AWARD NUMBER: W81XWH-18-1-0717

TITLE: Peptidylarginine Deiminase 2 and Citrullination of IgG in Immunity and Rheumatoid Arthritis

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CONTRACTING ORGANIZATION: University of Wisconsin System**,** Madison, WI

REPORT DATE: October 2020

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
October 2020	Annual	30Sep2019-29Sep2020

4. TITLE AND SUBTITLE

Peptidylarginine Deiminase 2 and Citrullination of IgG in Immunity and Rheumatoid Arthritis

5a. CONTRA-CT NUMBER
W81XWH-18-1-0717

5b. GRANT NUMBER
PR170847

5c. PROGRAM ELEMENT NUMBER

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

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MADISON WI 53715-1218

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

The purpose of this application is to identify mechanisms by which peptidylarginine deiminases (PADs) and citrullination regulate antibodies in immunity and rheumatoid arthritis. To this end, this project will (1) determine how PADs and IgG citrullination regulate a normal antibody response to immunization and normal antibody-based immunity to influenza, (2) identify how PADs and citrullinated IgG pathologically contribute to rheumatoid arthritis, and (3) determine if smoking increases IgG citrullination leading to autoimmune antibodies in genetically susceptible people. To date, we have discovered that PAD2 is not required for plasma cell numbers or arthritis severity in collagen-induced murine arthritis, but is required for anti-collagen IgG levels. Further, PAD2 is required for some antibodies formed in response to murine influenza. Experiments are ongoing to further understand these findings as well as to evaluate citrullination in autoimmune antibodies in humans.

15. SUBJECT TERMS

Rheumatoid arthritis, Antibodies, Anti-citrullinated protein antibodies, Rheumatoid factor, Citrullination, Peptidylarginine Deiminase 2

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	15	19b. TELEPHONE NUMBER *(include area code)*

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1. INTRODUCTION:

Rheumatoid arthritis, an autoimmune arthritis with a lifetime risk of about 3 percent, can lead to pain, disability, and early mortality despite lifelong treatment. Moreover, many of the treatments are unpleasant to inject and extremely costly. With about 1 percent of Veterans affected by rheumatoid arthritis often costing more than $15,000 per year, this is a major problem. Many people with rheumatoid arthritis produce antibodies against immunoglobulin (Ig) G, called rheumatoid factor (RF), and anti-citrullinated protein antibodies (ACPAs). These autoantibodies underpin the main diagnostic tests for rheumatoid arthritis. Unfortunately, about 25% of rheumatoid arthritis patients are seronegative for these tests, which delays diagnosis and treatment. In addition to these clinical dilemmas in rheumatoid arthritis, important pathophysiologic mysteries remain. Despite decades of research on ACPAs and RF, why these two different types of autoantibodies develop or why immune tolerance is broken against IgG is unknown. Further, the peptidylarginine deiminases (PADs) catalyze citrullination, the post-translational conversion of arginines to citrullines, and PAD2 and PAD4 are found in immune cells. However, our understanding of how citrullination and PADs regulate immunity and arthritis beyond simply generating the targets for ACPAs is rudimentary at best. Identifying the mechanisms by which the PADs and citrullination impact the immune system is critical to define fundamental pathways in immunity and aberrant pathways in rheumatoid arthritis. Moreover, gaps in our understanding of pathophysiology hinder the development of optimal diagnostics and treatments. The objective of this application is to identify mechanisms by which PADs and citrullination regulate antibodies in immunity and rheumatoid arthritis. The central hypothesis is that PAD2 regulates antibody-secreting plasma cells and citrullinates IgG, enhancing immunity and exacerbating rheumatoid arthritis. To test this hypothesis, Aim 1 will determine how PADs and IgG citrullination regulate a normal antibody response to immunization and normal antibody-based immunity to influenza. Aim 2 will identify how PADs and citrullinated IgG pathologically contribute to rheumatoid arthritis as well as determine how smoking, a major problem among Veterans, may increase IgG citrullination leading to autoimmune antibodies in genetically susceptible people. The successful completion of these Aims, in the short term, will establish a new mechanistic basis for how PAD2 and IgG citrullination regulate immunity and drive inflammation through immune cell function and citrullinated antigen generation. In the long term, these advances will usher in new translational opportunities to innovate diagnostics incorporating novel autoantibodies and therapeutics targeting the PADs ultimately to allow for faster diagnosis and more effective treatment of rheumatoid arthritis.

2. KEYWORDS:

Rheumatoid arthritis
Antibodies
Anti-citrullinated protein antibodies
Rheumatoid factor
Citrullination
Peptidylarginine Deiminase 2

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Major Task 1: Obtain appropriate approvals
- Target completion Date: January 31, 2019
- Completed: April 19, 2019

Major Task 2: Determine which arginines in IgG are citrullinated by PAD2 to extend IgG half-life.
- Target completion Date: September 29, 2021
- Percent Completed: 15%

Major Task 3: Determine how PAD2 regulates plasma cell numbers.

- Target completion Date: January 31, 2021
- Percent Completed: 50%

Major Task 4: Define the role of PAD2 in antibody-based immunity to influenza.
- Target completion Date: September 29, 2021
- Percent Completed: 50%

Major Task 5: Define the pathologic role of PAD2 in autoantibody-dependent inflammatory arthritis.
- Target completion Date: September 29, 2021
- Percent Completed: 60%

Major Task 6: Determine how anti-citrullinated IgG antibodies develop including the role of smoking and HLA variants.
- Target completion Date: September 29, 2021
- Percent Completed: 15%

Major Task 7: Prepare/publish manuscripts
- Target completion Date: September 29, 2021
- Percent Completed: 30%

What was accomplished under these goals?

Major Task 1:
1. Specific Objectives: Obtain appropriate approvals
2. Major activities: ACURO protocol submitted and approved. HRPO protocol submitted and approved.
3. Significant results: All protocols approved.
4. Other achievements: None
5. Goals not met: None

Major Task 2:
1. Specific Objectives: Determine which arginines in IgG are citrullinated by PAD2 to extend IgG half-life.
2. Major activities:
    a. Mass spectrometry optimization and identification of some citrullines of murine IgG. Methods were optimized for IgG purification from PAD2 WT and KO mice and human subjects **without** protein G, which we determined to be a potential issue in a previous reporting period. Project was switched from Dr Herbert and Dr Coon to Bin Wang in Dr Li's lab to use their new mass spec methodology in January, 2020. Work began but had limited success and was stopped in part due to COVID-19.
    b. As part of the experiments to rescue IgG half-life by in vitro citrullination of IgG with PAD2, we performed additional IgG transfer experiments.
3. Significant results:
    a. Several citrullines were identified.
    b. With the increased sample size in IgG half-life experiments, there was a less apparent difference in IgG half-life between PAD2-/- and WT mice.
4. Other achievements: None
5. Goals not met: Mass spectrometry experiments to identify arginines and citrullines of murine IgG were not completed in the predicted timeframe.

Major Task 3:
1. Specific Objectives: Determine how PAD2 regulates plasma cell numbers.
2. Major activities: Backcrossing to the C57BL/6 background was completed. Flow cytometry was optimized and experiments to determine why plasma cells are reduced in PAD2-/- mice were initiated. To complement the flow cytometry experiments, ELISpot and limiting dilution assays (LDAs) were optimized and experiments performed.

3. Significant results: With a larger sample size than our preliminary data, ELISpot and LDAs showed no difference in plasma cell numbers in immunized PAD2-/- vs WT mice. Similar findings were seen in arthritis (See Task 5)
4. Other achievements: None
5. Goals not met: None

Major Task 4:

1. Specific Objectives: Define the role of PAD2 in antibody-based immunity to influenza.
2. Major activities: Dosing of influenza was optimized for DBA1/J mice and 3 sets of PAD2 WT/KO mice were infected with influenza and challenged with re-infection using a lethal dose. Weights were tracked and sera were collected. Hemagglutination inhibition (HI) assays and anti-hemagglutination (HA) ELISA were performed.
3. Significant results: There was no difference in weight loss (a sign of flu severity) in PAD2 KO vs WT mice in the primary influenza infection. However, there was more weight loss in PAD2 KO mice upon secondary influenza infection (challenge), suggestive of incomplete immunity after the primary infection. Further, PAD2 KO mice had lower HI titers. Interestingly, anti-HA IgG titers were normal, but anti-HA IgM titers were reduced in PAD2 KO mice. IgA titers also appeared reduced in PAD2 KO mice, but the experiments were underpowered. See below.

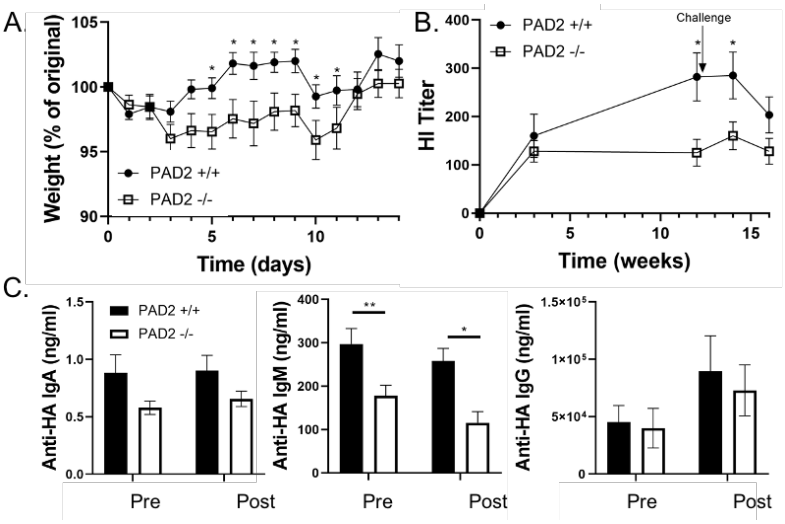


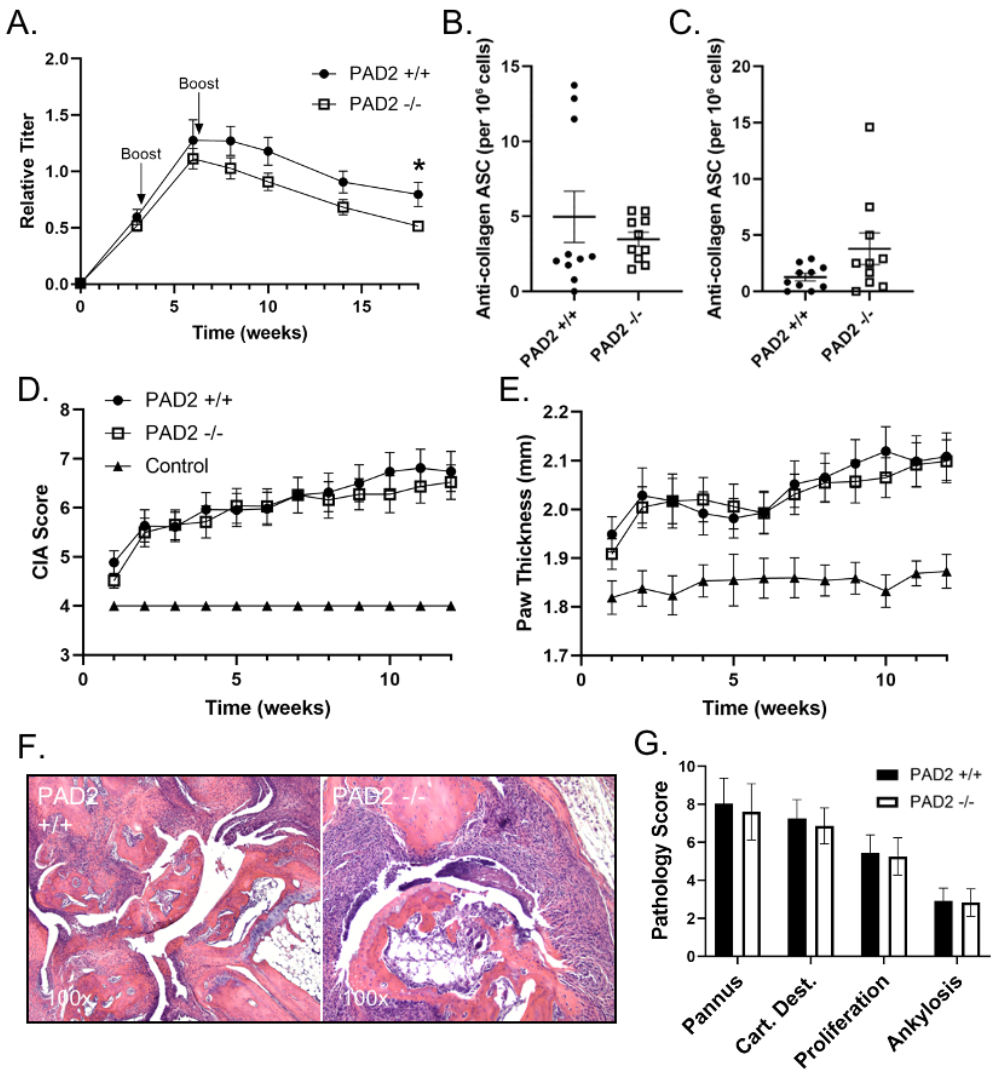
Figure 3. PAD2 is required for normal anti-hemagglutinin (HA) IgM titers after influenza infection and normal recovery after high dose influenza re-challenge. Mice were infected with 10 PFU of PR8-OT1 followed by lethal challenge with 3,000 PFU of PR8 12 weeks later. A. Mouse weights were obtained daily for 14 days after re-challenge and reported as percent of weight on the day of infection (n=11). B. Sera were collected at the indicated time points and used in a hemagglutination inhibition (HI) assay (n=11). Sera from 12 week (pre-challenge) and 14 week (2 weeks post-challenge) time points were used for anti-HA ELISA to detect IgG (n=11), IgM (n=9), and IgA (n=9) that bind to HA. Data are representative of 3 separate experiments. All graphs depict mean ± SEM with *p<0.05, **p<0.01.

4. Other achievements: None.
5. Goals not met: The primary infection studies have not been completed.

Major Task 5:

1. Specific Objectives: Define the pathologic role of PAD2 in autoantibody-dependent inflammatory arthritis.
2. Major activities: Scoring of collagen induced arthritis (CIA) was completed and serum/tissue collected for 23 pairs of mice (9 more pairs than proposed initially). Histological processing and pathological scoring was completed. Anti-collagen ELISA, ELISpot, flow cytometry, and a complementary limiting dilution assay were optimized and performed. ELISA to detect IgG against citrullinated collagen were attempted, but the collagen did not reliably citrullinate, likely due to the low pH needed to keep the collagen in solution. We attempted to perform ELISA against citrulline-containing and native peptides from type II collagen known to be pathogenic in CIA, but these were also unsuccessful to date.

3. Significant results:
    a. Despite the increased sample size, CIA by clinical and pathological score is no different between PAD2 KO and WT mice.
    b. Anti-collagen IgG levels are lower in PAD2 KO than WT mice 12 weeks after CIA induction.
    c. Anti-collagen plasma cell numbers are no different between PAD2 KO and WT mice using either ELISPOT or LDA assays.



In collagen-induced arthritis (CIA), loss of PAD2 causes lower anti-collagen IgG titers, but no loss of anti-collagen antibody secreting cells or reduction in arthritis severity. A. Serum was collected at indicated time points relative to CIA induction (first injection at time 0). IgG levels against collagen were quantified by ELISA and are graphed as a value relative to a serum standard (n=23). Bone marrow derived anti-collagen IgG antibody secreting cells (ASCs) were quantified by (B) enzyme-linked immunospot (ELISpot) and (C) limiting dilution assay 18 weeks after the first collagen injection (n=9). Following the last injection to induce CIA, (D) clinical scores and (E) paw thickness were measured at all 4 paws and averaged to create a single value for each mouse at each time point (n=23). Controls (n=9), which were scored and measured as above, received no injections and were co-housed with experimental mice. At 18 weeks after the first injection, front paws were fixed, embedded, sectioned, and stained with hematoxylin and eosin. F. Representative images are shown. G. The extent of pannus development, cartilage destruction, bony proliferation, and ankylosis were scored in a blinded manner (n=23). Data are representative of 6 separate experiments. All graphs depict mean ± SEM with *$p<0.05$.

4. Other achievements: None.
5. Goals not met: Some anti-collagen ELISAs were not completed.

Major Task 6:

1. Specific Objectives: Determine how anti-citrullinated IgG antibodies develop including the role for smoking and HLA variants.

2. Major activities: Mass spectrometry was initiated and is being optimized as discussed above. Experiments to identify the different epitopes of anti-IgG antibodies in clinical subsets have been initiated. Based on limited array data, a library of peptides derived from IgG has been selected, ordered, and optimized in ELISA. We began to test subjects from different clinical groups using these peptides. Early data suggest that different epitopes are bound by different individuals.
3. Significant results: None.
4. Other achievements: None.
5. Goals not met:
    a. HLA typing has not been completed.
    b. Correlation between HLA type and IgG peptides bound by IgG has not been determined.

Major Task 7:

1. Specific Objectives: Publish manuscripts
2. Major activities:
    a. An invited editorial was written and accepted for publication that includes many of Dr. Shelef's theories described in the grant application that led to the experiments in this project. (Shelef. Arthritis Rheum. 2019)
    b. A manuscript that contains a small amount of data funded by this award related to anti-citrullinated IgG antibodies was accepted for publication. (Zheng et al, Arthritis Rheum. 2020)
    c. A manuscript about the murine studies has been initiated.
    d. A manuscript about one of Zihao Zheng and Michael Newton's new statistical methods has been written and will soon be submitted for publication.
3. Significant results: Publication
4. Other achievements: None

What opportunities for training and professional development has the project provided?

All trainees and Michael Denny received one-on-one mentoring approximately weekly and have attended seminars at the UW.

How were the results disseminated to communities of interest?

Nothing to report.

What do you plan to do during the next reporting period to accomplish the goals?

Major Task 1: Completed

- Nothing to do.

Major Task 2: Determine which arginines in IgG are citrullinated by PAD2 to extend IgG half-life.

- Optimize mass spectrometry and identify citrullines in IgG either with Dr Lingjun Li and her trainees or a new collaborator such as Dr Jennifer Van Eyk or Dr Eranthie Weerapana, who have extensive experience in detecting citrullinated proteins, but are not at UW.
- We will continue to investigate the difference in IgG half-life in PAD2-/- vs WT mice to understand this phenomenon prior to continuing with PAD2 rescue experiments and mutation studies. Mass spec will help with this problem.

Major Task 3: Determine how PAD2 regulates plasma cell numbers.

- Since plasma cell numbers were unaltered in the absence of PAD2, we will no longer plan to perform experiments to assess survival vs formation of plasma cells (SubTasks 1 and 3). We will continue to explore why IgG levels are lower, potentially through the originally proposed gene expression analysis of plasma cells (SubTask 2) as well as through revealing the role of PAD2 in macrophages (see below).

Major Task 4: Define the role of PAD2 in antibody-based immunity to influenza.

- More mice will be infected with influenza to better understand the abnormal antibody response and reduced immunity in PAD2 KO mice.
- HI assays and HA ELISAs will be performed to quantify antibody response to flu in PAD2-/- and PAD2+/+ mice.
- Serum transfer experiments will be initiated.

Major Task 5: Define the pathologic role of PAD2 in autoantibody-dependent inflammatory arthritis.

- Given the anti-HA IgM results in flu experiments, ELISA will be performed to quantify anti-collagen IgM levels in PAD2-/- and PAD2+/+ mice with CIA. Also, we will continue to work on anti-citrullinated collagen ELISA.
- IgG will be evaluated by mass spectrometry.
- According to the original plan, we would induce CIA in more mice to use the sera from PAD2 KO and WT mice to induce arthritis in naïve mice to evaluate a potentially pathogenic role of PAD2 in arthritis-inducing IgG. However, such experiments may not be worthwhile since PAD2 KO mice with CIA have lower anti-collagen IgG levels, but no reduction in arthritis. Thus, the role of PAD2 in antibodies may not affect CIA severity. We will continue to consider whether or not these experiments will be helpful.

Major Task 6: Determine how anti-citrullinated IgG antibodies develop including the role for smoking and HLA variants.

- Identify citrullines in purified IgG from clinical subsets by mass spectrometry
- Identify specific epitopes bound by anti-IgG antibodies in clinical subsets including smokers and lupus.
- Determine which HLA types bind which IgG peptides bound by IgG

Major Task 7: Prepare manuscripts

- We are starting to write up murine findings for IgG levels, plasma cells, CIA, and influenza.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

Nothing to Report. Almost all data is still preliminary.

What was the impact on other disciplines?

Nothing to Report.

What was the impact on technology transfer?

Nothing to Report.

What was the impact on society beyond science and technology?

Nothing to Report.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

There were no significant changes in objectives or scope.

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

Major Task 1: Obtain appropriate approvals

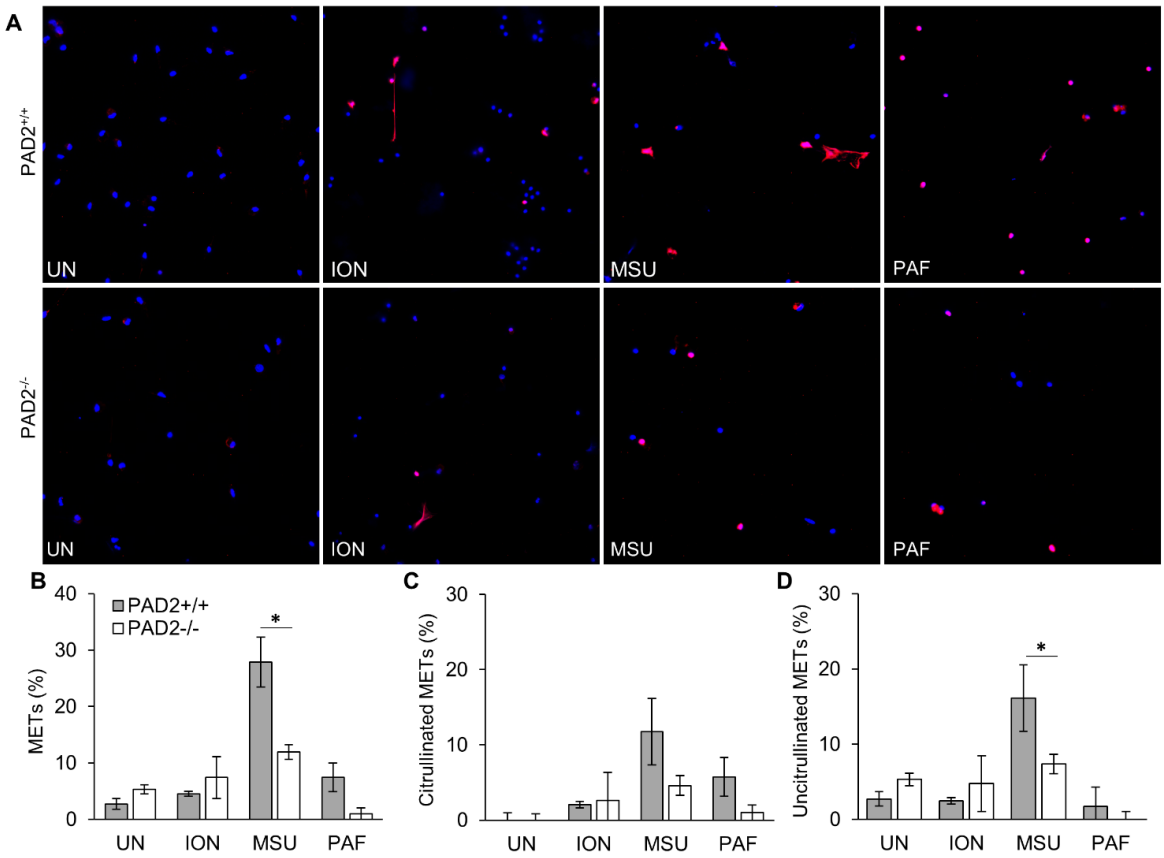
- **Problem/Delay:** HRPO protocol was not approved by the projected time.
- **Action/Plan:** Protocol was ultimately approved and work has been initiated, but some time was lost.

Major Task 2: Determine which arginines in IgG are citrullinated by PAD2 to extend IgG half-life.

- **Problem/delay:** Mass spectrometry experiments to identify arginines and citrullines of murine IgG were not completed with two different collaborative groups.
- **Action/Plan:** If these experiments continue to be unsuccessful with Dr Li's group, we will contact Dr Eranthie Weerapana, with whom we have published in the past, or Dr Jennifer Van Eyk about collaborating on this project given their expertise in detecting citrullines by mass spectrometry.
- **Problem/delay:** As part of the experiments to rescue IgG half-life by in vitro citrullination with PAD2, we performed additional IgG transfer experiments. With the increased sample size, there was a less apparent difference in IgG half-life between PAD2-/- and WT mice.
- **Action/Plan:** We will continue to investigate the difference in IgG half-life in PAD2-/- vs WT mice to understand this phenomenon prior to continuing with PAD2 rescue experiments and mutation studies. We will review all of our studies to identify any methodological or other discrepancies. We will continue to evaluate these data to determine if sample size needs to be increased further or if the differences are not sufficient to warrant further study.

Major Task 3: Determine how PAD2 regulates plasma cell numbers.

- **Problem/delay:** PAD2 does not appear to be required for plasma cell numbers in a response to immunization or CIA (Task 5), just IgG levels.
- **Action/Plan:** In addition to transcription studies on plasma cells to understand why Ig production might be lower, we will focus on how PAD2 in macrophages might be regulating IgG levels unrelated to plasma cell numbers. We selected macrophages for several reasons.
    1. We evaluated T cells in the CIA mice and saw no defect in the absence of PAD2.
    2. We saw a reduction in TNF induced arthritis in PAD2 deficient mice (Bawadekar et al. J Autoimmunity. 2017), a model of rheumatoid arthritis driven by innate immune cells. However, we did not see a reduction in CIA severity in the absence of PAD2 (above), an antibody-driven arthritis (Svensson et al. Clin Exp Immunol. 1998). Therefore, PAD2 is likely to be important in an innate immune cell like neutrophils or macrophages.
    3. Neutrophils are unlikely to be the cell by which PAD2 acts in arthritis since PAD4 is the major PAD expressed in neutrophils (Darrah et al. Ann Rheum Dis. 2012) playing a major functional role in the formation of citrullinated, but not uncitrullinated neutrophil extracellular traps (NETs) (Li et al. JEM. 2010; Holmes et al. J Immunol Res. 2019).
    4. We observed a reduction in joint citrullination, which is thought to be mediated by NETs, in TNF-induced arthritis in PAD2 KO mice (Bawadekar et al. J Autoimmunity. 2017). However, we found that PAD2 was not required for the formation of NETs in response to a variety of stimulants (Holmes et al. J Immunol Res. 2019; Bawadekar et al. J Autoimmunity. 2017), although a subsequent study did find a small role for PAD2 in NETs induced by PMA (Tian et al. JCI Insight. 2020).
    5. PAD2 is found in monocytes and macrophages (Vossenaar et al. Annals Rheum Dis. 2004) and is required for optimal inflammasome assembly, IL-1β release, and pyroptosis (Mishra et al. J Immunol. 2019; Tian et al. JCI Insight. 2020).
    6. We have preliminary data that PAD2 is required for macrophage extracellular trap (MET) formation. See figure below.
    7. Macrophages can regulate memory B cells, plasma cells, and IgG levels (Wu and Banchereau. Frontiers in Immunology. 2014).
- Based on all of these factors, we believe that PAD2 is important in macrophages and METs driving citrullination and antibody levels in inflammatory arthritis. Thus, we believe that PAD2 indirectly regulates plasma cells via its role in macrophages and we will perform experiments to evaluate the role of PAD2 in METs and other macrophage functions.



PAD2 is required for normal MET formation in response to some stimuli. Macrophages from PAD2+/+ and PAD2-/- mice were untreated (UN) or treated with ionomycin (ION), monosodium urate crystals (MSU), or platelet activating factor (PAF), fixed, and stained with DAPI (blue) and anti-citrullinated histone antibody (pink). (A) Representative images at 400x. The number of macrophages and METs were quantified. Graphs depict the average and SEM for percent macrophages that formed total METs (B), citrullinated METs (C), or uncitrullinated METs (D) with percent METs for each stimulant compared for PAD2+/+ versus PAD2-/- mice. For all images: n=3, *p<0.05.

Major Task 4: Define the role of PAD2 in antibody-based immunity to influenza.

- **Problem/delay:** Some mice died during infection reducing sample size. Moreover, we had to limit mouse breeding and we were unable to infect more mice due to COVID-19 from March to June.
- **Action/Plan:** Breeding was re-initiated and more mice were recently infected.

Major Task 5: Define the pathologic role of PAD2 in autoantibody-dependent inflammatory arthritis.

- **Problem/delay:** We discovered that we could not reliably citrullinate type II collagen in vitro and ELISA assays using collagen peptides were unsuccessful so far.
- **Action/Plan:** We will continue to optimize both peptide and protein ELISA assays for collagen type II.

Major Task 6: Determine how anti-citrullinated IgG antibodies develop including the role for smoking and HLA variants.

- **Problem/Delay:** HRPO protocol was not approved by the projected time.
- **Action/Plan:** Work has been initiated, but time was lost.
- **Problem/Delay:** Challenges with mass spectrometry (as above)
- **Action/Plan:** As above.
- **Problem/Delay:** Roche dissolved Nimblegen so new arrays could not be performed.
- **Action/Plan:**
    - Prior to Nimblegen dissolving, we were able to include IgG-derived peptides into a different array for projects unrelated to this award that broadly evaluate antibody binding in lupus and Sjogren's Syndrome. Although these data are less than proposed in this award, we were able to design a library of peptides to dissect the binding patterns of antibodies against IgG in rheumatoid arthritis vs lupus by ELISA, a second method originally proposed to characterize

anti-IgG antibodies in this award. Thus, we expanded the ELISA portion of this Task. Additionally, we will more deeply analyze the different binding patterns to IgG-derived peptides in the available disease group array data using new statistical methods developed by Zihao Zheng (graduate student) and Prof Michael Newton. To date, these individuals have not participated in the current project as proposed, but will start to analyze the arrays in hand with their new statistical methods to differentiate binding patterns to IgG peptides in lupus vs controls vs rheumatoid arthritis.

- **Problem/Delay:** HLA typing initially had not been completed due to the late approval of the HRPO protocol. However, based on our epitope analysis of anti-IgG antibodies by ELISA, we believe that we will need a larger sample size for the epitope analysis and thus for the HLA typing. Thus, we will complete the anti-IgG ELISA experiments prior to the HLA typing in order to determine the correct subjects and correct number of subjects on which to perform HLA typing.
- **Action/Plan:** HLA typing will be completed after anti-IgG experiments.

Major Task 7: Prepare/publish manuscripts

- No delays

All Tasks:

- **Problem/Delay:** From March to June, all work in the lab was stopped except for a single influenza experiment that has already been started due to COVID-19-related restrictions. Additionally, Michael Denny was forced to leave his job due to family responsibilities related to COVID.
- **Action/Plan:** Time at home was optimized with data analysis, manuscript preparation, and planning for the return to the lab. Michael Denny was replaced by Janna Bashar.

Changes that had a significant impact on expenditures

The HRPO protocol was not approved by the projected time and there have been delays due to COVID-19. These delays have delayed spending. These expenditures are expected to be made in subsequent years and thus total expenditures are expected to be unchanged over the whole period of the grant.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Significant changes in use or care of human subjects

No significant changes were made.

Significant changes in use or care of vertebrate animals

No significant changes were made.

Significant changes in use of biohazards and/or select agents

No significant changes.

6. PRODUCTS:

- **Publications, conference papers, and presentations**

Journal publications.

1. **Shelef MA.** New Relationships for Old Autoantibodies in Rheumatoid Arthritis. Arthritis Rheumatol. 2019 Sep;71(9):1396-1399. Federal support acknowledged.

2. Zheng Z, Mergaert AM, Fahmy L, Bawadekar M, Holmes CL, Ong I, Bridges AJ, Newton MA, **Shelef MA.** Disordered antigens and epitope overlap between anti-citrullinated protein antibodies and rheumatoid factor in rheumatoid arthritis. Arthritis Rheumatol. 2020 Feb;72(2):262-272. Federal support acknowledged.

Books or other non-periodical, one-time publications.

None

Other publications, conference papers and presentations.

None.

- **Website(s) or other Internet site(s)**

None.

- **Technologies or techniques**

None. All are still to preliminary to share.

- **Inventions, patent applications, and/or licenses**

None.

- **Other Products**

None.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	*Miriam Shelef*
Project Role:	*PI*
Researcher Identifier (e.g. ORCID ID):	*N/A*
Nearest person month worked:	*4*
Contribution to Project:	*Dr Shelef has been leading the projects, coordinating all scientists, mentoring trainees, reviewing ongoing experiments, participating in data analysis, and writing manuscripts.*
Funding Support:	*UW-Madison, Wisconsin Partnership Program*

Name:	*Marulasiddappa Suresh*
Project Role:	*Co-Investigator*
Researcher Identifier (e.g. ORCID ID):	*N/A*
Nearest person month worked:	*<1*
Contribution to Project:	*Dr Suresh oversees and guides influenza experiments.*
Funding Support:	*NIH, UW-Madison*

Name:	*Aisha Mergaert*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	*N/A*

Nearest person month worked:	*8 (90% effort on this award, but starting 7/1/2020 that effort was primarily cost-shared to a T32 position)*
Contribution to Project:	*Ms. Mergaert has been performing experiments related to CIA, flu, plasma cells, and anti-IgG antibodies.*
Funding Support:	*Hematology NIH T32*

Name:	*Michael Denny*
Project Role:	*Scientist*
Researcher Identifier (e.g. ORCID ID):	*N/A*
Nearest person month worked:	*10 (100% effort, leaving his job due to COVID demands on his family on 7/31/2020)*
Contribution to Project:	*Dr. Denny worked on purifying IgG for mass spec as well as plasma cell and anti-IgG antibody experiments.*
Funding Support:	*None*

Name:	*Janna Bashar*
Project Role:	*Graduate student*
Researcher Identifier (e.g. ORCID ID):	*N/A*
Nearest person month worked:	*4 (70% effort, joining the project on 4/1/2020)*
Contribution to Project:	*Ms. Bashar has been working on CIA, flu, and anti-IgG antibody experiments.*
Funding Support:	*UW-Madison*

Name:	*Bin Wang*
Project Role:	*Graduate student*
Researcher Identifier (e.g. ORCID ID):	*N/A*
Nearest person month worked:	*5*
Contribution to Project:	*Mr. Wang performed and analyzed mass spec experiments.*
Funding Support:	*none*

Name:	*Zihao Zheng*
Project Role:	*Graduate student*
Researcher Identifier (e.g. ORCID ID):	*N/A*
Nearest person month worked:	*1 (100% effort joining 9/1/2020)*
Contribution to Project:	*Mr. Zheng analyzes array data and develops improved statistical methods to do so.*
Funding Support:	*none*

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Miriam Shelef: Additional funds from the Wisconsin Partnership Program and UW have been obtained for COVID-related research. No overlap.

What other organizations were involved as partners?

Nothing to Report.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

N/A

QUAD CHARTS:

Quad chart and generic award chart are included.

APPENDICES:

Publications were included last year.