AWARD NUMBER: W81XWH-19-1-0538

TITLE: Therapeutic Targeting of Pattern Recognition Scavenger Receptor for Treatment of Rheumatoid Arthritis

PRINCIPAL INVESTIGATOR: Xiang-Yang Wang, PhD

CONTRACTING ORGANIZATION: Virginia Commonwealth University, Richmond, VA

REPORT DATE: October 2022

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 2022	Annual	30Sep2021-29Sep2022

4. TITLE AND SUBTITLE

Therapeutic Targeting of Pattern Recognition Scavenger Receptor for Treatment of Rheumatoid Arthritis

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-19-1-0538

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Xiang-Yang Wang, PhD

E-Mail: Xiang-Yang.Wang@vcuhealth.org

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Virginia Commonwealth University
3200 East Leigh St, Suite 3200,
Box 980568
Richmond, VA 23298-0568

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

Rheumatoid arthritis (RA) is a chronic inflammatory disease that causes irreversible joint damage and significant disability. The pathogenesis of RA is often associated with activation of immune cells and osteoclasts that cascade into a vicious cycle of inflammation and bone erosion. The objective of this project is to mechanistically understand scavenger receptor A (SRA), an innate pattern recognition receptor primarily expressed in innate myeloid cells, in promoting excessive inflammation and bone erosion during RA development. Using clinically relevant mouse models, we showed that lack of SRA in mice resulted in reduced inflammation, characterized by decreased IL-1β-expressing monocytic myeloid-derived suppressor cells (M-MDSCs), Th17 cells as well as Th1 cells following induction of collagen-induced arthritis. Additionally, SRA can promote Th17 cell-triggered production of IL-1β by M-MDSCs during the cellular interplays, which does not involve classical inflammasome activation. Furthermore, our proof-of-concept study showed that antibody blockade of SRA on M-MDSCs resulted in potent inhibition of M-MDSC differentiation into osteoclasts that can mediate bone loss. These data reveal a previously underappreciated autoimmune mechanism involving the myeloid cell-lymphocyte cross-talk that is regulated by SRA. Strategically targeting SRA to reduce inflammation and bone erosion may provide a novel approach to the treatment of RA.

15. SUBJECT TERMS
None listed.

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

TABLE OF CONTENTS

1. INTRODUCTION

Rheumatoid arthritis (RA) is a common autoimmune disease that is characterized by chronic inflammation, progressive joint destruction, progressive disability and systemic complications. RA is the most common type of inflammatory arthritis and affects 1% of the US population overall, rising to 2% in those 60 years of age and older. It causes both significant morbidity and increased mortality that is associated with socioeconomic costs. Although the management of RA has advanced significantly, there is still no cure for RA. Treatment for RA is unsatisfactory because some patients do not respond to the existing therapies and certain drugs cause serious side effects. Therefore, it remains an unmet need for more efficient and safe treatment options.

Inflammation is a tightly regulated and complex process that occurs in response to tissue injury or infection. Inflammation is triggered mainly by the recognition of microbes or other 'danger' signals by pattern recognition receptors (PRRs) expressed by innate immune cells (e.g., cells of the myeloid lineages).[1] These myeloid cells and other innate immune cells, together with inflammatory mediators (e.g., IL-1β, IL-17, and prostaglandins) mount an appropriate immune response that is vital to restore tissue homeostasis.[2] While inflammation is a normal physiological response, malfunction of the elements in this system and nonresolving or persistent inflammation can lead to tissue damage and inflammatory disorders, such as RA.[3] There is a close interactive relationship between bone destruction and immune activation in RA.[4,5] Understanding the fundamental mechanisms responsible for abnormal inflammatory cascades and bone absorption in RA and identifying key elements that perpetuate the vicious cycle of these dysregulated processes could provide new opportunities to develop novel therapeutic strategies for disease intervention.

Scavenger receptor A (SRA), also called CD204, is an innate pattern recognition molecule primarily expressed on the cells of myeloid origin. Using a mouse model that mimics human RA, we showed that genetic ablation of SRA renders mice highly resistant to collagen-induced arthritis (CIA), which correlated with reduced levels of MDSCs, IL-17A, and Th17 cells. During this funding period, we aim to determine the SRA effect on M-MDSC enhanced Th17 response and bone erosion well as the underlying mechanisms. These data together with our previous findings indicate that SRA is involved in pro-inflammatory processes of autoimmune arthritis and provide a scientific rationale for targeting SRA to potentially mitigate the symptoms of RA.

2. KEYWORDS

Rheumatoid arthritis, autoimmune inflammation, scavenger receptor A, myeloid derived suppressor cells, Th17 cell, osteoclasts differentiation, bone erosion, TNF-α, inflammasome activation.

3. ACCOMPLISHMENTS

The major goal of the project for the period from Sep. 30th, 2021 through Sep. 29th, 2022 is to determine the SRA effect on M-MDSC enhanced Th17 response and bone erosion as well as the underlying cellular and molecular mechanisms.

Accomplishments during this funding period:

During this funding period, we have examined the pro-inflammatory activity of SRA in autoimmune inflammation and RA progression. We made new findings that monocytic MDSCs (M-MDSCs), not granulocytic MDSCs (G-MDSCs) produced IL-1β in response to activated T cell, which was impaired in the absence of SRA. More importantly, MDSCs from Wild-type (WT) mice were more efficient than those derived from $SRA^{-/-}$ counterparts in producing IL-1β when co-culturing with Th17 cells, which did not require classical inflammasome activation. Inhibition of SRA using monoclonal antibodies impairs the differentiation of MDSCs into OCs.

Specific Aim 1: Determine the functional relevance of SRA in the pathogenic inflammation of autoimmune arthritis using animal models.

Major Task 4: Determine the SRA effect on M-MDSC enhanced Th17 response and arthritic progression.

Subtask 1. Demonstrate SRA-expressing M-MDSCs acquire enhanced capacity to promote a Th17 response after arthritic induction.

Our previous work reported that human MDSCs were significantly elevated in RA patients and correlated with the level of inflammatory cytokine IL-17A.[6] Therefore, we compared M-MDSCs with or without SRA expression from mice with collagen-induced arthritis (CIA) in producing the cytokine IL-1β driven by Th17 cells. Intracellular staining analysis showed that SRA^{+} M-MDSCs were more potent in producing IL-1β as compared with their $SRA^{-/-}$ counterparts (**Fig. 1A**). In line with enhanced activation of M-MDSCs, there were higher frequencies of Th17 cells and Th1 cells in the peripheral blood of WT CIA mice (**Fig. 1B**), suggesting that the correlation between SRA promoted M-MDSC activation and autoimmune inflammation in RA development and that the potential cross-talk between MDSCs and Th17 cells may further amplify the pro-inflammatory phenotype of myeloid cells (i.e., MDSCs).

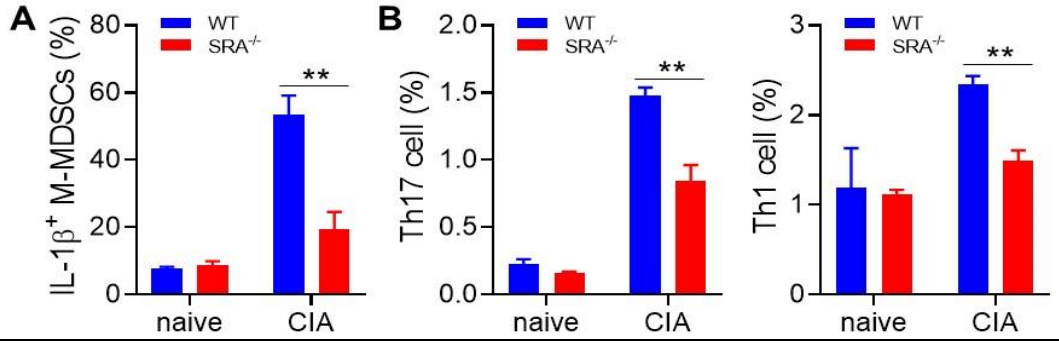

Figure 1. SRA exacerbates autoimmune inflammation in rheumatoid arthritis. CIA was induced in WT and $SRA^{-/-}$ mice by immunization with collagen emulsified in CFA. The frequencies of IL-1β expressing M-MDSCs (**A**), Th17 cells ($CD4^{+}IL\text{-}17A^{+}$) and Th1 cells ($CD4^{+}IFN\text{-}\gamma^{+}$) (**B**) in peripheral blood was determined by intracellular staining and flow cytometry. Cells from naïve mice were used as controls. **, $p < 0.01$.

IL-1β production is triggered by two-step mechanisms. Induction of pro-IL-1β requires activation of the transcription factor nuclear factor (NF)-κB by pattern recognition receptors (PRRs) such as the Toll-like receptors (TLRs).[7] To become biologically active, pro-IL-1β is

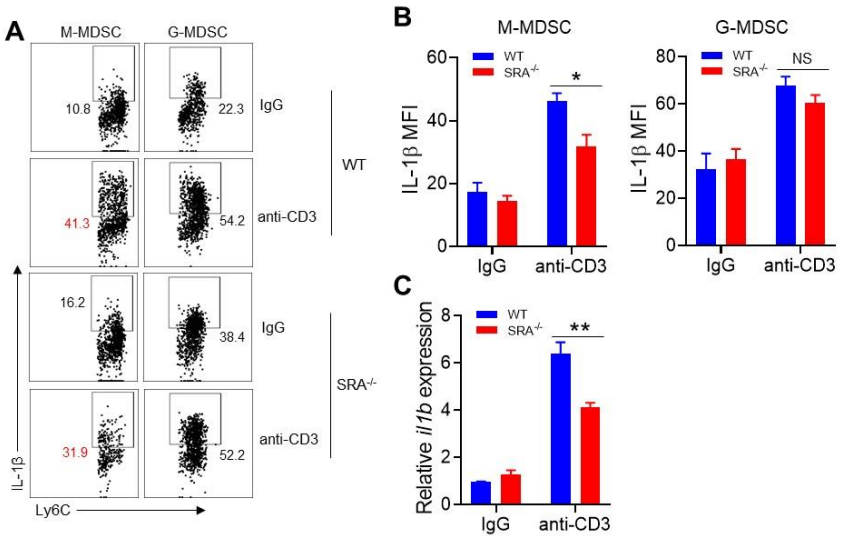

Figure 2. SRA enhances IL-1β expression in response to T cell activation. WT and SRA$^{-/-}$ mice were injected with anti-CD3 mAb (20 μg, i.p.) or control IgG. IL-1β expression in M-MDSC and G-MDSC was determined by intracellular staining and flow cytometry (**A** and **B**) 20 h after antibody injection. Transcription of il1b in the spleen was assayed by realtime PCR (**C**). *, $p < 0.05$. **, $p < 0.01$. NS, not significant.

proteolytic cleavaged following activation of the inflammasomes by damage-associated molecules or microbial virulence factors.[8] It is increasingly acknowleged that bioactive IL-1β production could be attributed to cognate interaction between T cells and myeloid cells in T cell driven autoimmune diseases.[9] To test the hypothesis that SRA facilitates IL-1β production from myeloid cells in response to activated T cell derived signals, we administered anti-CD3 mAb (20 μg, i.p.) in WT and SRA$^{-/-}$ mice. Upregulation of pro-IL-1β protein was detected by intracellular staining and flow cytometry in M-MDSCs (CD11b^{+}Ly6C^{high}Ly6G^{-}) and G-MDSCs (CD11b^{+}Ly6C^{low}Ly6G^{+}) in the spleen 3 hours after administration of anti-CD3 mAb (**Fig. 2A** and **B**). Intriguingly, induction of pro-IL-1β was impaired in SRA$^{-/-}$ M-MDSCs, not in G-MDSCs that do not express SRA (**Fig. 2A** and **B**). Additionally, induction of pro-IL-1β mRNA transcripts in splenocytes was also significantly reduced in SRA$^{-/-}$ mice compared to WT controls 20 hours after anti-CD3 Ab injection. Therefore, these results suggest that SRA absence impairs the compacity of these myeloid cell to produce IL-1β excecebated by activated T cells, thereby attenuating systemic inflammation.

To investigate the SRA activity in governing IL-1β production from M-MDSCs in the presence of Th17 cell-associated autoimmune inflammation, we co-cultured differentiated Th17 cells with M-MDSCs from WT and SRA$^{-/-}$ mice. The expression of pro-IL-1β was readily detected by immunoblotting analysis of the whole-cell lysates after 24 h of co-culture in a Th17 cell number dependent manner, which was reduced in the absence of SRA (**Fig. 3A**). SRA also imparied M-MDSCs-Th17 cell interaction triggered maturational cleavage of caspase 8 in the cell lysates (**Fig. 3A**), which has been reported to cause cleavage of pro-IL-1β in macrophages

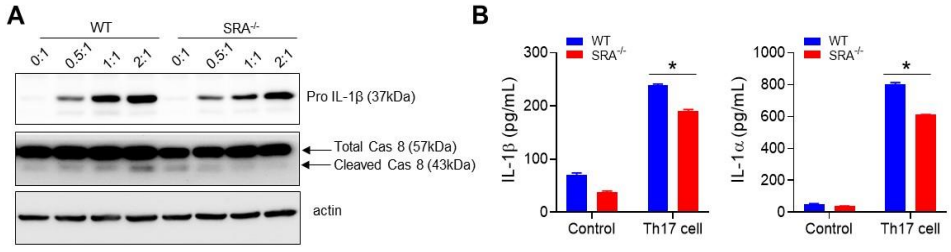

Figure 3. SRA promotes Th17 cell instructed IL-1β production by MDSCs that is independent of inflammasome activation. M-MDSCs from WT and SRA$^{-/-}$ mice were co-cultured with differentiated Th17 cells at the indicated ratios (Th17: M-MDSC) for 24 h followed by examination of pro-IL-1β and caspase 8 cleavage using immunoblotting (**A**). Levels of IL-1β and IL-1α were assayed by ELISA (**B**). *, $p < 0.05$.

and dendritic cells.[9,10] Consistent with the data from immunoblotting analysis, WT M-MDSCs produced higher levels of IL-1β and IL-1α/β in the presence of Th17 cells as compared with SRA$^{-/-}$ M-MDSCs (**Fig. 3B**), indicating that disrution of SRA activity can abrogate the pro-inflammatory phenotype of MDSCs during their interaction with pro-arthritic and pathogenic Th17 cells.

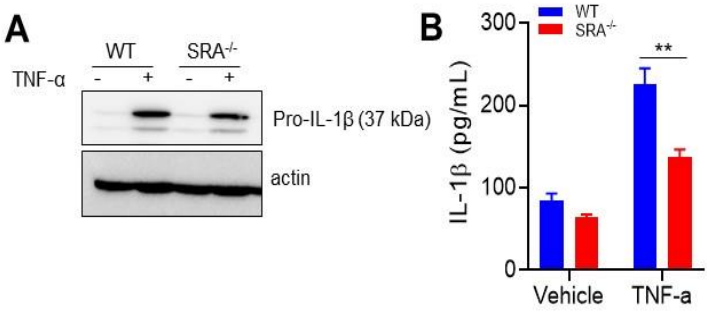

Figure 4. SRA promotes IL-1β production induced by TNF-α in M-MDSCs. M-MDSCs from naïve WT and SRA$^{-/-}$ mice were cultured in the presence TNF-α (50 ng/mL) for 24 h. IL-1β level in the culture media was determined by ELISA (**A**). Pro-IL-1β in the cells was assayed by immunoblotting. **, $p < 0.01$.

We have made efforts to identify the potential signals derived from Th17 cells that induces IL-1β maturation in M-MDSCs. We found that TNF-α, another pro-inflammatory cytokine significantly elevated in SRA WT mice during RA development, stimulated pro-IL-1β expression in M-MDSC lysates as well as secretion of IL-1β into the culture supernatant (**Fig. 4**). While additional studies are necessary to validate the involvement of this cytokine signaling pathway, our data suggest that SRA exhibits unique activity in modulating the cross-talk of this myeloid cell (i.e., M-MDSC)-T cell (i.e., Th17) axis and conseuquent autoimmune inflammation associated with RA. This myeloid-T cell pathway that is governed by SRA provide a new opportunity to develop novel approaches to improved treatment of this disease. We have completed 50% of the proposed work in Specific Aim 1/Major Task 4.

Specific Aim 3: Test the feasibility of targeting SRA in the inflamed joints using SRA monoclonal antibodies to reduce Th17 response and bone erosion.

Major Task 2: Demonstrate the potential therapeutic benefit in attenuation of bone erosion by targeting SRA during arthritis progression.

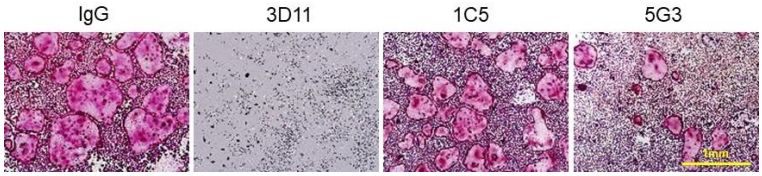

Fig. 5. Inhibition of SRA impairs OC differentiation from MDSCs. MDSCs from WT CIA mice were cultured in the presence of M-CSF (50 ng/mL) for 3 days. Cells were then cultured in the presence of anti-SRA monoclonal antibodies (20 ng/mL, clones 3D11, 1C5 and 5G3) under OC differentiation conditions for additional 6days. OC differentiation was assayed by TRAP staining.

Subtask 1: Determine SRA blocking can reduce the capability of M-MDSCs in differentiation into OCs in vitro.

Our previous work showed that MDSCs can serve as precursors of OCs and lack of SRA on MDSCs resulted in inhibition of OC differentiation. To test the feasibility of targeting SRA to achieve potential therapeutic benefits in treatment of SRA-relevant diseases, we recently generated multiple hybridoma clones (i.e., 3D11, 1C5 and 5G3) that can specifically react with SRA. For evaluation of their biological activities, we cultured CIA-associated MDSCs under OC differentiation conditions for 6 days in the presence of SRA mAbs. It was shown that blockade of SRA substantially impaired OC differentiation from MDSCs, with clone 3D11 being the most effective (**Fig. 5**). This finding suggests that strategically blocking SRA function using monoclonal antibodies against SRA could alleviate bone erosion in arthritic mice. 30% of the work proposed in Specific Aim 3/Major Task 2 has been completed during the past funding period.

REFERENCES

1. Barton, G.M. A calculated response: control of inflammation by the innate immune system. *J Clin Invest* **118**, 413-420 (2008).
2. Nathan, C. & Ding, A. Nonresolving inflammation. *Cell* **140**, 871-882 (2010).
3. Karsdal, M.A.*, et al.* Biochemical markers of ongoing joint damage in rheumatoid arthritis--current and future applications, limitations and opportunities. *Arthritis research & therapy* **13**, 215 (2011).
4. Sato, K. & Takayanagi, H. Osteoclasts, rheumatoid arthritis, and osteoimmunology. *Current opinion in rheumatology* **18**, 419-426 (2006).
5. Boyle, W.J., Simonet, W.S. & Lacey, D.L. Osteoclast differentiation and activation. *Nature* **423**, 337-342 (2003).
6. Guo, C.*, et al.* Myeloid-derived suppressor cells have a proinflammatory role in the pathogenesis of autoimmune arthritis. *Annals of the rheumatic diseases* **75**, 278-285 (2016).
7. Cogswell, J.P.*, et al.* NF-kappa B regulates IL-1 beta transcription through a consensus NF-kappa B binding site and a nonconsensus CRE-like site. *Journal of immunology (Baltimore, Md. : 1950)* **153**, 712-723 (1994).
8. Martinon, F., Burns, K. & Tschopp, J. The Inflammasome: A Molecular Platform Triggering Activation of Inflammatory Caspases and Processing of proIL-β. *Molecular Cell* **10**, 417-426 (2002).
9. Jain, A.*, et al.* T cells instruct myeloid cells to produce inflammasome-independent IL-1β and cause autoimmunity. *Nature Immunology* **21**, 65-74 (2020).
10. Bossaller, L.*, et al.* Cutting edge: FAS (CD95) mediates noncanonical IL-1β and IL-18 maturation via caspase-8 in an RIP3-independent manner. *Journal of immunology (Baltimore, Md. : 1950)* **189**, 5508-5512 (2012).

Opportunities for training and professional development has the project provided:

This funding provided a post-doctoral training opportunity for Dr. Zheng Liu.

The results disseminated to communities of interest:

A manuscript is being prepared for publication.

Plan to do during the next reporting period:

We will continue to investigate the SRA regulated cellular and molecular mechanisms that amplify autoimmune inflammation, which involves cross-talk between myeloid cells and Th17 cells associated with RA. We also plan to test the potential therapeutic activity of anti-SRA antibodies. We will focus on dissecting the relative contribution of myeloid cell subsets in conferring SRA promoted RA progression and autoimmune inflammation.

4. IMPACT

We also provide compelling evidence that SRA, as a pattern recognition receptor, can modulate the interactive communications between pro-inflammatory and pathogenic myeloid cells and lymphocytes during RA development. Given its important role in promoting autoimmune inflammation and osteogenesis that cause bone erosion, strategically targeting SRA may provide a novel approach to the treatment of RA.

The impact on the development of the principal discipline(s) of the project:

Nothing to Report

The impact on other disciplines:

Nothing to Report

The impact on technology transfer:

Nothing to Report

The impact on society beyond science and technology:

Nothing to Report

5. CHANGES/PROBLEMS

Nothing to Report

6. PRODUCTS:

PUBLICATIONS:

Nothing to Report

ABSTRACTS AND PRESENTATIONS:

Nothing to Report

INVENTIONS, PATENT, AND LICENSES

Nothing to Report

OTHER PRODUCTS

Nothing to Report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

Name	Xiang-Yang Wang	Chunqing Guo	Christopher Wise	Zheng Liu	Luiza Bretas
Project Role	Principal Investigator	Co-Investigator	Co-Investigator	Post-doc	Nurse Coordinator
Nearest person month worked	3	2.2	0.6	12	1
Contribution to Project	Oversaw the overall project and executed the research plan. Supervised Dr. Liu to carry out proposed studies.	Work with Dr. Wang to oversee the progress of the project and supervise Dr. Liu to perform the proposed studies.	Work with Dr. Wang for research progress of the project and provide clinical expertise in RA.	Performed work in arthritis induction, histology and pathology analysis of arthritic mice, evaluate autoimmune inflammation in arthritic mice.	Assist Dr. Wise with clinical research involving specimens from patients with RA.
Funding Support	NIH/DOD	NIH/DOD	DOD	DOD	DOD

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

Nothing to Report

What other organizations were involved as partners?

Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

N/A

9. Appendices