total BAL cell numbers at day 7 after influenza infection and (D) relative expression of influenza M gene from lung lysates harvested from the various IL-15Tg mice and non-Tg mice are crossed with CD8KO mice and infected when they were at 8-10 weeks of age.

Analysis/Interpretation: IL-15Tg and non-transgenic mice were treated with antibodies to deplete NK cell populations. Mice were injected i.p. with 100 µl of anti-asialo GM1 or control rabbit sera (Wako Chemicals, Richmond, VA, USA) diluted 1:10 in PBS. IL-15Tg and non-transgenic mice were also crossbred with CD8KO mice to generate IL-15TG x CD8KO mice. These results show that CD8 T cells were partially responsible for the increased inflammatory responds during influenza virus infection. These did not affect the viral load in the lung. In addition, NK cells were partially are needed for the inflammatory changes in the lungs of IL-15Tg mice during influenza viral infections.

CD8 gene induction in Flu-infected IL-15 TG mice

Gene	WT + Flu	IL15tg + Flu	Fold Increase
IL-15	203.16	6266.87	30.85
Nkg7 – NK cell group 7	305.68	7506.54	24.56
Ctsw – cathepsin W, lymphopain (CD8, NK)	189.47	3922.21	20.70
CD8b	470.33	9501.74	20.20
Thy1	435.68	8233.14	18.90
CXCL9 – MIG	140.72	2414.21	17.16
OAS1g – 2'-5- oligoadenylate synthetase2	151.58	2191.17	14.46
CCL5 – RANTES	724.78	8716.53	12.03
CX3CR1 – fractalkine receptor	131.87	1494.76	11.33
Lck – lymphocyte protein tyrosine kinase	407.03	3724.48	9.15
Birc5 – survivin	329.01	2261.56	6.87
Slc26a4 – pendrin – Cl^-/I^- transporter	361.80	2205.20	6.10

Table 1. Microarray analyses of WT versus IL-15Tg mice infected with influenza virus. Non-transgenic C57BL6 and IL-15 transgenic mice are infected with influenza virus with 0.05 LD50 A/PR8/34 H1N1 influenza virus for 7 days. 5 mice per group are used to harvest lungs for RNA for microarray analyses using the Illumina platform.

Analysis/Interpretation: IL-15Tg infected with influenza had genes related to CD8 T cells such as CD8b, Thy1, Ctsw, and Lck to be increased compared to non-transgenic wildtype mice with influenza virus suggesting the role of IL-15 in driving CD8 pathways. In addition, NK cell makers such as Nkg7 was also increased. Other inflammatory genes increased in the infected transgenic mice include RANTES and CXC3CR1 the fractalkine receptor. Birc5 or surviving has been shown to be important in the survival and anti-apoptosis and maintenance of T cells.
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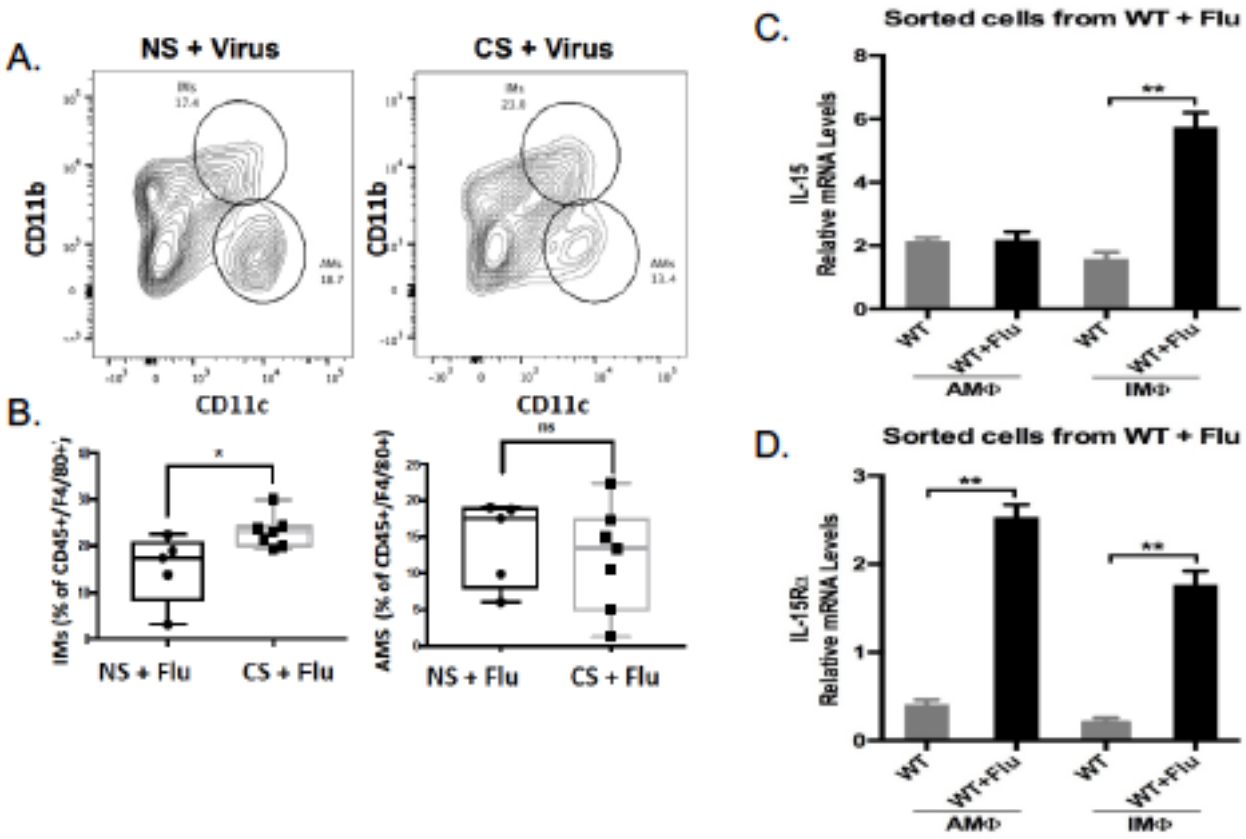

Figure 5. Lung macrophages in mice exposed to cigarette smoke and influenza virus. The mice were exposed to CS or room air for 1 months (2 cigarettes a day for 5 days / week) and were infected with 0.05 LD50 A/PR8/34 H1N1 influenza virus for 7 days. 5-7 mice are in each treatment groups. (A) FACS gating strategy to identify CD45+F4/80+ cells that are CD11b+CD11c (interstitial macrophage) and CD11b-CD11c+ (alveolar macrophages). (B) Percentages of CD45+F480+ cells that are IMs or AMs in mice treated with room air and influenza compared to CS and influenza. (C) Expression of IL15 in sorted populations of macrophages. (D) Expression of IL15Ra in sorted populations of macrophages. Data are representative of three experiments. $*P < 0.05$; $**P < 0.01$

Analysis/Interpretation: Mice exposed to cigarette smoke and influenza virus results in increased numbers of interstitial macrophages in the lung compared to mice exposed to room air (no cigarette smoke: NS) and influenza virus. Moreover, IL15Ra expression was increased in both the alveolar macrophage and interstitial macrophage population. Importantly, specifically interstitial macrophages expressed more IL-15 after influenza infection, compared to uninfected controls, and not seen in the alveolar macrophage population.

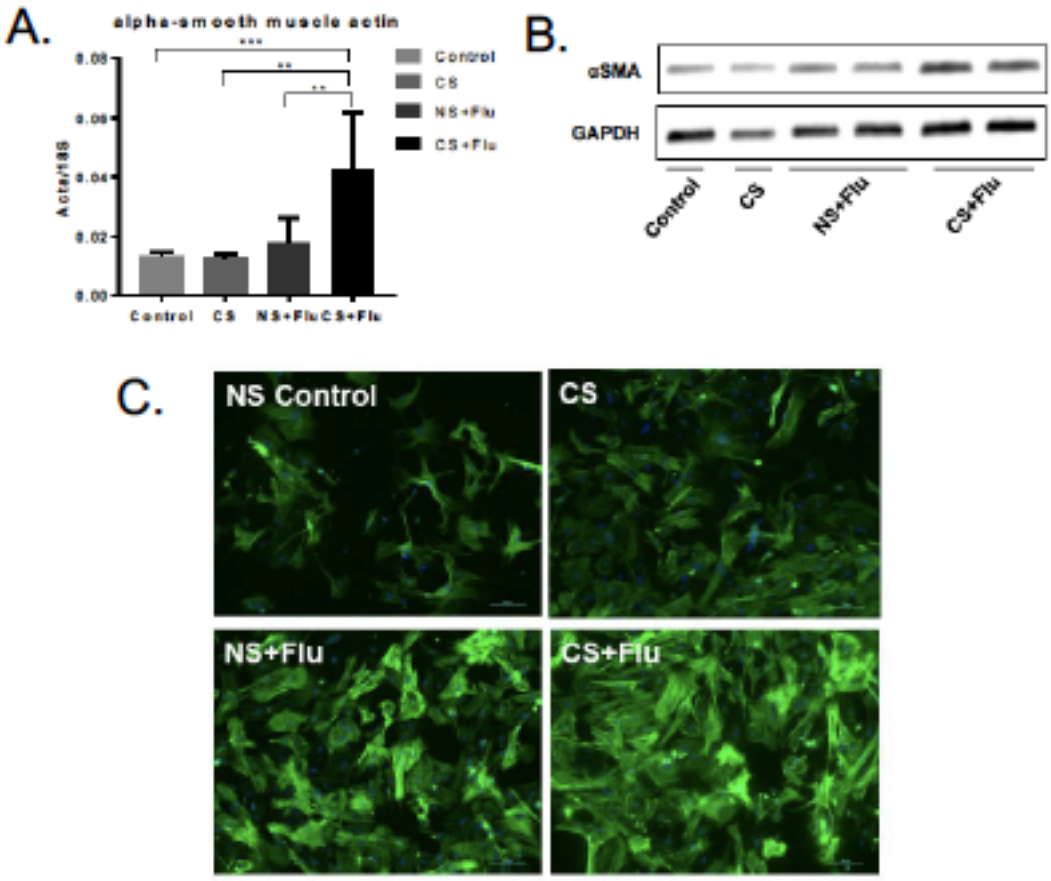

Figure 6. Lung fibroblasts isolated from lungs of mice exposed to cigarette some and influenza virus. The mice were exposed to CS or room air for 1 months (2 cigarettes a day for 5 days / week) and were infected with 0.05 LD50 A/PR8/34 H1N1 influenza virus for 7 days. (A) Lung fibroblasts are isolated for ex-vivo culture and measurements of alpha smooth muscle actin as determined by quantitative RT-PCR. (B) Western blot analyses of alpha smooth muscle actin proteins in lungs of control mice, mice exposed to CS, room air (NS) + influenza virus and CS + influenza virus. (C) Immunofluorescence staining of α-SMA is shown in lung fibroblasts obtained from different groups at ×40 magnification. **P < 0.01; ***P < 0.001

Analysis/Interpretation: Mice exposed to room air (NS) or cigarette smoke and influenza virus as described above. Primary lung fibroblasts were isolated from two mice in each group with or without influenza infection or smoking. The third passage of cells was used for all experiments to achieve sufficient cell numbers for in vitro studies. Total RNA was isolated from the fibroblasts using the RNeasy Plus Mini Kit (Qiagen). After synthesis of cDNA, quantitative PCR was performed using SsoAdvanced Universal SYBR Green Supermix (Bio-Rad), and the specific primers for α-smooth muscle actin (α-SMA). We used 18S ribosomal RNAs as housekeeping genes. Fibroblast lysates were prepared and the total protein concentration was determined. Equal amounts of sample proteins were used for Western blot analysis. Isolated primary fibroblasts were cultured in chamber slides, fixed, permeabilized, and incubated with the rabbit anti-mouse α-SMA antibody (Abcam). Ex vivo fibroblasts obtained from CS-exposed mice with influenza show increased markers of myofibroblasts including alpha smooth muscle actin. All figures are from representative data of three or more independent experiments. In vitro experiments were performed in triplicate wells. **P < 0.01; ***P < 0.001.

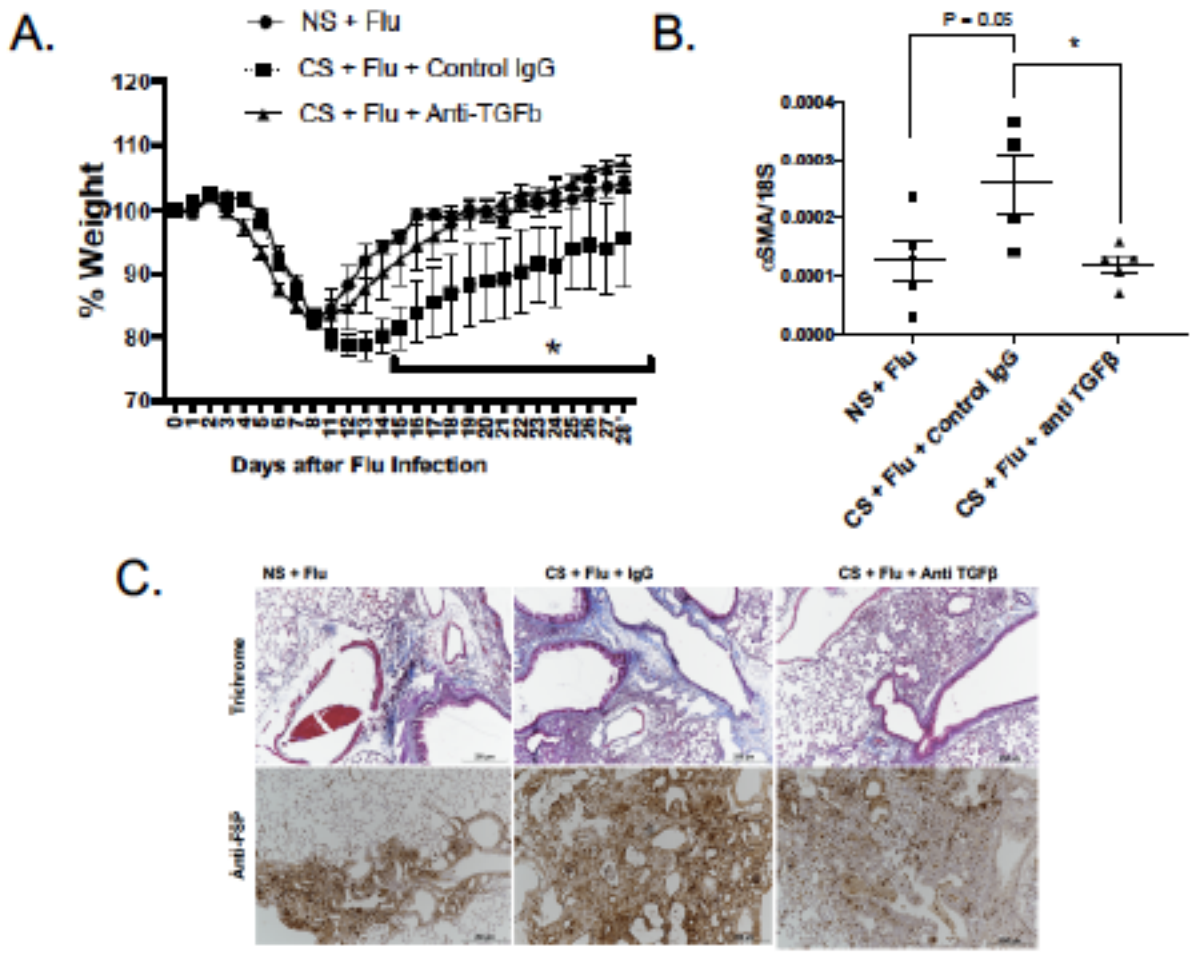

Figure 7. Anti-TGF β antibody treatment of mice exposed to CS and influenza infections. Mice were exposed to CS or NS for 2–3 weeks and then infected with influenza virus. The mice continued to receive the respective exposure even after infection. The CS-exposed mice were then divided into two groups to receive either anti–TGF-β antibody (1 mg per mouse every other day starting from Day 12 after infection) or the same amount of control IgG. (A) Weight changes over time of mice exposed to room air and later infected with influenza virus, compared with mice exposed to CS and influenza virus. Anti-TGF β antibody or control IgG were given from day 14 post infection and on. (B) Lung alpha smooth muscle actin expression by qRT-PCR. (C) Histological evaluation of lungs from mice exposed to NS + influenza and CS + influenza. Upper panel: Trichrome staining. Lower panel: immunohistochemistry anti-fibrotic specific properties. Mice are treated with control IgG versus antibodies against TGF- β. Data are from one experiment performed with n = 4–5 mice per each group.

Analysis/Interpretation: Mice exposed to room air (NS) or cigarette smoke and influenza virus. Mice were observed daily for weight changes and sacrificed at Day 28 after infection to harvest the lungs. The overall morbidity measured as weight changes. Lung sections were subjected to histological examination using Trichrome staining for collagen deposition and FSP. The extent of fibrosis on Trichrome-stained sections was quantified. The use of antibodies against TGF β was able to result in improved disease course after influenza virus infection in mice that were exposed with CS. This resulted in decreased in weight loss of mice infected with virus that were treated with antibodies against TGF β. Moreover, antibody treatment resulted in decreased alpha smooth muscle actin expression as well as Trichrome and FSP fibrotic specific protein staining suggesting that inhibiting TGF β can ameliorate the fibrogenic process as a result of CS and influenza virus infection of the lung.

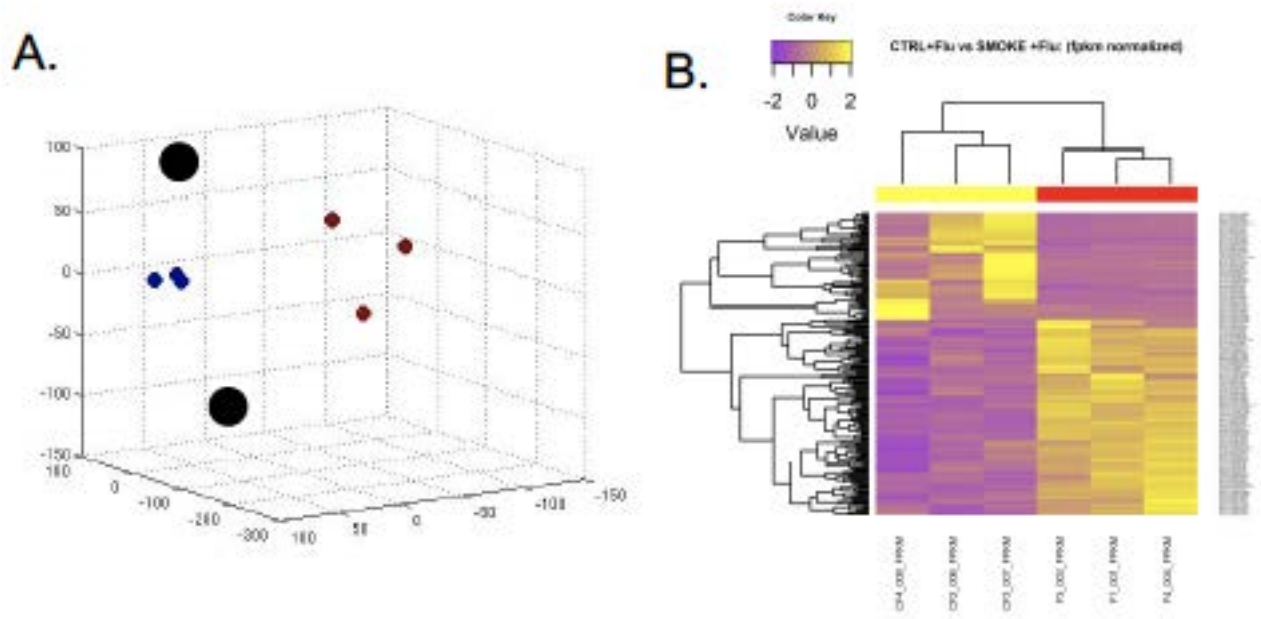

Figure 8. Ex vivo fibroblasts isolated from mice exposed to CS or NS and infected with influenza virus. Fibroblasts were cultured ex vivo from the mouse groups. RNA was isolated from the fibroblasts and RNA sequencing analyses were performed. (A) Principal component analysis (PCA) of the normalized RNAseq data transcripts per million (TPM) of fibroblast cells isolated from mice exposed to CS + influenza or influenza alone. (B) Heat map of RNAseq differential gene expression analysis between CS + Flu and NS + Flu exposed fibroblasts. Heat map shows log2 fold transformed differences in mRNA relative abundances in selected genes.

Analysis/Interpretation: Fibroblasts are isolated from mice exposed to room air (NS) or cigarette smoke and influenza virus. Results show that fibroblast gene expression from infected lungs exposed to CS vs NS was different and exhibit differential changes across a series of genes that were identified. These identify a set of genes that are expressed in CS-exposed fibroblasts that potentially explain their fibroblastic potential and impact on lung fibrosis during cigarette smoke exposure and viral infections.

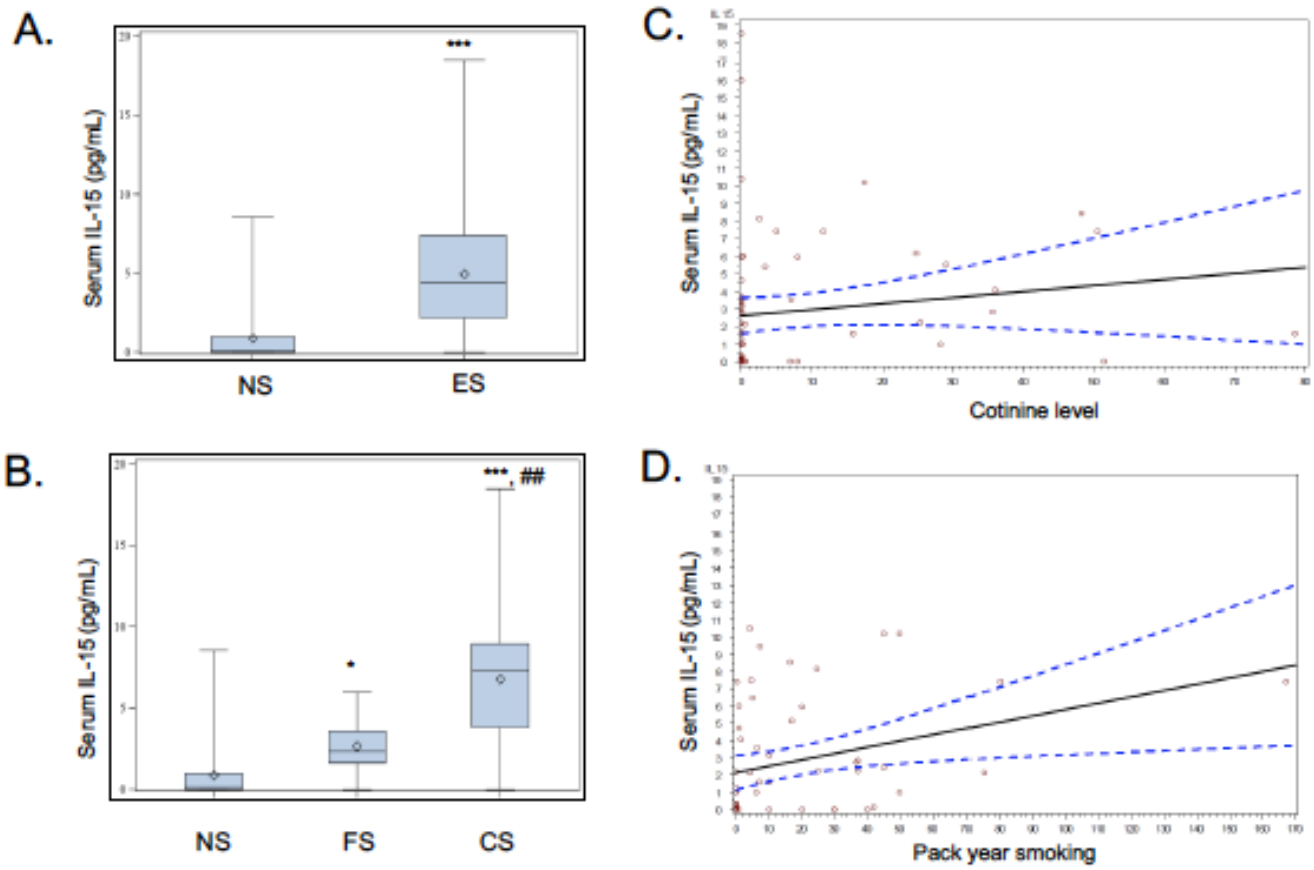

Figure 9. Smokers and levels of circulating IL-15. Subjects with varying smoking history were previously recruited for serum sample collections. Subjects were either never smokers (NS) or ever smokers (ES) which are comprised for former smokers (FS) and current smokers (CS). All these patients were hospitalized with influenza virus infections. (A) Serum IL-15 levels of NS and ES patients. (B) Serum IL-15 of NS, FS and CS patients. (C) Relation of serum IL-15 and cotinine level in the blood. (D) Relation of serum IL-15 and pack year smoking.

Analysis/Interpretation: The serum IL-15 were analyzed of subjects hospitalized with influenza virus who are non-smokers or smokers. Results show increased serum levels of IL-15 in ever smokers with a dose response with patients who are current smokers having more IL-15 than former smokers. Pearson correlation coefficient studies show no significant relation between serum IL-15 and cotinine levels (r=0.168, p=0.1768). However, there was statistically association of serum IL-15 and subjects pack year smoking (r=0396, p=0.0173). There were 48 ever smokers and 18 smokers with 29 current smokers and 11 former smokers in the samples analyzed.

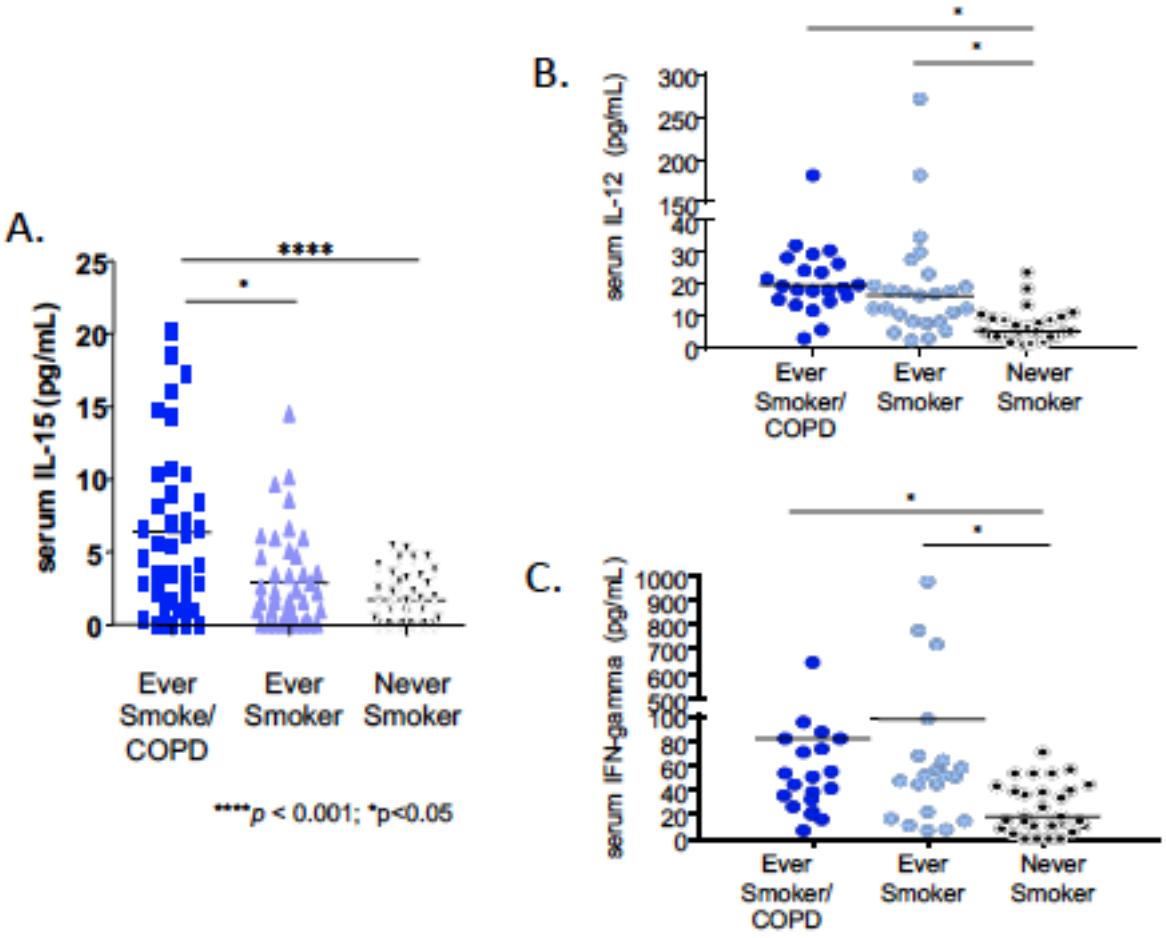

Figure 10. Circulating IL12 and IFN-gamma in patients infected with influenza virus. Subjects with varying smoking history were previously recruited for serum sample collections. Subjects were either never smokers (NS) or ever smokers (ES) where some have diagnosis of COPD. All these patients were hospitalized with influenza virus infections. (A) Serum IL-15 levels of NS and ES (with or without COPD) patients. (B) Serum IL-12 of NS and ES (with or without COPD) patients. (C) Serum IFF-gamma of NS and ES (with or without COPD) patients.

Analysis/Interpretation: The serum IL-15 were analyzed of subjects hospitalized with influenza virus who are non-smokers or smokers with or without COPD. Results show increased serum levels of IL-15 were elevated in ever smokers who had COPD compared to ever smokers without the diagnosis of COPD; these levels were much higher than infected never smokers. Moreover, although ever smokers had higher levels of IFN-gamma and IL-12, COPD patients did not have higher levels of these cytokines as it was with IL-15. This suggests that IL-15 could possibly be elevated specifically for patients who are smokers with COPD and have the potential to contribute to the pathogenesis of disease.

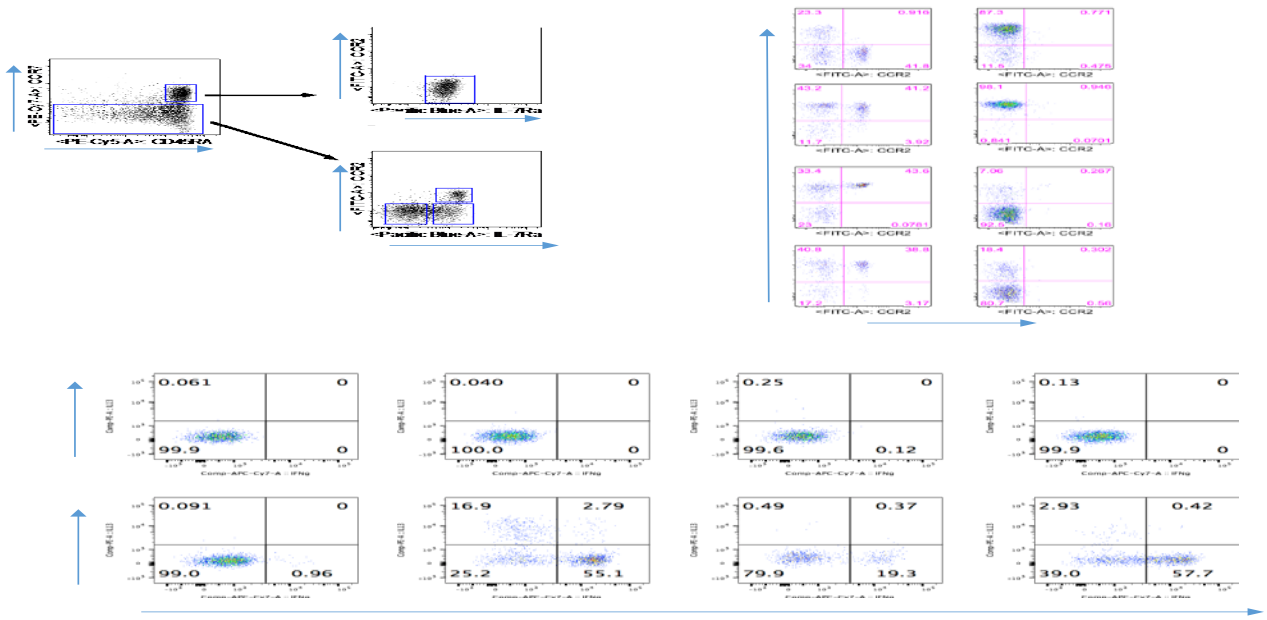

Figure 11. Identification of CCR2+ effector memory (EM) CD8+ T cells that produce high levels of IL-13 in human peripheral blood. (A-B). Peripheral blood mononuclear cells (PBMCs) were purified from blood of a healthy human subject. Cells were stained with antibodies to CD3, CD8, CCR7, CD45RA, IL-7Ra, CD57, 2B4, CD27, and CD28 followed by flow cytometric analysis. (A) IL-7Rahigh EM CD8+ T cells have two distinct subsets with or without expressing CCR2. (B) CCR2+ and CCR2- IL-7Rahigh EM CD8+ T cells have differential expression of CD57, 2B4, CD28 and CD27. (C) Naïve, CCR2+IL-7Ra+, CCR2-IL-7Rahigh and IL-7Ralow EM CD8+ T cells were FACS purified and stimulated for 4 hours with or without PMA/ionomycin. Cells were analyzed for intracellular expression of IL-13 and IFN-g using flow cytometry. Representative data from >5 (A-B) and 2 (C) independent experiments.

Analysis/Interpretation: Identification of CCR2+ effector memory (EM) CD8+ T cells that produce high levels of IL-13 in human peripheral blood. Our previous study reported increased production of IL-13 by human peripheral effector memory (EM, CCR7-CD45RA+/-) CD8+ T cells that express high levels of IL-7 receptor alpha (IL-7Ra) and IL-6Ra. In IL-7Rahigh EM CD8+ T cells, we have found two distinct cell subsets with and without expressing the chemokine receptor CCR2 (Fig 1A). CCR2+IL-7Rahigh EM CD8+ T cells express high levels of the costimulatory molecules CD27, CD28 and 2B4 without the expression of the senescent marker CD57 (Fig 1B). CCR2+IL-7Rahigh EM CD8+ T cells produce increased levels of IL-13 and IFN-g that can play a role in COPD compared to naïve, CCR2-IL-7Rahigh EM and IL-7Ralow EM CD8+ T cells (Fig 1C). We will further these results in patients with COPD and healthy subjects by analyzing additional phenotypic and functional characteristics of CCR2+IL-7Rahigh EM CD8+ T cells.

What opportunities for training and professional development have the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

These studies resulted in new training opportunities by 1) promoting proficiency in immunophenotyping in the lung and blood compartment using both the mouse and human samples; 2) proficiency in in vitro co-culture systems; 3) proficiency in mouse handling and 4) proficiency in lung physiologic, cellular and molecular assays.
Professional development activities include broadening of scientific knowledge in lung biology, evaluations of IL15 pathways and the presentation of the results at Pulmonary research lab meetings and national and international conferences.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to Report

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

The next set of goals and milestones to achieve are the following:

1. Additional evaluations of macrophage populations modified by IL-15
2. Evaluations of effector memory CD8 T cells in mouse model experiments
3. Continued study of co-culture studies to evaluate macrophage and fibroblast, and macrophage and T cell interactions
4. Continued recruitment of patients with or without respiratory viral infections.

4. **IMPACT:** Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Our initial studies show that cigarette smoke exposures result in elevated cytokine expressions in the lung after respiratory viral infections. We have also identified subsets of macrophages with elevated expressions of interleukin-15 receptors in response to these exposures. Exposures to both cigarette smoke and viral infections results in lung fibroblasts to exhibit more proliferative activity. These studies highlight important features of COPD of chronic inflammation and lung damage and fibrosis that is seen in these patients.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Our studies will impact the field of COPD by better understanding the role of respiratory viral infections in the lung and dissecting the mechanisms that drive a subset of these patients at risk of developing lung injury and fibrosis. Respiratory viral infections with or without smoking exposure can likely results in worsening of underlying lung disease, it could also result in long-term lung pathology. Given that respiratory viruses such as influenza, and now given the pandemic with COVID-19, in addition to lung pathologies, these infections could result in systemic inflammation that can be exacerbated by smoking. Our study in the IL-15 pathway could have the potential of having broader effects on many other disciplines.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Not applicable

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

If these studies identify the IL-15 and effector memory CD8 T cell pathway as potential mechanisms that drive chronic lung inflammation and fibrotic response, this can help identify antibodies or antagonists as a therapy or as a diagnostic tool in identifying at risk patients who will develop chronic fibrosis in these COPD patients.

CHANGES/PROBLEMS: The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:

Changes in approach and reasons for change

Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Nothing to Report

Actual or anticipated problems or delays and actions or plans to resolve them

Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Because of COVID-19 since March 2020, research activities in both basic science and clinical translational study recruitment were place on hold until this past July. During this time, we were able to process samples previously collected for the grant prior to COVID-19. We have since re-started the smoking exposure and infection studies for more samples for analyses.

Changes that had a significant impact on expenditures

Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

There were no significant changes that impacted expenditures.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Nothing to Report

Significant changes in use or care of vertebrate animals

Nothing to Report

Significant changes in use of biohazards and/or select agents

Nothing to Report

PRODUCTS: List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

1. Lee SW, Sharma L, Kang YA, Kim SH, Chandrasekharan S, Losier A, Brady V, Bermejo S, Andrews N, Yoon CM, Liu W, Lee JY, Kang MJ, **Dela Cruz CS**. Impact of Cigarette Smoke Exposure on the Lung Fibroblastic Response after Influenza Pneumonia. Am J Respir Cell Mol Biol. 2018 Dec;59(6):770-781. PMID: 30110182
2. Zhang D, Lee H, Wang X, Groot M, Sharma L, **Dela Cruz CS**, Jin Y. A potential role of microvesicle-containing miR-223/142 in lung inflammation. Thorax. 2019 Sep;74(9):865-874. doi: 10.1136/thoraxjnl-2018-212994. Epub 2019 Jul 22. PMID: 31331947
3. Sharma L, Rebaza A, **Dela Cruz CS**. When "B" becomes "A": the emerging threat of influenza B virus. Eur Respir J. 2019 Aug 15;54(2). pii: 1901325. doi: 10.1183/13993003.01325-2019. PMID: 31416813
4. Sharma L, Rebaza A, **Dela Cruz CS**. When "B" becomes "A": the emerging threat of influenza B virus. Eur Respir J. 2019 Aug 15;54(2). pii: 1901325. doi: 10.1183/13993003.01325-2019. PMID: 31416813
5. Mihaylova VT, Kong Y, Fedorova O, Sharma L, **Dela Cruz CS**, Pyle AM, Iwasaki A, Foxman EF. Regional Differences in Airway Epithelial Cells Reveal Tradeoff between Defense against Oxidative Stress and Defense against Rhinovirus. Cell Rep. 2018 Sep 11;24(11):3000-3007. PMID: 30208323
6. Chang D, Mo G, Yuan X, Tao Y, Peng X, Wang F, Xie L, Sharma L, **Dela Cruz CS*,** Qin E. Time Kinetics of Viral Clearance and Resolution of Symptoms in Novel Coronavirus Infection. AJRCCM, 2020 Mar 23. PMID:32200654.
7. Torres A, Chalmers JD, **Dela Cruz CS**, Dominedò C, Kollef M, Martin-Loeches I, Niederman M, Wunderink RG. Challenges in severe community-acquired pneumonia: a point-of-view review. Intensive Care Med. 2019 Feb;45(2):159-171. PMID: 30706119
8. Gautam S, Sharma L, **Dela Cruz CS**. Personalizing the Management of Pneumonia. Clin Chest Med. 2018 Dec;39(4):871-900. PMID: 30390755
9. Gautam S, Stahl Y, Young GM, Howell R, Cohen AJ, Tsang DA, Martin T, Sharma L, **Dela Cruz CS**. Quantification of bronchoalveolar neutrophil extracellular traps and phagocytosis in murine pneumonia. Am J Physiol Lung Cell Mol Physiol. 2020 Aug 12. doi: 10.1152/ajplung.00316.2020. Online ahead of print.PMID: 32783617
10. Ramirez JA, Musher DM, Evans SE, **Dela Cruz C**, Crothers KA, Hage CA, Aliberti S, Anzueto A, Arancibia F, Arnold F, Azoulay E, Blasi F, Bordon J, Burdette S, Cao B, Cavallazzi R, Chalmers J, Charles P, Chastre J, Claessens YE, Dean N, Duval X, Fartoukh M, Feldman C, File T, Froes F, Furmanek S, Gnoni M, Lopardo G, Luna C, Maruyama T, Menendez R, Metersky M, Mildvan D, Mortensen E, Niederman MS, Pletz M, Rello J, Restrepo MI, Shindo Y, Torres A, Waterer G, Webb B, Welte T, Witzenrath M, Wunderink R. Treatment of Community-Acquired Pneumonia in Immunocompromised Adults: A Consensus Statement Regarding Initial Strategies. Chest. 2020 Jun 16:S0012-3692(20)31681-0. doi: 10.1016/j.chest.2020.05.598. PMID: 32561442
11. [illegible]

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to Report

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

- 2020: American Thoracic Society, Philadelphia, CA (Virtual 2020), "Unlocking Immunity to Fight Infection"
- 2019: American Thoracic Society, San Diego, CA, "Antimicrobial Immunity and Chronic Lung Diseases"
- 2019: International Respiratory Infections Conference, Dallas, TX, "Consequences of Respiratory Infections on Lung Diseases"
- 2019: 15º Curso Internacional Infecciones Pulmonares, Buenos Aires, Argentina, "Integrated clinical approach to pneumonia in the immunocompromised host"
- 2019: 15º Curso Internacional Infecciones Pulmonares, Buenos Aires, Argentina, "Personalized Approach to Pneumonia"
- 2019: BIRDS Lecture, Brown University, Providence, RI, "Regulation of Antiviral Responses in the Cigarette Smoke Exposed Lung - Implications for COPD Endotypes"
- 2019: Mayo Clinic, Department of Immunology Grand Rounds, Rochester, MN, "Immunopathology and Interleukin-15"
- 2019: Pittsburg International Lung Conference, Pittsburg, PA, "Host-Pathogen Interactions in ARDS"

Website(s) or other Internet site(s)

List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to Report

Technologies or techniques

Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Nothing to Report

Inventions, patent applications, and/or licenses

Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to Report

Other Products

Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment

and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

- *data or databases;*
- *physical collections;*
- *audio or video products;*
- *software;*
- *models;*
- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

Nothing to Report

5. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Name:	*Charles Dela Cruz, MD*
Project Role:	*Principal Investigator*
Researcher Identifier (e.g. ORCID ID):	*n/a*
Nearest person month worked:	*5*
Contribution to Project:	*Dr. Dela Cruz supervised the overall design, experimental planning and data interpretation for all the studies.*
Funding Support:	*N/A*

Name:	*Insoo Kang, MD*
Project Role:	*Co-Investigator*
Researcher Identifier (e.g. ORCID ID):	*n/a*
Nearest person month worked:	*3*
Contribution to Project:	*Dr. Kang supervised the studies with T cells*
Funding Support:	*N/A*

Name:	*Min Jong Kang, MD,MPH, PhD*
Project Role:	*Co-Investigator*
Researcher Identifier (e.g. ORCID ID):	*n/a*
Nearest person month worked:	*2*
Contribution to Project:	*Dr. Kang assists in the exposure experiments and is the director of the Core..*
Funding Support:	*N/A*

Name: *Naftali Kaminski, MD*
Project Role: *Co-Investigator*
Researcher Identifier (e.g. ORCID ID): *n/a*
Nearest person month worked: *2*
Contribution to Project: *Dr. Kaminski assists with experiments related to single cell analyses*
Funding Support: *N/A*

Name: *Lokesh Sharma, PhD*
Project Role: *Postdoctoral Fellow*
Researcher Identifier (e.g. ORCID ID): *n/a*
Nearest person month worked: *8*
Contribution to Project: *Dr. Sharma worked on experiments related to mouse experiments, processing of samples and assays*
Funding Support: *N/A*

Name: *Min Shin PhD*
Project Role: *Associate Research Scientist*
Researcher Identifier (e.g. ORCID ID): *n/a*
Nearest person month worked: *8*
Contribution to Project: *Dr. Shin worked on the analyses of human immune samples*
Funding Support: *N/A*

Name: *Martin Slade*
Project Role: *Biostatistician*
Researcher Identifier (e.g. ORCID ID): *n/a*
Nearest person month worked: *1*
Contribution to Project: *Mr Slade assist with biostatistical and data analyses*
Funding Support: *N/A*

Name: *Santos Bermejo*
Project Role: *Research Assistant*
Researcher Identifier (e.g. ORCID ID): *n/a*
Nearest person month worked: *6*
Contribution to Project: *Mr Bermejo worked on patient recruitment and human sample processing*
Funding Support: *N/A*

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of

effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Nothing to Report

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Nothing to Report

6. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to **https://ers.amedd.army.mil** for each unique award.

Not applicable

QUAD CHARTS: If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.

Not applicable

7. APPENDICES: Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.

None