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:** Temple University – of the Commonwealth System of Higher Education

2. **Reporting Period (start and end date of grant award period):** 01/1/2009 -12/31/2012

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** Germaine Calicat, MLA

4. **Grant Contact Person's Telephone Number:** 215.204.7655

5. **Grant SAP Number:** 4100047651

6. **Project Number and Title of Research Project:** *1 - Resolving Inflammation in Arthritis*

7. **Start and End Date of Research Project:** 7/1/2009 – 6/30/2010

8. **Name of Principal Investigator for the Research Project:** Marion M. Chan, PhD

9. **Research Project Expenses**.

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

   $ $21,962.47

   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
Evans	Graduate Student	100	$21,962.47

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
Chan	Associate Professor	20

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:
Cyclooxgenase 2 in Resolution of Inflammation	■NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)	01-Apr-2009 And 01-Dec-2009	$ 1,875,000	$ None
Cyclooxgenase in Resolution of Arthritis	☐NIH ☐ Other federal (specify:_____) ■ Nonfederal source (specify: American College of Rheumatology RESEARCH)	01-Jul-2009	$ 400,000	$ None
Resolution of Inflammation by the Integrative Actions of Bioactive Lipids Inflammation	■NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)	01-Jul-2011	$1,875,000	$ None
Prostaglandins in the Controlling of Arthritis	☐NIH ☐ Other federal (specify:_____) ■ Nonfederal source (specify: American College of Rheumatology RESEARCH)	01-Jul-2011	$400,000	$ None

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:

I will keep the project active through collaboration. Generate more preliminary data to continue applying for funding from NIH, Arthritis Foundation or other non-federal sources.

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

I plan to continue to pursue 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			1	
Female				
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 agreement). Summarize the progress made in achieving these goals, objectives and aims for the period that the project was funded (i.e., from project start date through end date). 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.

Based on the fact that inflammation is resolved by an active process, and considering the fact that patients seek care after symptoms become apparent, a strategy to promote resolution is more effective than ones that prevent initiation of inflammation disorder. To develop such a therapy, there is a definite need to understand the "resolution" mechanism. The **objectives** of this project are to compare and contrast the events that operate in the resolution phase, and identify molecules that are essential for resolving inflammation in arthritis. Since we found that COX-2 was up-regulated during resolution and blocking its activity impeded resolution, our strategy is to set forth from the COX pathway, with the prostaglandins PGE_2 and 15d-PGJ_2 as our foci. The **specific aims** are as follow:

1. Determine if loss of these prostaglandins is associated with "failure to resolve".
2. Determine if replenishing the identified prostaglandin(s) would restore resolution of inflammation.

Results and Methods:

Kinetic studies revealed a resolution phase in the pathogenesis of arthritis.

Swelling of the footpads was found to follow three phases - induction, inflammation and resolution, analogous to those in acute inflammation (Fig. 1A). From days 0-30, arthritis had not developed; the mice were asymptomatic and their footpads

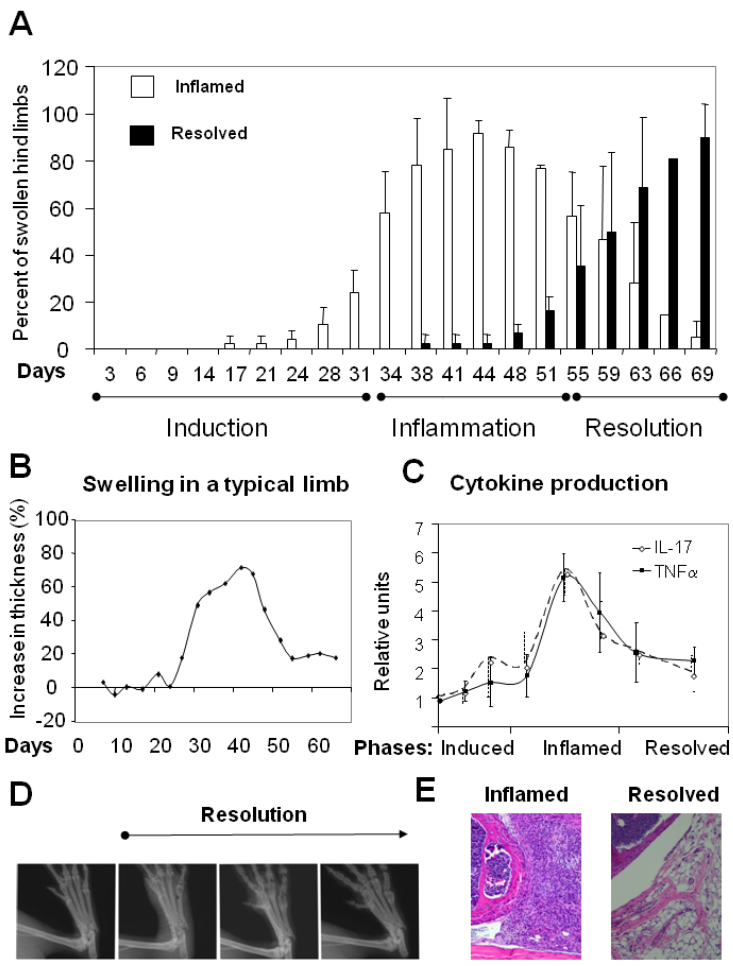

Fig. 1 Three phases of inflammation in arthritis.
[A] Induction phase, d1-30 - the limbs were not swollen; inflamed phase, d30-55 - the open bars indicate the percent of limbs (Y-axis) that have swollen by 20%, and resolution phase, d55-70 - the filled bars indicate the percent of swollen limbs that have decreased by more than 20% in footpad thickness. The sample size ranged from 100 to 200 in the progression phase**, and** 50 to 100 in the resolution phase, as mice were sacrificed for cytokine analyses. [B] Progression of inflammation in a typical arthritic limb. The Y-axis indicates the degree of swelling in the limbs, calculated with the formula: (thickness on day of measure - thickness on day 0)/(thickness on day 0). [C] Temporal profile of pro-inflammatory cytokine across the three phases. The levels of IL-17 and TNFα mRNA expression were analysis by real-time RT-PCR, according to the method in Adapala & Chan. Levels of mRNA expression were normalized against 18S RNA and then shown as the fold induction relative to day 0 expression (assigned the value of '1'). Each data point is averaged from three independent experimental repeats. In each of the **repeat**s, 4–6 limbs, each harvested from a different mouse was pooled for analyzing each time point. Statistic analysis by unpaired, two way ANOVA showed that for both IL-17 and TNFα, the levels at peak of inflammation are significantly different from when the joints are normal (day 0) or resolved (day 70), $p < 0.02$, Their levels in normal and resolved joints are not significantly different, $p>0.05$. [D] Sequential x-ray of representative metatarsal joints of a footpad is shown; note the subsiding of soft tissue swelling and the return of joint integrity and bone density in the fifth phalange. [E] H&E shows a highly cellular synovium in the inflamed (70% swollen at day 40) but not in the resolved (subsided from 91% to 12% swelling at day 70) knee joints (original magnification ×40).

were not swollen. Footpad swelling became increasingly prevalent beginning at day 30 and continued to do so until around day 45 when over 95% the footpads were swollen. Subsequently the footpad swelling began to progressively decrease with 40% resolved at day 55 and 90% at day 69. The progression from induction through inflammation to resolution is illustrated by a representative footpad (Fig. 1B).

The cytokines, IL-17 and TNFα drive progression in inflammatory response. Therefore, groups of mice were sacrificed at weekly intervals and the joints were collected for real-time PCR analysis to verify the state of inflammation by their expression in the joints. The mRNA expression of IL-17 and TNFα increased by 4-6 fold during the inflammatory phase from days 35 to 45, then subsided as inflammation resolved by day 70 (Fig. 1C). Thus, in the course of pathogenesis their expression corresponded to the phases of the inflammatory process. Furthermore, resolution is associated with the clearance of neutrophils. In a single cycle of disease, tissue repair occurs as indicated by the return of joint alignment, space and bone density in X-ray and by immuohistochemistry (IHC).

<u>COX2 was expressed in the resolution phase</u>.

Whereas the gene expression of IL-17 and TNFα subsided with resolution, COX-2 mRNA showed a bimodal pattern of expression (Fig. 2A). This bimodal pattern corresponds with the nature of COX-2 expression in acute inflammation. Kinetic studies demonstrated that COX-2 was up-regulated by 3-4 fold during the inflammatory phase between days 35 to 45, experienced a slight decline around day 55, then the level resurged during resolution through day 70. Comparisons by unpaired ANOVA analysis demonstrated that the expression level on day 70 was significantly higher than at baseline ($p < .005$). However, there were no significant differences between levels on day 70 and the peaks observed at days 35 and 45 ($p > .05$). The presence of COX-2 was confirmed at the protein level by <u>immunohistochemical</u> staining of the resolving knees (Fig. 2B). The enzyme was detected in the <u>synovium</u> as well as the infiltrating cells. The enzymatic product of COX-2, PGE_2, was also detected within the phase of resolution

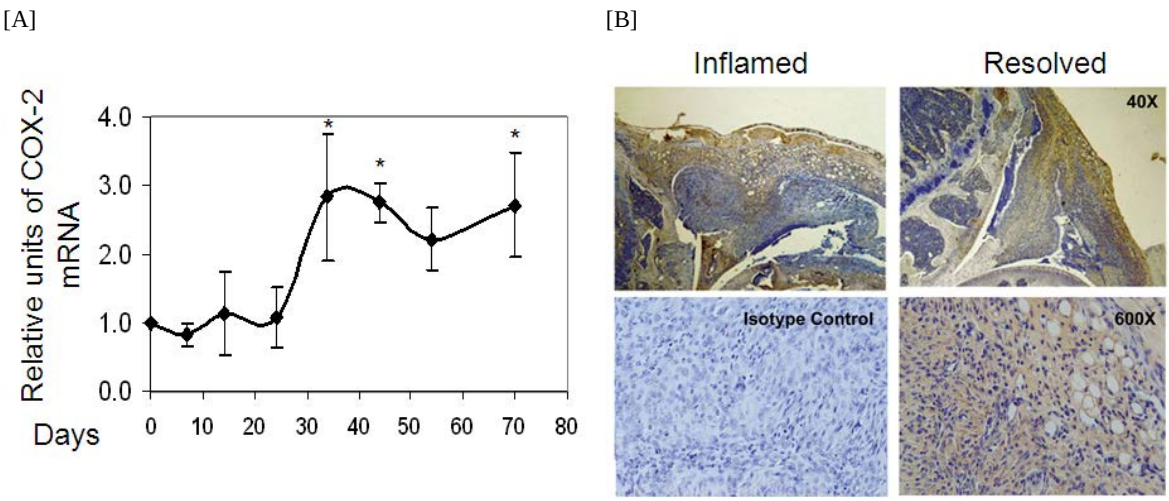

Fig. 2 COX2 expression in the three phases.

Panel to the left shows the kinetics of COX2 mRNA expression. X-asis indicates days. It shows the results of three totally independent trials. RNA was extracted and real-time PCR was performed as described in legend to Fig. 1. Each data point was derived using extracted from 4-6 limbs, each from a different mouse. Panel to the right shows immunohistochemical staining of a representative joint at the inflammation [A] and resolution phases [B]. Positive staining for COX2 appears as brown. The experiment was repeated three times and the results are shown as averages derived by combining the repeats.

(see Fig. 4D below).

<u>The COX2 inhibitor, NS398, interfered with resolution</u>. COX-2 and PGE_2 are dual acting agents that are known to have anti-inflammatory effects. We found that they are important for switching off the inflammatory response in the resolution phase. Giving NS398 [N-[2-(cyclohexyloxy)-4-nitrophenyl]-methanesulfonamide], a COX-2 specific inhibitor, prior to symptom manifestation (initiating NS398 feeding at day 23) prevented footpad swelling during the inflammation phase (Fig 3). However, the action of NS398 in the inflammation and the resolution phases is opposite (Fig. 4). During the resolution phase, blocking COX-2 led to failure to resolve. Using resolution index criteria described in animal model, 79% of the footpads in the vehicle control group had experienced resolution (negative slope), only 21% of them remained unchanged or continued to swell. In contrast, inflammation progressed (positive or '0' slope) in 76% of the footpads in the group that was fed NS398 preventively and 60% the footpads in the group that was fed NS398 therapeutically (Fig. 4A). The degree of inflammation corresponded to the levels of pro-inflammatory cytokines in the harvested limbs. Real-time PCR analysis confirmed that the levels of TNFα and IL-17 mRNA were significantly higher in the NS398-treated groups, and the differences are statistically significant as determined by ANOVA (Fig. 4B). Fig. 4C illustrates how NS398 affects the course of resolution using a representative limb from each group. Kinetically, the footpads in the vehicle control swelled and then subsided. COX-2 expression was important for resolution and production of PGE_2 (Fig. 4D).

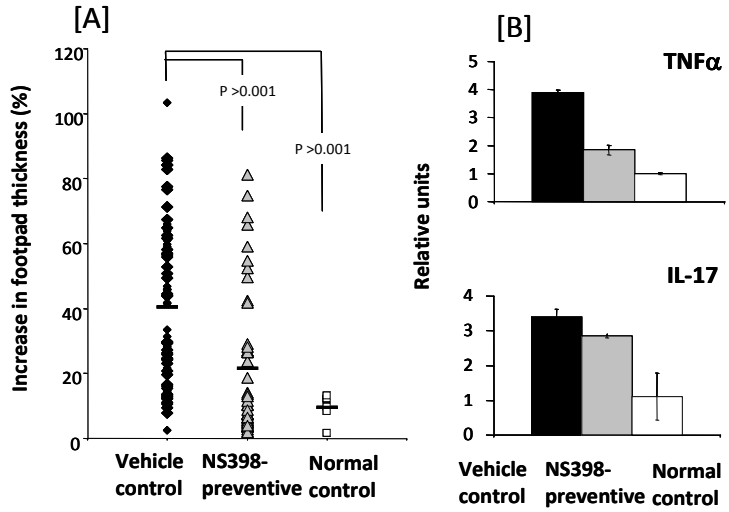

Fig. 3 NS398 inhibited footpad swelling in the inflamed phase.

Panel [A] depicts the relative increase in thickness of footpad (mm) due to arthritis induced by chicken collagen. The maximum thickness obtained within days 30-55, the inflamed phase, was plotted for each foot. A black diamond represents a footpad of mice that were induced for arthritis but fed vehicle as control. The grey triangles represent footpads of experimental mice that were fed NS398 prior to manifestation of symptoms. Most of them are congregated at the bottom. The open squares represent footpads of normal mice that were not induced for arthritis, and thus not swollen. The horizontal bars indicate the average value in each group. The results are from a total of three independent experimental repeats. The groups were compared using the Kauskal-Wallis method, and $p < 0.05$ is considered significant. Panel [B] shows the levels (fold change) of mRNA in a subgroup of limbs that were harvested at day 45. RNA was extracted with Trizol reagent and then real-time RT-PCR analyses were performed. The levels of mRNA expression were normalized against 18S RNA and then relative units deduced by comparing to normal control group (white). The data shown are the mean ± SEM from 4–6 mice and representative of three experimental repeats. Statistical analysis by ANOVA showed the differences are significant when the NS398-fed groups were compared with the vehicle control. $p < 0.05$ value is considered significant.

The consequence of failure of inflammation to resolve is exacerbated tissue destruction. Histological staining showed that the knee joints of the NS398-fed mice were infiltrated with more leukocytes (H&E) and sustained more severe cartilage damage than those of the vehicle controls (Safranin O). In terms of joint destruction, the advantage provided by NS398 is restricted to delaying the onset of inflammation.

<u>PGE analogs, misoprostol and dmPGE$_2$ restores the resolution of inflammation in NS398-treated mice.</u>

Based on the finding that the NS398-mediated decrease in PGE$_2$ production is associated an exacerbation of inflammation, we explored whether reconstituting the prostaglandins will restore resolution. Since PGE$_2$ is unstable, we employed misoprostol, a stable analog in the PGE$_1$ form that resists degradation in vivo and has many of the properties of the PGE$_2$ counterpart. Clinically, misoprostol has been prescribed for preventing

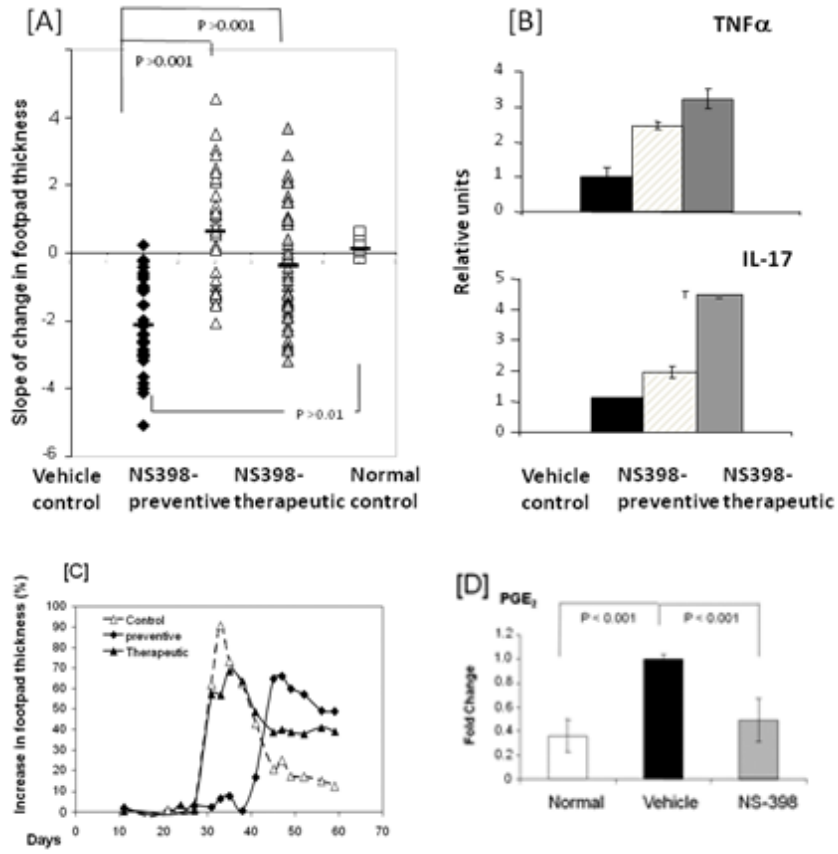

Fig. 4 NS398 increased the incidence and severity of swelling in the resolution phase. Panel [A] depicts the relative change in thickness of footpads during the resolution. <u>A 'slope' was derived by linear regression</u> from the peak of swelling (e.g. day 45) or from when NS398 is administered (day 23 preventive and day 45 therapeutic) to the point of sacrifice at end of the experiment (days 66-70). Each open square represents a footpad of normal mice that were not induced for arthritis by collagen injection. Each black diamond (n=28) represents the footpad of a mouse that was induced for arthritis but fed vehicle as control. The triangles represent footpads of experimental mice that were fed NS398, feeding began prior (open triangles, n=38) and after (grey triangles, n=48) symptom manifestation. The black bars indicate the average value in each group. Change in thickness was not observed in normal controls that had not been induced for arthritis (open squares). The results are from a total of three independent experimental repeats. The groups were compared using the <u>Kauskal-Wallis method, and $p < 0.05$</u> is considered significant. Panel [B] shows the relative levels of TNF and IL-17 mRNA in the footpads from each treatment group. The footpads were harvested at days 66-70 and real-time RT-PCR analyses were performed as described in legend to Fig. 3. The data shown are the mean ± SEM from 4–6 mice and representative of three experimental repeats. Statistical analysis by ANOVA showed the differences are significant when the NS398-fed groups were compared with the vehicle control group (black filled and assigned the value of 1). $p < 0.05$ is considered significant. [C] <u>Kinetics of pathogenesis when COX2 was blocked</u>. Each line shows the kinetics of pathogenesis in a representative limb. Shown are the change in thickness of the footpad from the vehicle control group (dashed line with open triangles), the footpad from a mouse fed NS398 preventively at day 23 (solid line with filled diamonds) and therapeutically at day 40 (solid line with filled triangles). Panel [D] shows NS398 inhibited production of PGE$_2$. The relative levels of PGE$_2$ in footpads that were harvested at day 70 is shown. Lipid was extracted and ELISA was performed. The data points were averages derived by combining data from three independent repeats. The total number of footpads analyzed were n=24 for normal, n=33 for vehicle controls and n=46 for NS398-fed. Statistic analysis by ANOVA followed by Bonferroni procedure was performed. The *p* values indicated that there was significant difference when the vehicle control group was compared to the normal basal level and when vehicle control group was compared with NS398-fed group. The concentration of PGE$_2$ metabolites before normalization ranged from 600-1900 pg/ml.

NSAID-induced stomach ulcers. Chemically, misoprostol is structurally a prostacyclin although it is also a PGE_1 that binds EP1-4 receptors. Therefore, we studied whether the analog would restore the resolution of inflammation in NS398-treated mice. Mice were induced for arthritis. As inflammation developed, some were fed NS398 so resolution of inflammation was inhibited and others were fed vehicle only so resolution would occur. The group of arthritic mice that were not fed NS398 served as the vehicle controls. The swollen limbs in the NS398 mice were then divided into two subgroups as depicted in Fig. 5. One of the subgroups were injected with misoprostol and the others were injected with PBS.

In Fig. 5, from the right, footpads of normal mice did not show any change in thickness as they were never swollen. The swollen footpads from the mice in the vehicle group resolved naturally (- slope) and those from the NS398 fed mice that did not make PGE_2 failed to resolve (+ slope). However, when PGE was replenished by injection of misoprostol, resolution was restored (- slope). The degree of swelling subsided in 92% of the swollen footpads in the reconstituted group (NS398 + PGE). It is similar to the vehicle control that was not fed NS398, where 86% of footpads resolved naturally. Comparatively, those footpads from NS398 fed mice and were given PBS (NS398 + PBS) had failed to resolve. In this group, 50% of the footpads remained swollen and their thickness increased beyond the inflamed phase. Statistical comparison by the Kauskal-Wallis method verified that the reconstituted group and vehicle control group were similar, but they were different from the group that received PBS instead of misoprostol. Henceforth, the study supported the idea that PGE_2 restores resolution in the NS-398 mice. It also serves as a pilot experiment conducted to lay out a strategy and reconstitution protocol for subsequent mechanistic studies.

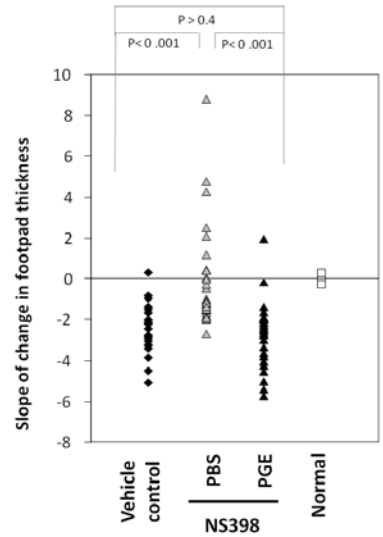

Fig. 5 Loss of PGE_2 with NS398 treatment and restoration of resolution with a PGE analog.

The change in thickness of footpads during the resolution is depicted by the 'slope' calculated by linear regression as described in the text and Fig.3. Each open square represents a footpad of normal mice that were not induced for arthritis by collagen injection. Each black diamond (n=22) represents the footpad of a mouse that was induced for arthritis but fed vehicle. Open triangles represent footpads of experimental mice that were fed NS398, and injected with PBS (n=28) or grey triangle, represent footpads of mice injected with misoprostol (n=28). Change in thickness was not observed in normal controls that had not been induced for arthritis (open squares). The groups were compared using the Kauskal-Wallis method, and $p < 0.05$ is considered significant.

Conclusion:

Therefore, our goal was accomplished. Upon completion of this project, we gained an understanding on how prostaglandins play roles in the inflammation resolving mechanisms. Chronic inflammation, such as arthritis, reduces the quality of life for many individuals. To date, the knowledge on the resolution mechanisms is still at its infancy. By studying the newly discovered machinery of inflammation resolution in murine collagen-induced model, which closely resembles human arthritis in etiology, we hope to deduce a therapeutic strategy for treating arthritis by enhancing resolution. Ultimately, we anticipate the findings may precipitate a paradigm shift in the therapeutic approaches to inflammation disorders, from stopping induction to promoting resolution

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

Note: Studies that fall dramatically short on recruitment are encouraged to provide the details of their recruitment efforts in Item 17, Progress in Achieving Research Goals, Objectives and Aims. For example, the number of eligible subjects approached, the number that refused to participate and the reasons for refusal. Without this information it is difficult to discern whether eligibility criteria were too restrictive or the study simply did not appeal to subjects.

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 or paper submitted for publication, listed in the table, in a PDF version 5.0.5 (or greater) 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. None				☐Submitted ☐Accepted ☐Published

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

Yes_____ No___x____

If yes, please describe your plans:

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.

The study indicates that promoting resolution is a new way for treating inflammation.

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.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Marion M. Chan	Associate Professor
eRA COMMONS USER NAME MCHAN001	

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 Maryland, College Park, MD	B.S.	1975	Microbiology
University of Maryland, College Park, MD	M.A.	1978	Immunology
Cornell University Medical College, NY, NY	Ph.D.	1986	Immunology

Positions and Honors.

Position and Employment

Jun 86 - Sep 88 Post-doctoral Fellow, Laboratory of Dr. Max D. Cooper (Member of the National Academy of Sciences), University of Alabama Medical School, Birmingham, AL.

Oct 88 - Sep 91 Post-doctoral Fellow, Laboratory of Dr. Dunne Fong, Rutgers University, Piscataway, NJ.

Spring 90 and 92 Lecturer, Immunology, Department of Biological Sciences, Rutgers University, Piscataway, NJ.

Oct 91 - Sep 95 Assistant Research Professor, Department of Biological Sciences, Rutgers University, Piscataway, NJ.

Jan 93 -Sep 98 Adjunct Member, Graduate Program in Microbiology and Molecular Genetics, Rutgers University/University of Medicine and Dentistry of New Jersey, New Brunswick, NJ.

Sep 95 - Jun 98 Associate Professor, Pennsylvania College of Podiatric Medicine, Philadelphia, PA.

July 98 - June 03 Assistant Professor, Department of Microbiology & Immunology, Temple University School of Medicine, Philadelphia, PA.

June 03 - Present Associate Professor, Department of Microbiology & Immunology, Temple University School of Medicine, Philadelphia, PA.

Other Experience and Professional Membership

- Member of NIH study sections on grants for "Tropical Medicine Research Center", 95; for "Botanical Drug Interaction", 00; for "Cancer Etiology", 06; for "Arthritis, Connective Tissue and Skin", 07 & 09; for "Clinical Trial Planning Grant, NIAID", 07, 08 & 09; for "Mechanistic Research on CAM Natural Products", 12.
- Grant Reviewer - Inflammatory Bowel Disease Society, Eli and Edythe L. Broad Foundation, 02.
- Grant Reviewer - Research Councils Grant of Hong Kong, 02 - 12.
- Grant Reviewer - U.S. Civilian Research & Development Foundation (CRDF). Targeted Research Competition "Leishmaniasis: Collaborative Research Opportunities in North Africa and the Middle East", 09 &10.
- Member of Editorial Advisory Board for *Biochemical Pharmacology*, 02 - 04; Editorial Review Board of the *Journal of Applied Research in Clinical and Experimental Therapeutics*, 07 - Present.

- Editorial Board for *Journal of Autacoids*, 11 – Present.

Awards and Honors

1. American Society for Microbiology, 33rd Interscience Conference on Antimicrobial Agents and Chemotherapy, Young Investigator Award, 93.
2. Peninsula Laboratories Poster Award, 5th International Symposium and Workshop, Society of Chinese Bioscientists in America, 93.

Selected Peer-reviewed publications. (Total of 47, 35 as first or corresponding author)

1. Chan, M.M., and D. Fong. Edited 2012. Special Issue "The Interplay of PPARs with Parasites and Related Intracellular Pathogens" *PPAR Research.* 2012. Hindawi Publishing Corporation http://www.hindawi.com/journals/ppar/si/540762/
2. Chan, M.M., and D. Fong. 2011. Chapter 28, Anti-inflammatory Therapeutics. In "*Development of Therapeutic Agents Handbook*, First Edition" ed. Gad SC. John Wiley & Sons, Inc., pp. 655-671.
3. Chan, M.M., and Moore, A.R., 2010. Resolution of inflammation in murine autoimmune arthritis is disrupted by cyclooxygenase-2 inhibition and restored by prostaglandin E_2-mediated lipoxin A_4 production. *Journal of Immunology*. 184: 6418-6426. PMID: 20435922.
4. Adapala, N., and M.M. Chan, 2008. Long-term use of an anti-inflammatory, curcumin, suppressed type 1 immunity and exacerbated visceral leishmaniasis in a chronic experimental model. *Laboratory Investigation* 88:1329-1339. PMID:18794851.
5. Chan, M. M., and J.A Mattiacci. 2001. Nitric oxide: Actions and roles in arthritis and diabetes. *The Foot*. 10: 1-7.
6. Chan, M. M., H.-I. Huang, M.R. Fenton and D. Fong. 1998. *In vivo* inhibition of inducible nitric oxide synthase gene expression by curcumin, a cancer preventive natural product with anti-inflammatory properties. *Biochemical Pharmacology.* 55:1955-1962. PMID: 9714315
7. Chan, M. M., N. Tada, S. Kimura, M. K. Hoffmann, R. A. Miller, O. Stutman and U. Hammerling. 1983. Characterization of lymphocyte subsets with monoclonal antibodies: Discovery of a distinct marker Ly-22 of T suppressor cells. *Journal of Immunology*. 130: 2075-2078. PMID: 6187835
8. Chan, M. M., U. Hammerling and O. Stutman. 1988. Ly-10 antigen is a marker of activated T lymphocytes. *Immunogenetics.* 28:425-432. PMID: 2972612.
9. Sowder J. T., C. H. Chen, L. L. Ager, M. M. Chan and M. D. Cooper. 1988. A large subpopulation of avian T cells express a homologue of the mammalian T□□ receptor. *Journal of Experimental Medicine*. 167: 315-322. PMID: 3258004
10. Chan, M. M., C. H. Chen, L. L. Ager and M. D. Cooper. 1988. Identification of the avian homologues of mammalian CD-4 and CD-8 antigens. *Journal of Immunology*. 140: 2133-2138. PMID: 3258329
11. Chan, M. M., and D. Fong. 1988. Expression of human cathepsin B protein in *Escherichia coli. FEBS Letters.* 239:219-222. PMID: 3053249
12. Chan, M. M., and D. Fong. 1990. Inhibition of *Leishmania* but not host macrophages by the antitubulin herbicide trifluralin. *Science*. 249:924-926. PMID: 2392684
13. Chan, M. M., M. Grogl, C-C. Chen, E. J. Bienen and D. Fong. 1993. Herbicides to curb human parasitic infections: *In vitro* and *in vivo* effects of trifluralin on the trypanosomatid protozoans. *Proceedings of the National Academy of Sciences, USA.* 90:5657-5661. PMID: 8516314

14. Chan, M. M., 1993. T cell response in murine *Leishmania mexicana amazonensis* infection: Production of □IFN by CD-8^{+} cells. *European Journal of Immunology*. 23:1181-1184. PMID: 8097471
15. Chan, M.M., and D. Fong. 1994. Anti-inflammatory and cancer preventive immuno-modulation through the diet: The effects of curcumin on T lymphocytes. In "Food Phytochemicals for Cancer Prevention", ed. Huang M.-T. & Ho C.-T., American Chemical Society Press, Washington D.C., pp. 222-230.
16. Chan, M. M., 1995. Inhibition of tumor necrosis factor by curcumin, a phytochemical. *Biochemical Pharmacology*. 49: 1341-1348. PMID: 7786295
17. Chan, M. M., C-T. Ho and H-I. Huang. 1995. Effects of three dietary anti-inflammatory phytochemicals from tea, rosemary and turmeric on nitrite production through the nitric oxide pathway. *Cancer Letters*. 96: 23-29. PMID: 7553604
18. Chan, M. M., D. Fong, C.-T. Ho and H.-I. Huang. 1997. Inhibition of inducible nitric oxide synthase gene expression and enzyme activity by epigallocatechin gallate, a natural product from green tea. *Biochemical Pharmacology*. 54:1281-12. PMID: 9393670
19. Chan, M. M., and D. Fong. 1999. Modulation of the nitric oxide pathway by natural products. In "Nitric Oxide in Inflammation and Tissue Injury", ed. Laskin J. & Laskin D., Marcel Dekker, Inc., New York, NY. pp. 333-351
20. Chan, M. M., J.A. Mattiacci, H.S. Hwang, A. Shah and D. Fong. 2000. Synergy between ethanol and grape polyphenols, quercetin and resveratrol, in the inhibition of inducible nitric oxide synthase pathway. *Biochemical Pharmacology* 60:1539-154 PMID: 11020457
21. Pooyan, S., B. Qiu, M.M. Chan., D. Fong, P.J. Sinko, M.J. Leibowitz, and S. Stein. 2002. Conjugates bearing multiple formyl-methionyl peptides display enhanced binding to but not activation of phagocytic cells. *Bioconjugate Chemistry* 13:216-233. PMID: 11906258
22. Chan, M.M., H.I. Huang, J. A. Mattiacci, and D. Fong. 2003. "Modulation of Cytokine Gene Expression by Curcumin In ", ed. Ho, C.T. In “Food Factors for Disease Prevention and Health Promotion” ed. Shahidi F., Ho C.-T. & S. Watanabe S. American Chemical Society Press, Washington D.C. pp. 86-99
23. Chan, M.M., D. Fong, K.J. Soprano, W.F. Holmes, and H. Heverling. 2003. Inhibition of growth and sensitization to cisplatin-mediated killing of ovarian cancer cells by polyphenolic chemopreventive agents. *Journal of Cellular Physiology*. 194:63-70. PMID: 12447990
24. Chan, M. M., N.S. Adapala, and D. Fong. 2005. Curcumin overcomes the inhibitory effect of nitric oxide on *Leishmania*. *Parasitology Research* 96: 49–56. PMID: 15772867.
25. Chan, M.M., K. J. Soprano, K. Weinstein and D. Fong. 2006. Epigallocatechin-3-gallate delivers hydrogen peroxide to induce death of ovarian cancer cells and enhances cisplatin susceptibility. *Journal of Cellular Physiology* 207:389-396. PMID:16402374.
26. Chan, M.M., K.W Evans, A.R. Moore, and D. Fong, 2010. Peroxisome proliferator-activated receptor (PPAR): balance for survival in parasitic infections. *J Biomed Biotechnol* 2010:(828951) PMID: 20169106
27. Fong, D., A. Yeh. R. Naftalovich, T.H. Choi and M.M. Chan. 2010. Curcumin inhibits the side population (SP) phenotype of the rat C6 glioma cell line: towards targeting of cancer stem cells with phytochemicals. *Cancer Lett.* 293:65-72. PMID: 20089354
28. Chan, M.M., and D. Fong 2012. The Interplay of PPARs with Parasites and Related Intracellular Pathogens. *PPAR Research*.;2012:624845. PMID: 22536211

29. D. Fong and M.M. Chan. 2012. Targeting cancer stem cells with phytochemicals: inhibition of the rat C6 glioma side population by curcumin. In "Stem Cells and Cancer Stem Cells. Volume 1", ed. M.A. Hayat, Springer, New York, pp. 61-68.
30. Chan, M.M., N.S. Adapala and C. Chen. 2012. Peroxisome Proliferator-Activated Receptor-γ-Mediated Polarization of Macrophages in Leishmania Infection. *PPAR Research.* 2012:796235 PMID: 22448168