Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format. Questions? Contact Health Research Program staff at 717-783-2548.

1. **Grantee Institution:** Drexel University

2. **Reporting Period (start and end date of grant award period):** 1/01/2009 – 12/31/2010

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** Anne Martella

4. **Grant Contact Person's Telephone Number:** (215) 895-6471

5. **Grant ME Number or SAP Number:** 4100047631

6. **Project Number and Title of Research Project**: 1- Triggers of Inflammation in Scleroderma

7. **Start and End Date of Research Project:** 01/01/2009 – 12/31/2009

8. **Name of Principal Investigator for the Research Project:** Carol M. Artlett, PhD

9. **Research Project Expenses**.

   9(A) Please provide the amount of health research grant funds spent on this project for the entire duration of the grant, including any interest earned that was spent:

   $ 112,500

   9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of **all** persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name	Position Title	% of Effort on Project	Cost
Artlett, Carol M.	Associate Professor	5	5,499
Katsikis, Peter D	Professor	5	11,031
Rieger Judith	Graduate Student	100	28,435
Norton, Jill	Technician	50	22,785

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name	Position Title	% of Effort on Project
None		

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment.

Type of Scientific Equipment	Value Derived	Cost
None		

10. Co-funding of Research Project during Health Research Grant Award Period. Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes_____ No___X_____

If yes, please indicate the source and amount of other funds:

11. Leveraging of Additional Funds

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes____X_____ No_____

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the application was submitted (column C), and the amount of funds requested (column D). If you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert "not funded" in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

A. Title of research project on grant application	B. Funding agency (check those that apply)	C. Month and Year Submitted	D. Amount of funds requested:	E. Amount of funds to be awarded:
Role of the inflammasome in systemic sclerosis	X NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify: _____)	February 2009	$2,668,378	$ unfunded
Role of the inflammasome in systemic sclerosis	X NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify: _____)	September 2009	$3,008,382	$ unfunded

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes___X_____ No_____

If yes, please describe your plans:

We plan to resubmit the grant based on the current criticisms that we received from NIH

12. Future of Research Project. What are the future plans for this research project?

We will continue to pursue this avenue of investigation to obtain more preliminary data that will allow us to get NIH funding.

13. New Investigator Training and Development. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

Yes____X_____ No_____

If yes, how many students? Please specify in the tables below:

	Undergraduate	Masters	Pre-doc	Post-doc
Male				
Female			1	
Unknown				
Total			**1**	

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic			1	
Unknown				
Total			**1**	

	Undergraduate	Masters	Pre-doc	Post-doc
White			1	
Black				
Asian				
Other				
Unknown				
Total			**1**	

14. Recruitment of Out-of–State Researchers. Did you bring researchers into Pennsylvania to carry out this research project?

Yes_____ No____X_____

If yes, please list the name and degree of each researcher and his/her previous affiliation:

15. Impact on Research Capacity and Quality. Did the health research project enhance the quality and/or capacity of research at your institution?

Yes_____ No____X_____

If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.

16. Collaboration, business, and community involvement.

16(A) Did the health research funds lead to collaboration with research partners outside of your institution (e.g., entire university, entire hospital system)?

Yes_____ No___X_____

If yes, please describe the collaborations:

16(B) Did the research project result in commercial development of any research products?

Yes_____ No____X_____

If yes, please describe commercial development activities that resulted from the research project:

16(C) Did the research lead to new involvement with the community?

Yes_____ No____X_____

If yes, please describe involvement with community groups that resulted from the research project:

17. Progress in Achieving Research Goals, Objectives and Aims.

List the project goals, objectives and specific aims (as contained in the grant application's strategic plan). Summarize the progress made in achieving these goals, objectives and aims for the entire grant award period. Indicate whether or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was submitted, please describe the changes. Provide detailed results of the project. Include evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a DETAILED report of the methods and findings. It is not sufficient to state that the work was completed. Insufficient information may result in an unfavorable performance review, which may jeopardize future funding. If research findings are pending publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a performance review by an expert panel of researchers and clinicians who will assess project work using this Final Progress Report, all project Annual Reports and the project's strategic plan. After the final performance review of each project is complete, approximately 12-16 months after the end of the grant, this Final Progress Report, as well as the Final Performance Review Report containing the comments of the expert review panel, and the grantee's written response to the Final Performance Review Report, will be posted on the CURE Web site.

There is no limit to the length of your response. Responses must be single-spaced below, no smaller than 12-point type. If you cut and paste text from a publication, be sure symbols print properly, e.g., the Greek symbol for alpha (α) and beta (ß) should not print as boxes (□) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.

The goal of the application was to understand the contribution of the inflammasome to dermal fibrosis in an effort to understand the contribution of the inflammasome to the autoimmune disease scleroderma/systemic sclerosis

<u>Specific Aim.</u> To understand the contribution of the inflammasome in early dermal fibrotic lesions and autoantibody production utilizing various knockout mouse models. These experiments will determine the precise role of the inflammasome in BLM-induced fibrosis. Knockout mice to be used include MyD88$^{-/-}$, TRIF$^{-/-}$, ASC$^{-/-}$, or NALP3$^{-/-}$ deficient mice and compared to C57BL/6J wild-type mice.

Fibroblasts stimulated with bleomycin express more collagen COL1A1 and COL3A1. First, we wanted to confirm by *in vitro* studies in our laboratory that bleomycin induced the expression of collagen molecules so that we could further study the expression of the inflammasome in mouse dermal cells. Fibroblasts were isolated from C57BL/6 skin, cultured until confluent and incubated with 10 nM bleomycin for 24 h. The RNA was isolated and assayed by real-time PCR for COL1A1 and COL3A1 transcripts and the expression of these transcripts was normalized to β-actin. We found that collagen COL1A1 mRNA levels increased by 13% and COL3A1 increased by 66% (Figure 1) with the bleomycin treatment. These differences were found to be statistically significant (P = 0.0018 for COL1A1 and P = 0.0004 for COL3A1).

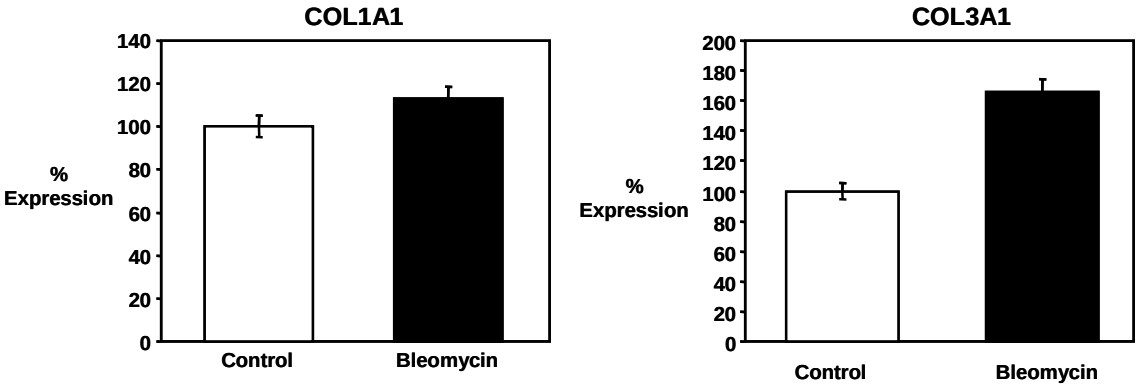

Figure 1. Bleomycin induced collagen expression in human fibroblasts. *Fibroblasts were stimulated overnight with 10nM bleomycin. RNA was extracted and made into cDNA and COL1A1 and COL3A1 transcripts were measure by real-time PCR. All transcripts were normalized to β-actin expression. Pooled data from n=5 mouse fibroblast lines. COL1A1, P = 0.0018; COL3A1 P = 0.0004*

Bleomycin induced TGF-β1 mRNA in mouse fibroblasts. We then further investigated the expression of fibrotic mediators in the bleomycin treated fibroblasts and found that TGF-β1 mRNA levels were also increased in a dose dependent manner (Figure 2). In these experiments we were trying to determine the best amount of bleomycin to use in these studies.

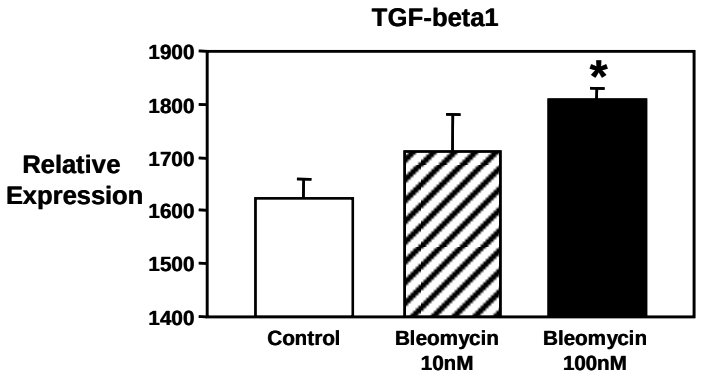

Figure 2. TGF-β1 expression is induced with bleomycin. *Fibroblasts were stimulated overnight with 10 nM or 100nM bleomycin. RNA was extracted and made into cDNA and TGF-β1 transcripts were measure by real-time PCR and corrected for β-actin expression. Pooled data from n=5 human fibroblast from healthy donors shown.* *P = 0.024.

Bleomycin induces inflammatory cytokines *in vitro* in human fibroblasts. It is now known that fibroblasts contribute to the innate immune signaling and these cells are no longer thought to be just involved in extracellular matrix expression and it has been suggested that bleomycin induced fibrosis must be mediated by the innate immune system. Recently it has been shown that the inflammasome is part of the innate immune signaling system as its activation takes place early in response to infection. Therefore, we cultured normal human fibroblasts to determine pertinent cytokine expression in response to bleomycin (Figure 3). We found that bleomycin induced mRNA increases for IL-1β, IL-18, IL-33, and IL-6. The increase in mRNA for IL-1β, IL-18, and IL-33 do not directly show inflammasome activation but may suggest increases in substrates of the inflammasome activation and also positive feedback loops originating from the inflammasome. For example, mature IL-1β is a known potent inducer of IL-1β mRNA in fibroblasts. Thus in our experiments of Figure 3, mRNA increases may suggest inflammasome activation and the induction of mature cytokines that induce mRNA upregulation in fibroblasts.

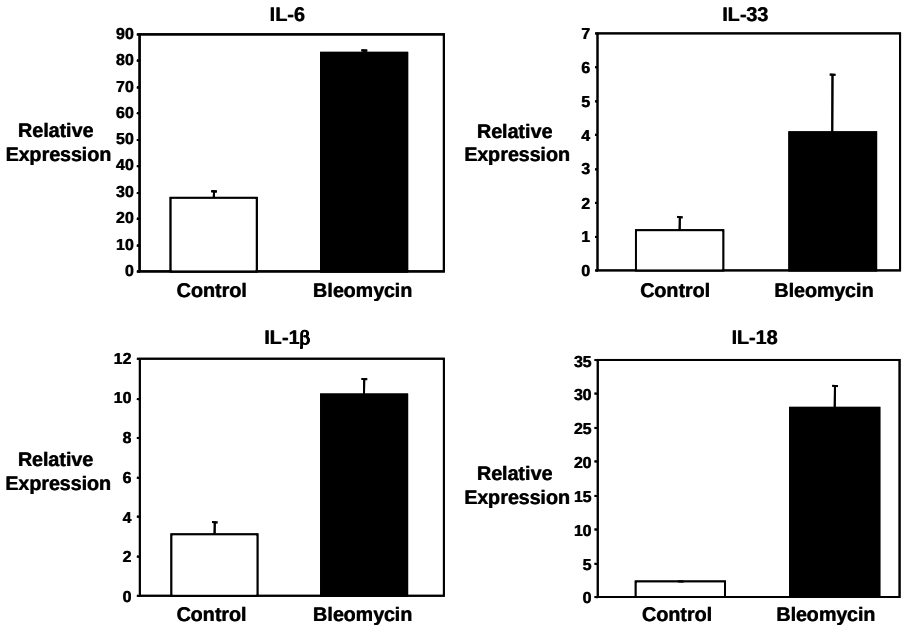

Figure 3. Bleomycin induces IL-1β, IL-6, IL-18 and IL-33 mRNA. *Fibroblasts isolated from normal skin were treated overnight with 100nM bleomycin. RNA was extracted and converted to cDNA and IL-1β, IL-6, and IL-33 transcripts were measured by Real Time PCR. mRNA transcripts were normalized to β-actin. We found that with bleomycin, there was almost a 3.2-fold increase in IL-1β, (P = 0.0085); a 3-fold increase in IL-6 (P = 0.0012); a 3.5-fold increase in IL-33 (P = 0.009); and a 12.6 fold increase in IL-18 (P= 0.0082). Pooled data from human fibroblast from n=5 healthy donors*

NALP3 is involved in bleomycin induced skin fibrosis in mice. Treated mice had both flanks shaved to allow for the relocation of the injection sites. Bleomycin was injected into the dermis of one flank and PBS (carrier) was injected into the other flank in C57BL/6 (n=6), NALP3-/- (n=6), ASC-/- (n=4), and MyD88-/- (n=2) mice for 28 days. The mice were sacrificed by CO_2 asphyxiation and cervical dislocation. Skin from the injection sites and lung was harvested and embedded in paraffin for sectioning. Sections were stained with Masson's trichrome to detect collagen (blue stain). The sections of skin and lung were photographed at 10X magnification (Figure 7). Measurements of skin thickness were taken at five random points across the image from the epidermis down to the pannus carnosus layer and the measurements in each mouse was averaged. In the C57BL/6 mice, we found that bleomycin induced a 1.75 +/- 0.29 fold increase (~75% increase) in the thickness of the dermal layer compared to NALP3-/- mice that showed no increase (1.04 +/- 0.24 fold increase in skin thickness) and MyD88-/- mice (1.03 +/- 0.25) (Figure 4).

However, we found that ASC was not absolutely necessary for dermal fibrosis, suggesting that there may be an alternative adaptor molecule in the skin. This is the first observation of this. Further, this alternative adaptor molecule may not be as efficient at activating CASP1, as induction of fibrosis was approximately half of that which was observed in the wild-type mice (Figure 4). We also harvested the lung from the C57BL/6, NALP3-/-, ASC-/-, and MyD88-/- mice and stained the sections with Masson's trichrome. It is known that bleomycin injected in the skin will also induce fibrosis in the lung and indeed we observed that the lung in the C57BL/6 mouse had altered architecture and increased collagen deposition, whereas

the NALP3, ASC, and MyD88 deficient mice did not. Of note, the lung was completely devoid of fibrosis in the ASC-/- confirming the previously published data. In summary, these results show that NALP3 and MyD88 play a critical role in bleomycin induced skin fibrosis and provides strong support for our hypothesis that the inflammasome may be involved in skin fibrosis.

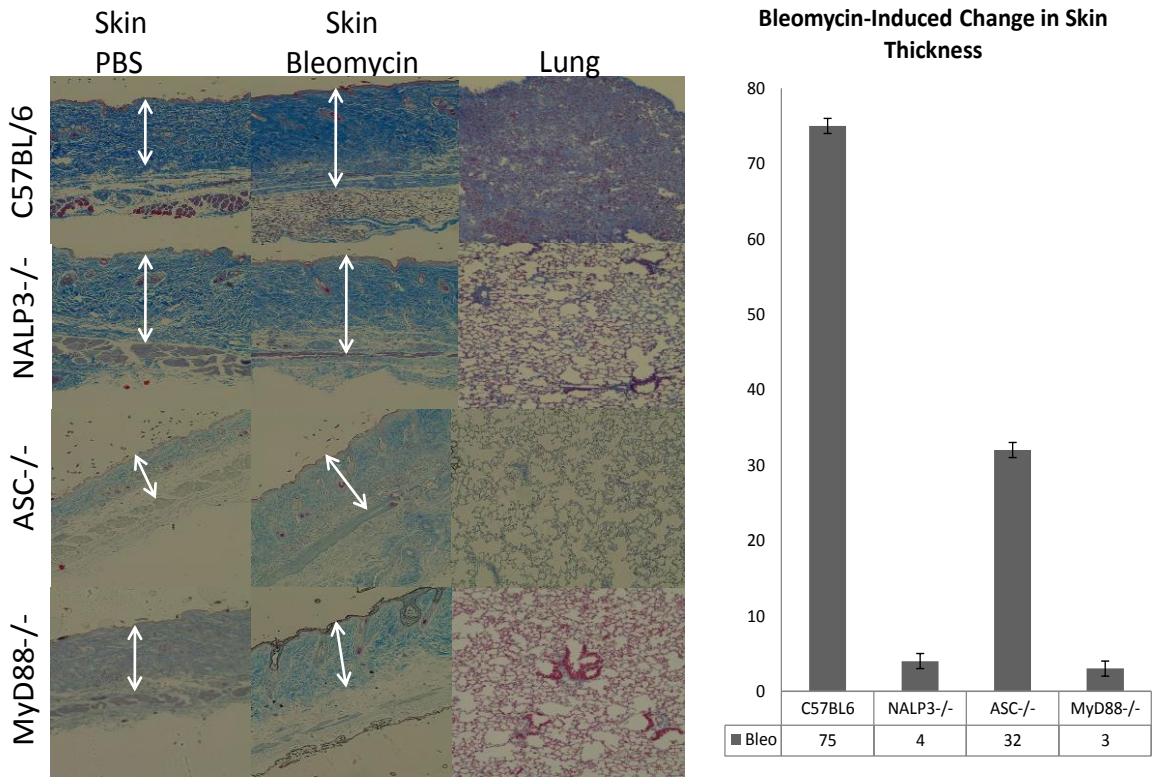

Figure 4. Bleomycin induced skin thickening is abrogated in NALP3-/- and MyD88-/-mice. *C57BL6 (n=4) and NALP3-/- (n=3) mice were injected intradermally for 28 days. Skin and lung were collected, embedded in paraffin and sectioned for staining. Note the increased skin thickness in the bleomycin treated C57BL/6 mice compared to the NALP3-/-, ASC-/-, and MyD88-/- mice. Also note the lung occlusion and collagen (blue) deposition in the C57BL/6 mice. The NALP3-/-, ASC-/-, and MyD88-/- had normal lung architecture and there was no increase in collagen, denoted by blue staining. The skin thickness was measure at 5 random places across the image and then averaged (e.g. white arrow). We found that the increase in skin thickness in the C57BL6 mice was 1.75 (+/- 0.29) fold more, whereas no increase was found in the NALP3-/- (1.04 +/- 0.24 fold increase) or MyD88-/- (1.03 +/- 0.25). These differences were statistically significant with P = 0.019. Fold increases were determined by comparing the bleomycin treated flank with PBS treated flank in each mouse. Left: Representative animals are shown. All images are at 10X magnification; Right: Pooled data from C57BL6 (n=6),NALP3-/- (n=6), ASC-/- (n=4), MyD88 (n=2) shown.*

Infiltrating cells in skin biopsies at the BLM or PBS injection sites. We investigated infiltrating cells in the C57BL6/J wild type mice and the NALP3-/-, ASC-/- mice to determine if any changes in inflammatory cell numbers were present. We found that in the wild type mice BLM induced a CD3 inflammatory infiltrate at 28 days with BLM. This was also confirmed with the NALP3-/- and ASC-/- mice. We observed no difference in the number of infiltrating cells in the 3 groups for CD3. We also investigated the presence of macrophages and observed that there were no differences in the pattern of infiltrate between the PBS injection site and the BLM injection site.

Bleomycin activation of the inflammasome is inhibited with KCl. It has previously been demonstrated that the activation of the NALP3 inflammasome can be specifically inhibited by potassium chloride (KCl). KCL does not inhibit other inflammasomes. Therefore, we

wanted to determine if inflammasome activation by bleomycin could be prevented when KCl was added to fibroblasts at the same time as 10 nM bleomycin. This experiment confirms that bleomycin does indeed activate the NALP3 inflammasome. Normal fibroblasts were incubated with 10 nM bleomycin and 100 nM KCl for 24 h. RNA was extracted and transcripts for IL-18, IL-33, and NALP3 were measured by real-time PCR. We found that with KCl, mRNA transcripts were reduced to below control levels (Figure 5). We also found that KCL decreased NALP3 transcripts but these were still elevated. This data confirms that bleomycin activates the NALP3 inflammasome.

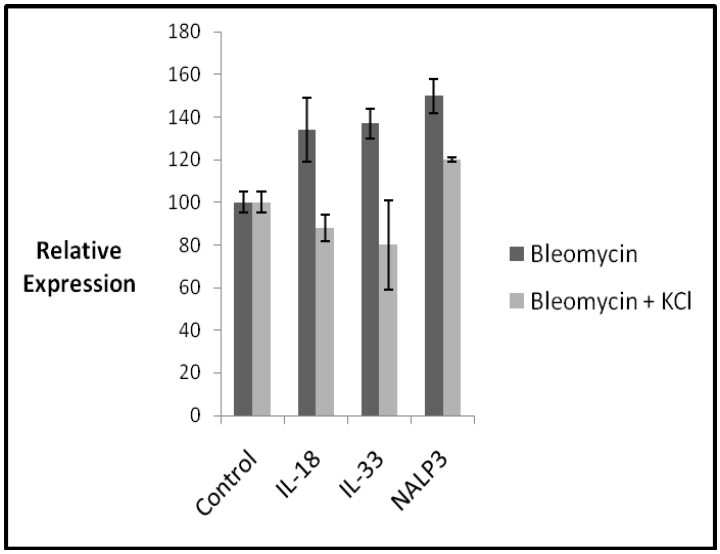

Figure 5. Bleomycin activation of the inflammasome is abolished with KCl. *Fibroblasts isolated from normal skin were treated overnight with 10nM bleomycin or 10nM bleomycin + KCl. RNA was extracted and converted to cDNA and IL-18, IL-33, and NALP3 transcripts were measured by Real Time PCR. mRNA transcripts were normalized to β-actin. We found that with the addition of KCl, bleomycin stimulation of inflammasome proteins was abolished further confirming that bleomycin activates the inflammasome.*

IL-33, IL-18, and NALP3 mRNA is elevated in SSc fibroblast cell lines. We investigated mRNA expression of IL-18, IL-33, and NALP3 to determine if the expression of these proteins are elevated in SSc. Low passage (passage 4) SSc primary cell lines (n=7) were cultured in DMEM supplemented with 10% FBS and antibiotics. RNA was purified and converted to cDNA and IL-18, IL-33 and NALP3 mRNA transcripts were measured by Real Time PCR and normalized to β-actin expression (Figure 6). Expression of the transcripts was compared to expression in normal fibroblasts (n=4).

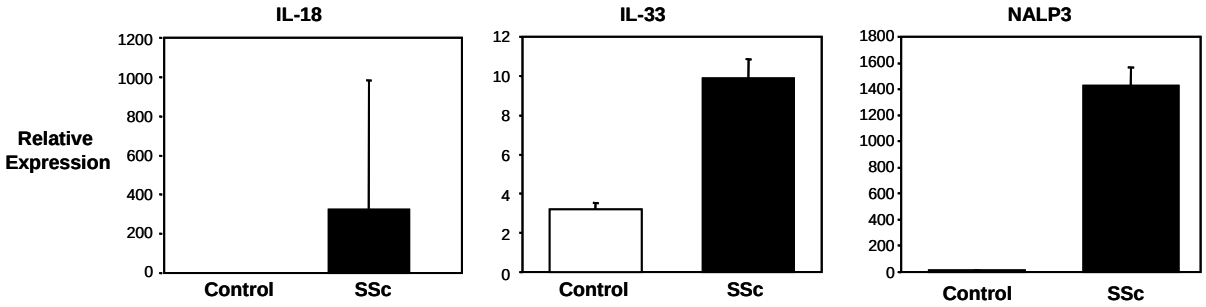

Figure 6. mRNA levels of IL-18, IL-33, and NALP3 are increased in SSc fibroblasts. *Primary cell lines from SSc patients (n=7) of low passage (4) were cultured and RNA was extracted, converted to cDNA. IL-18, IL-33, and NALP3 transcripts were measured by real-time PCR. All transcripts were normalized to β-actin and compared to control fibroblasts (n=4). We found that the average expression was IL-18: 323 in SSc vs. 0.41 in control (P<0.0001); IL-33: 9.85 in SSc vs. 3.21 in control (P<0.001); and NALP3: 1423 in SSc vs. 9.5 in control (P<0.0001). Bar graphs show pooled data.*

NALP3, Active CASP1, and IL-1β Proteins are elevated in scleroderma (SSc) dermal fibroblasts. The bleomycin mouse model which we utilized to understand the role of the inflammasome in fibrosis is a good model for scleroderma. Therefore we investigated the activation of the inflammasome in primary fibroblast cell lines. A critical step in the activation of CASP1 is the assembly of the inflammasome. CASP-1 is present in the cytosol as an inactive zymogen but becomes activated through the inflammasome, via self cleavage to produce a p20 subunit. CASP1 cleavage is dependent on the NALP3 inflammasome. The resulting activation of CASP1 then induces cleavage of IL-1β. We investigated the expression of the inflammasome protein NALP3 and active CASP1, the CASP1 p20 fragment in SSc fibroblast cell lines (n=4) established from the active lesions and compared these to normal cell lines (n=4). NALP3 was expressed 1.86 fold more (increase of 86%), active CASP1 cleaved protein fraction was found to be 5.4-fold more (increase of 540%) and cleaved IL-1β was expressed 1.52 (increase of 52%) than that expressed in normal fibroblasts (Figure 7). This data suggests that the inflammasome is activated and participating in disease in SSc and further validates our rationale for further studying this mechanism in SSc.

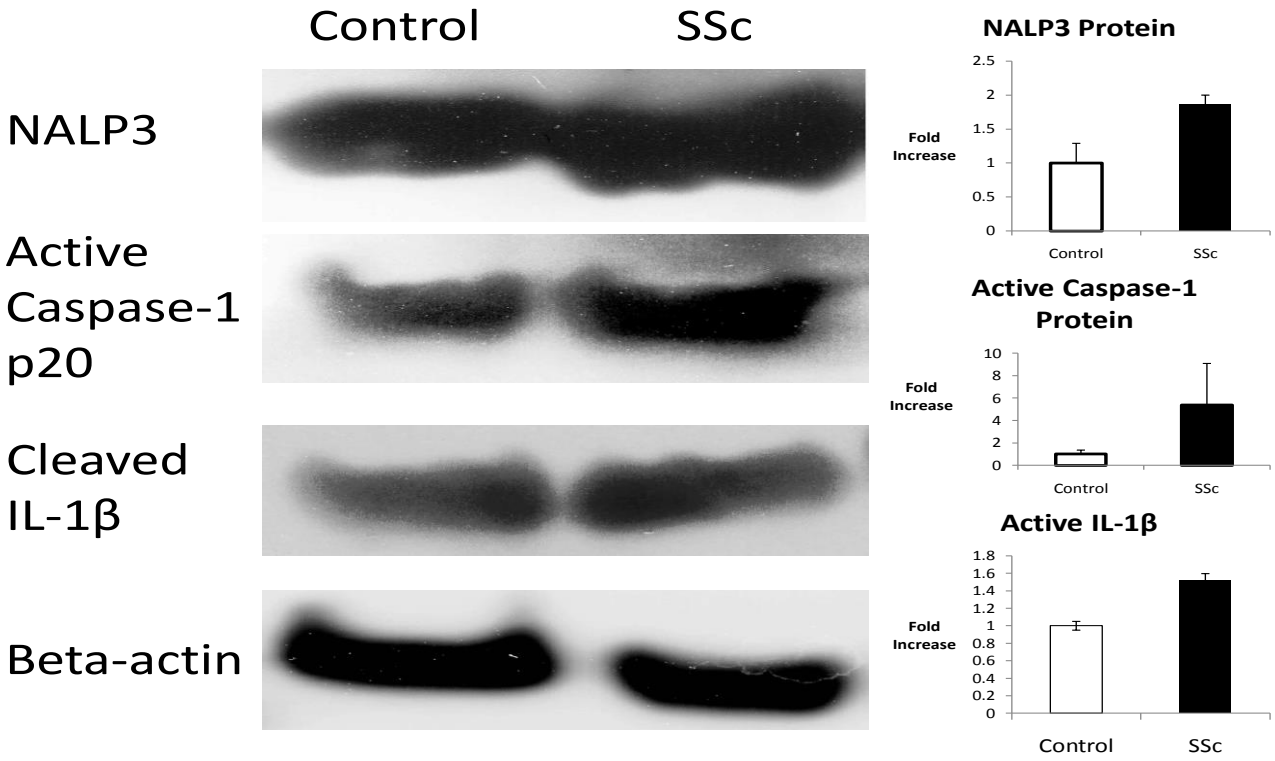

Figure 7. NALP3 and active CASP-1 proteins are elevated in SSc dermal fibrosis. *Early passage dermal fibroblasts were lysed and the proteins size fractionated on a polyacrylamide, transferred to PVDF, and probed with antibodies against NALP3, the active caspase-1 p20 fragment, and β-actin. Band intensities were measure by ImageJ and normalized to β-actin expression. In SSc dermal fibroblasts (n=2), more NALP3 and active CASP-1 protein is expressed suggesting that the inflammasome is activated and participating in fibrosis. Bar graphs show pooled data from 4 SSc fibroblast lines and 4 normal lines.*

Immunofluorescence of active CASP1 and NALP3 protein identify altered localization in SSc fibroblasts. We stained fibroblasts of low passage for active CASP1 (p20) protein and NALP3. Compared to control fibroblasts, active CASP1 was detected more in the SSc fibroblasts and appeared to aggregate in the cytoplasm (Figure 8). NALP3 protein in the controls was found close to the nucleus, whereas in SSc the protein appeared to localize to the cytoplasm and stress fibers. This data further supports the activation of the inflammasome in SSc.

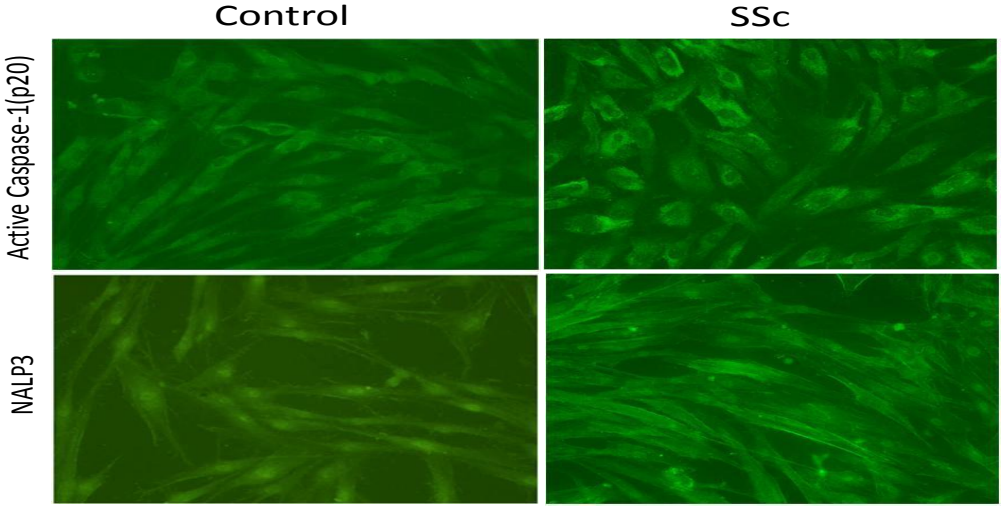

Figure 8. SSc Fibroblasts have increased expression and altered localization of active CASP1 and NALP3. *SSc and control fibroblasts were cultured on chamber slides until 90% confluent. Cells were fixed and stained with active CASP1 or NALP3. Cells were viewed with an epi-fluorescent microscope and images were taken at 200X magnification. Note the increased staining of both CASP1 and NALP3. Also note the altered localization of NALP3 protein from the nuclear region in the controls to what appears to be stress fibers in SSc.*

Active CASP1 p20 subunit is elevated in SSc lung tissue. We investigated the expression of the active CASP1 p20 subunit in paraffin embedded lung tissue from SSc (n=1) and normal (n=1). Briefly the sections were deparaffinized, boiled in 10 mM Citrate buffer, pH 6.0 for 30 min and blocked with 5% goat serum. Primary antibody rabbit-anti-CASP1 p20 was diluted 1:50 and applied to the section for 40 min at room temperature in a humid chamber. The unbound antibody was washed off and the secondary antibody goat-anti-rabbit-Cy2 was diluted and applied to the section for 40 min. Slides were washed and mounted with DaPI and a coverslip and viewed with a Nikon Eclipse epi-fluorescent microscope (Figure 9).

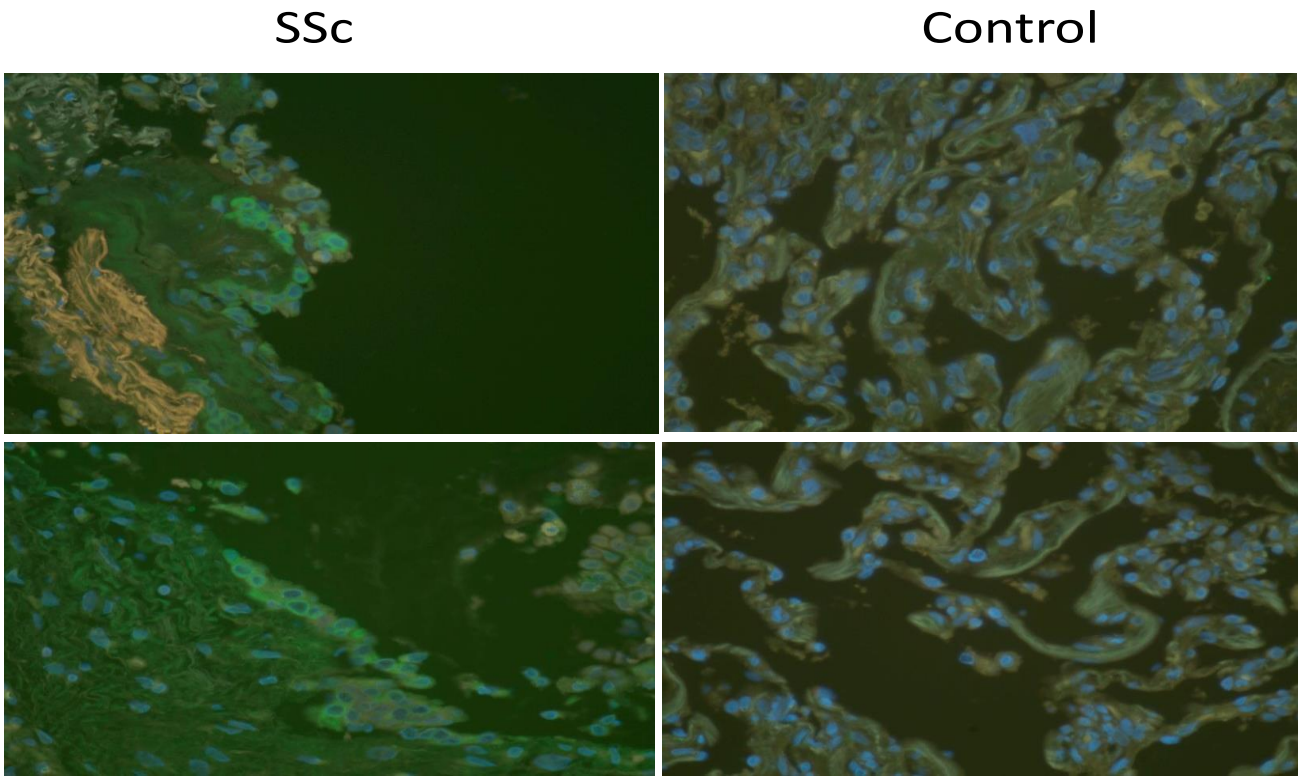

Figure 9. SSc lung tissue has increased expression of active CASP1 p20 subunit. *SSc and control lung tissue was stained for active CASP1 protein as described above. Tissues were viewed with an epi-fluorescent microscope and images were taken at 200X magnification. Note the increased green staining of active CASP1 in the tissue of the SSc lung.*

Bleomycin induces the secretion of IL-6 from normal human fibroblasts and IL-6 is elevated in SSc fibroblasts. We measured IL-6 protein in the culture media from human fibroblasts treated with bleomycin by ELISA. We found that 10nM bleomycin stimulated the fibroblasts to secrete 1.8 fold more IL-6 into the media (Figure 10). This was statistically significant P = 0.05. We further found that IL-6 protein is elevated in SSc fibroblasts.

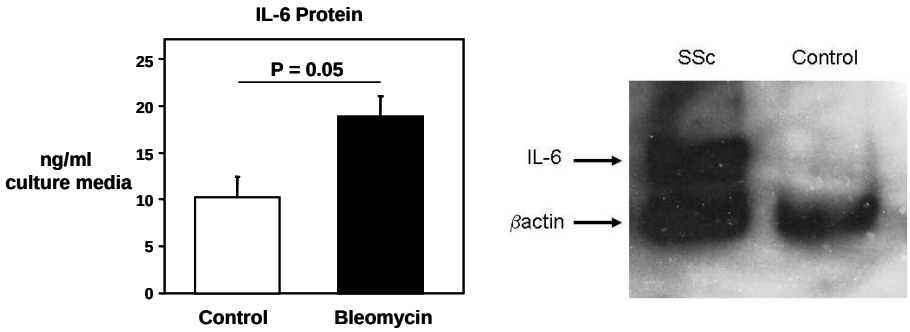

Figure 10. IL-6 production by human fibroblasts is enhanced by bleomycin. *Left: Fibroblasts from normal skin were treated overnight with 100nM bleomycin. IL-6 was assayed in the culture media by commercially available specific ELISA. Pooled data from human fibroblast from n=5 healthy donors shown. Right: Western blot for IL-6 in SSc and control fibroblasts.*

Summary: We found that bleomycin activates the NALP3 inflammasome utilizing a similar pathway to that which has been reported in lung fibroblasts; however, we found that ASC was not absolutely required for fibrosis in dermal fibroblasts suggesting for the first time that there is an alternative adaptor molecule that is present in the skin and not in the lung tissues. This data strongly supports the role of the inflammasome in scleroderma pathogenesis.

Outstanding Items: We were unable to complete the analysis of ANA titers on the mice that were injected with bleomycin. We have the serum currently stored at -80°C and this assay will be performed within the next 6 months.

18. Extent of Clinical Activities Initiated and Completed. Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be "No."

18(A) Did you initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
___X__No

18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
__X___No

If "Yes" to either 18(A) or 18(B), items 18(C) – (F) must also be completed. (Do NOT complete 18(C-F) if 18(A) and 18(B) are both "No.")

18(C) How many hospital and health care professionals were involved in the research project?

_____Number of hospital and health care professionals involved in the research project

18(D) How many subjects were included in the study compared to targeted goals?

_____Number of subjects originally targeted to be included in the study
_____Number of subjects enrolled in the study

18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

Gender:
_____Males
_____ Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics
_____Not Latinos or Hispanics
_____Unknown

Race:
_____American Indian or Alaska Native
_____Asian
_____Blacks or African American
_____Native Hawaiian or Other Pacific Islander
_____White
_____Other, specify:_____
_____Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in more than one county, list all of the counties where the research study was conducted.)

19. Human Embryonic Stem Cell Research. Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and 19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?
_____Yes
___x__ No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?
_____Yes
_____ No

19(C) Please describe how this project involved human embryonic stem cells:

20. Articles Submitted to Peer-Reviewed Publications.

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal abstracts or presentations at professional meetings; abstract and meeting presentations should be listed at the end of item 17. **Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source** (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic

copy of each publication, listed in the table, in a PDF version 5.0.5 format, 1,200 dpi. Filenames for each publication should include the number of the research project, the last name of the PI, the number of the publication and an abbreviated research project title. For example, if you submit two publications for PI Smith for the "Cognition and MRI in Older Adults" research project (Project 1), and two publications for PI Zhang for the "Lung Cancer" research project (Project 3), the filenames should be:

Project 1 – Smith – Publication 1 – Cognition and MRI
Project 1 – Smith – Publication 2 – Cognition and MRI
Project 3 – Zhang – Publication 1 – Lung Cancer
Project 3 – Zhang – Publication 2 – Lung Cancer

If the publication is not available electronically, provide 5 paper copies of the publication.

Note: The grant agreement requires that recipients acknowledge the Pennsylvania Department of Health funding in all publications. Please ensure that all publications listed acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the Commonwealth, do not list the publication.

Title of Journal Article:	Authors:	Name of Peer-reviewed Publication:	Month and Year Submitted:	Publication Status (check appropriate box below):
1. The Inflammasome and Caspase-1 Involvement in Systemic Sclerosis Fibroblasts Mediates Fibrosis and Myofibroblast Differentiation	Artlett, Carol M.; Sassi-Gaha, Sihem; Rieger, Judy; Boesteanu, Alina; Feghali-Bostwick, Carol; Katsikis, Peter	Arthritis and Rheumatism	Jan 2011	☐Submitted X Accepted ☐Published

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes___X_____ No_____

If yes, please describe your plans:

We are completing the final analysis of our studies on the inflammasome in the bleomycin mouse model. We anticipate that the manuscript will be submitted to a journal within the next couple of months.

21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project. Describe the outcome, impact, and effectiveness of the research project by summarizing its impact on the incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of outcome, impact or effectiveness of the research project. If there were no changes, insert "None"; do not use "Not applicable." Responses must be

single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None

22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment. Describe major discoveries, new drugs, and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project. If there were no major discoveries, drugs or approaches, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None

23. Inventions, Patents and Commercial Development Opportunities.

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance of work under this health research grant? Yes_____ No___x_____

If "Yes" to 23(A), complete items a – g below for each invention. (Do NOT complete items a - g if 23(A) is "No.")

a. Title of Invention:

b. Name of Inventor(s):

c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):

d. Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____

If yes, indicate date patent was filed:

e. Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____
If yes, indicate number of patent, title and date issued:
Patent number:
Title of patent:
Date issued:

f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes_____ No____

If yes, how many licenses were granted?_____

g. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes___ No____

If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents, or undertake any commercial development opportunities in the future?

Yes_____ No____X_____

If yes, please describe your plans:

24. Key Investigator Qualifications. Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. In place of narrative you may insert the NIH biosketch form here; however, please limit each biosketch to 1-2 pages. *For Nonformula grants only – include information for only those key investigators whose biosketches were not included in the original grant application.*

BIOGRAPHICAL SKETCH

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

NAME	POSITION TITLE
Artlett, Carol M.	Associate Professor
eRA COMMONS USER NAME cma102	

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 Sydney, Sydney Australia	B.Sc.	1986	Microbiology/Biochemistry
University of London, London United Kingdom	Ph.D.	1996	Medicine
Post-Doctoral Fellowship, Philadelphia PA		1996 - 1998	Scleroderma

B. Positions and Honors

Positions

1986 – 1990 Scientific Officer, Department of Medicine, Royal Alexandra Hospital for Children, Sydney Australia
1991 – 1994 Research Associate (Ph.D. Student), Tissue Typing, Oxford Transplant Center, Oxford UK
1994 – 1996 Research Associate, Division of Rheumatology, Thomas Jefferson University, Philadelphia, PA
1996 – 1998 Post-doctoral Fellow, Division of Rheumatology, Thomas Jefferson University, Philadelphia, PA
1998 - 2006 Research Assistant Professor of Medicine, Thomas Jefferson University, Philadelphia PA
2006 - 2007 Assistant Professor Microbiology & Immunology, Drexel University, Philadelphia PA
2007 - Associate Professor Microbiology & Immunology, Drexel University, Philadelphia PA

Honors & Awards

1997 Penn State, Finkelstein Award
1997 – 1999 American College of Rheumatology/Larry Freeman Physician Scientist Award
1997 – 1999 Post-Doctoral Fellowship Arthritis Foundation
1998 – 2003 FIRST Award (R29), National Institutes of Health
2001 – 2003 Scleroderma Foundation grant review committee member
2003 NICHD/NIH Special Emphasis Panel ZHD1 DRG-A (C5)
2002 – 2004 Thomas Jefferson University Dean's DORP Award
2004 – 2005 Children's Hospital of Michigan grant reviewer
2005 – 2006 Thomas Jefferson University Dean's DORP Award
2006 Welcome Trust grant reviewer
2008 – 2010 Scleroderma Foundation grant review committee member
2009 Drexelmed CURE Award
2009 EULAR grant review committee member
2010 Research Competitiveness Program, American Association for the Advancement of Science (AAAS) review committee member

C. Publications (most recent from 50 published papers)

1. Jimenez SA, Artlett CM, Sandorfi N, Derk C, Latinis K, Sawaya H, Piera-Velazquez S, Haddad R, Shanahan JC. Dialysis-associated systemic fibrosis (nephrogenic fibrosing dermopathy): a clinical description of nine new cases and study of inflammatory cells and transforming growth factor β1 expression in affected skin. Arthritis Rheum **50**:2660-6, 2004. PMID: 15334482
2. Sawaya HHB, Jimenez SA, Artlett CM. Quantification of fetal microchimeric cells in clinically affected and unaffected skin of patients with systemic sclerosis. Rheumatology **43**:965-8, 2004. PMID: 15199216
3. Kowalzick L, Artlett CM, Thoss K, Baum H-P, Ziegler H, Mischke D, Blum R, Ponnighaus J-M, Quietzsch J. Chronic graft versus host like dermopathy in a child with CD4+ cell microchimerism and successful treatment with UVA1 phototherapy. Dermatology **210**: 68-71, 2005. PMID: 15604551
4. Artlett CM. Immunology of systemic sclerosis. Frontiers in Bioscience **10**:1707 – 1719, 2005. PMID: 15769660
5. Gentiletti, J, McCloskey LJ, Artlett CM, Peters J, Jimenez SA, Christner PJ. Demonstration of autoimmunity in the tight skin-2 mouse: a model for scleroderma. J. Immunol. **175**:2418-26, 2005. PMID: 16081813
6. Derk C, Artlett CM, Jimenez SA. Morbidity and mortality of patients diagnosed with systemic sclerosis after the age of 75: a nested case-control study. Clin Rheumatol **25**:1-4, 2006. PMID: 16435160
7. Mendoza FA, Artlett CM, Sandorfi N, Latinis K, Piera-Velazquez S, Jimenez SA. Description of 12 cases of nephrogenic fibrosing dermopathy and review of the literature. Semin Arthritis Rheum **35**:238-249, 2006. PMID: 16461069
8. Del Galdo F, Artlett CM. T cells and B cells in the Pathogenesis of SSc: Recent Insights and Therapeutic Opportunities. Curr Rheumatol Rep **8**:123-30, 2006. PMID: 16569371
9. Del Galdo F, Jimenez SA, Artlett CM. Tissue expression of allograft inflammatory factor-1 in systemic sclerosis and in vitro differential expression of its isoforms in response to transforming growth factor-beta. Arthritis Rheum **54**: 2616-25, 2006. PMID: 16868985
10. Del Galdo G, Artlett CM, Jimenez SA. The role of allograft inflammatory factor 1 in systemic sclerosis. Curr Opin Rheumatol. **18**:588-93, 2006. PMID: 17053503
11. Otieno FG, Lopez AM, Jimenez SA, Gentiletti J, Artlett CM. Allograft inflammatory factor-1 and tumor necrosis factor single nucleotide polymorphism in systemic sclerosis. Tissue Antigens **69**: 583 – 91, 2007. PMID: 17498268
12. Mueller YM, Duc HD, Altork SR, Artlett CM, Graceley EJ, Katsetos CD, Legido A, Villinger F, Altman JD, Brown CR, Lewis MG, Katsikis PD. IL-15 treatment during acute SIV infection increases viral set point and accelerates disease progression. J. Immunol **180**:350-60, 2008. PMID: 18097036
13. Hoffman WH, Artlett CM, Zhang W, Kriepke CW, Passmore GG, Rafols JA, Sima AA. Receptor for advanced glycation end products and neuronal deficit in the fatal brain edema of diabetic ketoacidosis. Brain Res **1238**: 154-62, 2008. PMID: 18775683
14. "1-Peptidyl-2-arachidonoyl-3-stearoyl-*sn*-glyceride: An Immunologically Active Lipopeptide from Goat Serum (*Capra hircus*) is an Endogenous Damage-Associated Molecular Pattern", *J Nat Prod* **72**(11):1993-1999 (2009).
15. Loughlin DT, Artlett CM. 3-Deoxyglucosone-Collagen Alters Human Dermal Fibroblast Migration and Adhesion: Implications for Impaired Wound Healing in Patients with Diabetes. Wound Repair Regen **17**:739-749, 2009. PMID: 19769726
16. Sassi-Gaha S, Loughlin DT, Kappler F, Schwartz ML, Su B, Tobia AM, Artlett CM. Two dicarbonyl compounds, 3-deoxyglucosone and methylglyoxal, differentially modulate dermal fibroblasts. Matrix Biology (In Press). PMID: 19800404

BIOGRAPHICAL SKETCH

NAME Peter D. Katsikis	POSITION TITLE Professor,
eRA COMMONS USER NAME Katsikis1	Department of Microbiology & Immunology,

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of Thessaloniki, Thessaloniki, Greece	M.D.	1978-1985	Medicine
University of Ioannina, Ioannina, Greece	Ph.D.	1986-1990	Immunology

Positions and Honors.

Positions:

1988-1989 Research Fellow, Dept. of Immunology, University of Brest, Brest, France.
1990-1994 Postdoctoral Fellow, Kennedy Institute of Rheumatology Research, London, U.K.
1994-1997 Postdoctoral Scholar, Dept. of Genetics, Stanford University School of Medicine, Stanford CA.
1997-1999 Assistant Professor, Department of Microbiology and Immunology, Allegheny University of the Health Sciences, Philadelphia, PA.
1999-2005 Associate Professor, Department of Microbiology & Immunology, Drexel University College of Medicine, Philadelphia, PA.
2004- Professor, Department of Microbiology & Immunology, Drexel University College of Medicine, Philadelphia, PA.
2004- Director of Center for Immunology and Vaccine Sciences, Drexel University College of Medicine
2006- Founder and Chief Scientific Officer of Anosia Inc., Laguna Hills, CA.

Honors and Awards:

- Young Investigator Award, European League Against Rheumatism (EULAR), Xth European Workshop for Rheumatology research, 1990.
- First Panhellenic Medical Award, 16th Panhellenic Medical Congress, 1990
- Young Investigator Award of European League against Rheumatism (EULAR), 1994
- Basic Research Scientist Award, Drexel University College of Medicine, 2006

Other Experience and Professional Memberships:

Member of American Association of Immunologists (AAI)
Member of the American Association for the Advancement of Science (AAAS)
2002-2004: Member of American Heart Association (AHA) Peer Review Study Group Immunology & Microbiology I 2003: Ad Hoc Grant reviewer for the Association for International Cancer Research, Fife, UK.
2003: Grant Reviewer for Allegheny General Hospital (AGH) PA State Tobacco Formula Funds FY03-04
2004: Co-chair of block session "Regulation and evasion of the immune response by pathogens" at AAI 2004 meeting.
2004: Ad Hoc reviewer of Program Project (P01) for RPRB, DEA, National Cancer Institute (NCI).
2004: Ad Hoc reviewer for NIAID Special Emphasis Panel, "HIV Vaccine Design and Development Teams for Prevention and Treatment", NIAID ZAI1 DP-A (C1), NIH.
2004: Ad Hoc reviewer for NIH Inter-Institute Program for the Development of AIDS-Related Therapeutics (IIP), Developmental Therapeutics Program NCI/NIH

2004: Ad Hoc reviewer for U.S. Civilian Research & Development Foundation (CRDF) Cooperative Grants Program (CGP)
2005: Ad Hoc Member, General Secretariat of Research and Technology (GSRT), Ministry of Development, Greece, PENED grants, April 2005.
2005: Ad Hoc Member, National Cancer Institute (NCI), National Institutes of Health (NIH), P01 Review, Immunotherapy Cluster Meeting, May 25-27, 2005
2005: Ad Hoc Member, Veterans Administration (VA) Merit Awards Grant review,
2006: Ad Hoc reviewer for AIDS Immunology and Pathogenesis Study Section (AIP), NIH.
2006: Ad Hoc reviewer for Immunity and Host Defense Study Section (IHD), NIH.
2007: Member of AIDS Immunology and Pathogenesis Study Section (AIP), NIH.
2007: Associate Editor, Journal of Immunology

A. Selected peer-reviewed publications (in chronological order). Recent peer-reviewed publications (selected from 70 papers):

1. Fraietta JA, Mueller YM, Do HD, Holmes VM, Howett MK, Lewis MG, Boesteanu AC, Alkan SS, Katsikis PD: Phosphorothioate 2′ deoxyribose oligomers as microbicides that inhibit human immunodeficiency virus type 1 (HIV-1) infection and block Toll-like receptor (TLR)7/9-triggering by HIV-1 Antimicrob. Agents Chemother. 54:4064-73, 2010
2. Boesteanu AC, Babu NS, Wheatley MA, Papazoglou ES, Katsikis PD: Biopolymer encapsulated live influenza virus as a universal CD8+ T cell vaccine against influenza virus. Vaccine 29:314–322, 2010
3. Duttagupta PA, Boesteanu AC, Katsikis PD: Costimulation signals for memory CD8+ T cells during viral infections. Crit. Rev. Immunol. 29:469-86, 2009
4. Mueller YM, Do HD, Boyer JD, Kader M, Mattapallil JJ, Lewis MG, Weiner DB, Katsikis PD: CD8+ cell depletion of SHIV89.6P-infected macaques induces CD4+ T cell proliferation that contributes to increased viral loads. J. Immunol. 183:5006-12, 2009
5. Bucks CM, Norton JA, Boesteanu AC, Mueller YM, Katsikis PD: Chronic antigen stimulation alone is sufficient to drive CD8+ T cell exhaustion. J. Immunol 182:6697-6708, 2009.

C. Research Support.

Ongoing Research Support:

R01 AI046719-07 Katsikis (PI) 03/15/99 - 06/30/11
NIH/NIAID
Title: "In vitro stimulation of HIV specific $CD8^+$ T cells"
The objective of this project is to investigate the function of HIV specific $CD8^+$ T cells in HIV infection. The outcome of antigen-specific stimulation (activation, cytokine production, proliferation/expansion or apoptosis) of HIV Gag- and Pol-specific CD8+ T cells and what are the costimulation requirements for these cells is investigated. The long term goal of this study is to understand the survival and functional defects of HIV-specific CD8+ T cells.
Role: Principal Investigator.

R21 AI082680 Katsikis (PI) 05/01/10-04/30/12
NIH/NIAID
Principle Investigator: Peter D. Katsikis 20% Effort
Title: Phosphorothioate oligonucleotides as microbicides against HIV transmission.
The goal of this grant is to develop a novel dual acting microbicide against HIV infection. Studies for the optimization, testing toxicity and identifying the mechanism of action for the phosphorothioate based microbicide are planned.
Role: Principal Investigator.