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PRINCIPAL INVESTIGATOR: Jeoung-Sook Shin

CONTRACTING ORGANIZATION: University of California San Francisco
San Francisco 94143

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Jeoung-Sook Shin

E-Mail:jeoung-sook.shin@ucsf.edu

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Regents of the University of California
Controller's office
1855 Folsom St STE 420
San Francisco CA 94103-4241

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14. ABSTRACT

Asthma is a serious economic and health concern in the United States. Although multiple controlling medications exist, many of them exert significant side effects while treatment is not sufficiently achieved. Therefore, development of better drugs by identifying new molecular targets is in urgent need. The purpose of this project to is to assess the candidacy of a molecule named MARCH1 as a novel therapeutic target for treatment of asthma. By using a mouse model of asthma, we found that MARCH1 plays a significant role in evoking type 2 T helper cell-driven inflammation in asthmatic airways. We also found that MARCH1 activity can be inhibited by the membrane trans-passing domain of CD83 involving the tyrosine-containing helical face. These findings suggest that one could develop a small molecule inhibitor of MARCH1 by exploiting the CD83 transmembrane domain and utilize this inhibitor as a therapeutic for treatment of asthma.

15. SUBJECT TERMS
Asthma, MARCH1, Inhibitor, Airway, Inflammation, Allergen

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1. INTRODUCTION:

Asthma is a serious economic and health concern in the United States. Although multiple controlling medications exist, many of them exert significant side effects while treatment is not sufficiently achieved. Therefore, development of better drugs by identifying new molecular targets is in urgent need. We have recently found that mice deficient in a protein named membrane-anchored RINC-CH1 (MARCH1) were resistant to developing asthmatic airway inflammation to house dust mite allergens, a major cause of asthma. This novel finding strongly suggests that MARCH1 plays an essential role in the development and possibly exacerbation of asthma. In this application, we aimed to assess the candidacy of MARCH1 as a therapeutic target for treatment of asthma. First, we examined whether ablating MARCH1 expression in mice with established asthma retards progression of the disease. Secondly, we investigated a key structural element of CD83 capable of inhibiting MARCH1.

2. KEYWORDS:

Asthma, MARCH1, inhibitor, airway, inflammation, allergen

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Specific Aim 1: Determine whether MARCH1 ablation ameliorates memory response to allergens.

Milestone - We aimed to find out whether MARCH1 is essential for memory response to allergen by the 13th month of the study – this aim was completed at the 16th month of the study.

Specific Aim 2: Identify a key structural element capable of inhibiting MARCH1.

Milestone - We aimed to define the key structural element of CD83 capable of inhibiting MARCH1 by the 18th month of the study – this aim was completed at the 18th month of the study.

What was accomplished under these goals?

Specific Aim 1: Determine whether MARCH1 ablation ameliorates memory response to allergens.

To test whether activation of memory Th2 cells during allergy recall responses is dependent on MARCH1, we generated mice in which MARCH1 could be deleted in an inducible manner by administering tamoxifen (MARCH1$^{fl/fl}$ UBC$^{ERT2\text{-}Cre}$). These mice and UBC$^{ERT2\text{-}Cre}$ control mice were exposed to house dust mite allergen (HDM), allowed to recover at least three weeks, administered with tamoxifen, and re-exposed to HDM (Fig. 1A). Strikingly, MARCH1$^{fl/fl}$ UBC$^{ERT2\text{-}Cre}$ mice had fewer CD69^{+} Th2 cells in the lungs than the UBC$^{ERT2\text{-}Cre}$ control mice (Fig. 1B). MARCH1$^{fl/fl}$ UBC$^{ERT2\text{-}Cre}$ mice also were less capable of accumulating CD44^{+} Th2 cells in the mLN than the control mice (Fig. 1C). This finding indicates that MARCH1 supports activation of memory Th2 cells both in the lungs and the lymph node. Next, we measured airway hyper-reactivity of the mice to determine whether MARCH1 also supports development of airway-hyperreactivity during memory allergic responses. MARCH1$^{fl/fl}$ UBC$^{ERT2\text{-}Cre}$ mice developed airway hyper-reactivity to a similar degree to UBC$^{ERT2\text{-}Cre}$ control mice (Fig. 1D), indicating that airway hyper-reactivity developing during memory allergic responses is independent of MARCH1. Taken together, **we found MARCH1 plays a significant role in supporting activation of memory Th2 cells in allergic asthma but not for the development of airway-hyperreactivity.** All experimental procedures and protocols were approved by the IACUC and ACURO.

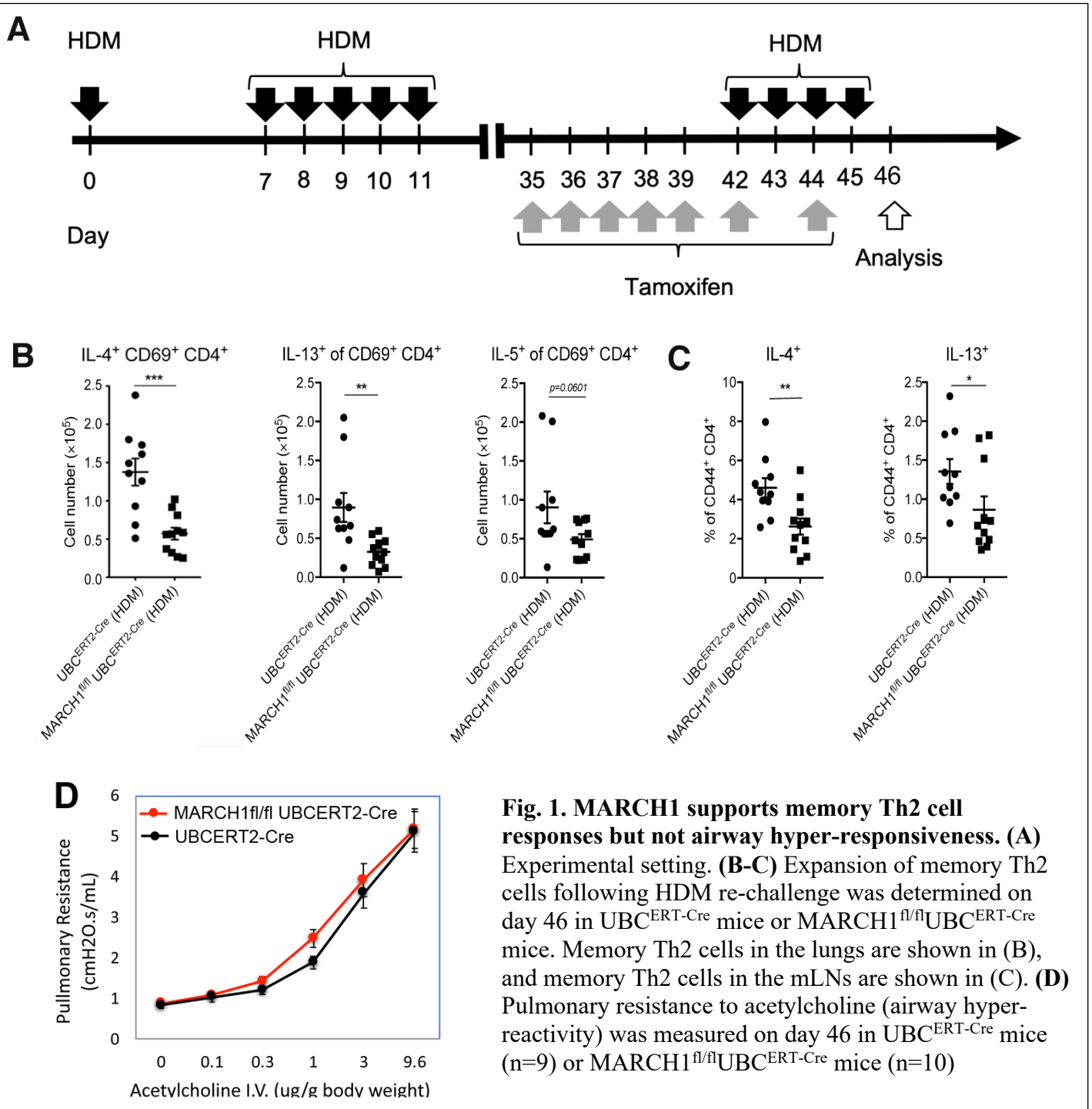


Fig. 1. MARCH1 supports memory Th2 cell responses but not airway hyper-responsiveness. (A) Experimental setting. **(B-C)** Expansion of memory Th2 cells following HDM re-challenge was determined on day 46 in $UBC^{ERT\text{-}Cre}$ mice or $MARCH1^{fl/fl}UBC^{ERT\text{-}Cre}$ mice. Memory Th2 cells in the lungs are shown in (B), and memory Th2 cells in the mLNs are shown in (C). **(D)** Pulmonary resistance to acetylcholine (airway hyper-reactivity) was measured on day 46 in $UBC^{ERT\text{-}Cre}$ mice (n=9) or $MARCH1^{fl/fl}UBC^{ERT\text{-}Cre}$ mice (n=10)

Specific Aim 2: Identify a key structural element capable of inhibiting MARCH1.

CD83 is a membrane protein that binds MARCH1 through its transmembrane (TM) domain. Importantly, CD83 binding inhibits MARCH1 to interact with its substrates, thus acting as a competitive inhibitor. We aimed to determine which face of CD83 TM helical domain binds MARCH1 and interferes MARCH1 interaction with one of its substrates, MHCII. We generated cDNAs that encode a series of CD83 TM helical mutants, each of which had mutation in the nucleotides that encode three amino acids consisting of a given helical face. The DNA nucleotides that encode amino acids smaller than valine in size (alanine, glycine, alanine, serine, threonine, and cysteine) were replaced with the nucleotides that encode the larger amino acid phenylalanine. Vice versa, the nucleotides that encode amino acids equal to or larger than valine (valine, leucine, isoleucine, phenylalanine, tyrosine) were replaced with the nucleotides that encode the smaller amino acid alanine. Each of the generated mutants had one amino acid overlapped with another mutant. In this way, total 9 mutants were generated (Fig. 2).

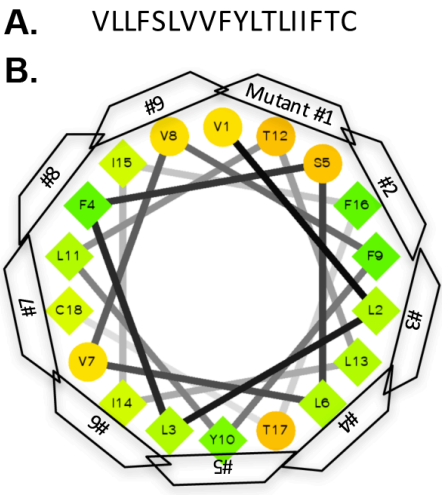
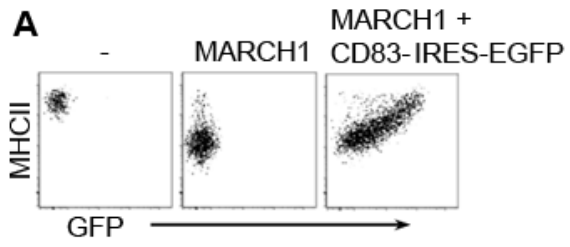
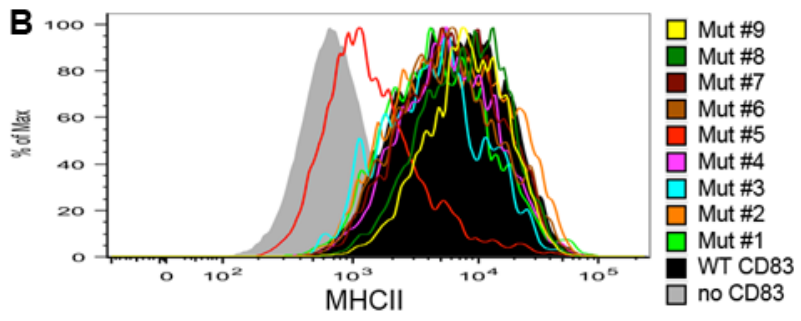


Fig. 2. (A) Amino acid sequence of CD83 transmembrane domain. (B) Helical structure of CD83 transmembrane domain, and 9 helical faces to be mutated (mutant #1~ #9).

To determine which mutant(s) fail(s) to inhibit MARCH1, we first transduced MelJuSo cells, a human melanocyte cell line expressing MHCII, with retrovirus that encodes MARCH1. The transduction resulted in a marked reduction in the surface expression of MHCII, consistently with the role of MARCH1 in ubiquitinating and down-regulating MHCII from cell surface (Fig. 3A). Transduced cells were then subsequently transfected with the cDNA encoding wild type CD83 linked to IRES-GFP. The CD83-transfected cells were readily distinguished by the expression of GFP, and these cells showed a marked increase in the surface level of MHCII compared to the untransfected GFP$^-$ cells (Fig. 3A) indicating that CD83 indeed inhibits MARCH1. Then, we transfected the MARCH1-transduced cells with CD83 TM mutants #1 to #9 and screened for the mutant (s) that failed to increase MHCII. Interestingly, we found that #5 mutant failed to increase MHCII to the degree to which wild type CD86 did (Fig. 3B). All the other mutants increased MHCII as much as wild type CD86 did (Fig. 3B). This finding suggests that the helical face comprised of L3, Y10, and T7 plays an essential role in CD83 binding to and inhibiting of MARCH1. Because tyrosine is not commonly found





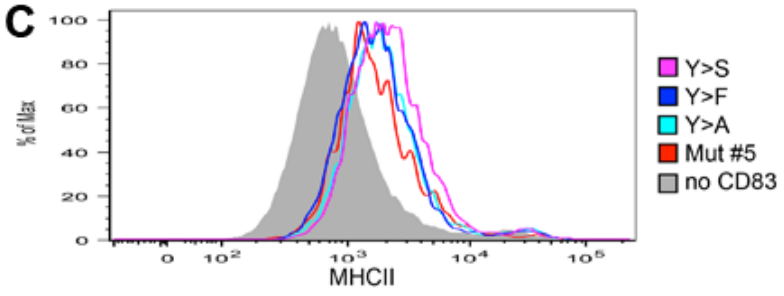


Fig. 3. MARCH1 is antagonized by CD83 involving the single tyrosine residue localized in the middle of transmembrane domain. (A) Flow cytometry of MelJuSo cells transduced with retrovirus encoding mock (-), MARCH1, or MARCH1 together with CD83 linked to IRES-EGFP. **(B-C)** A histogram showing the surface expression of MHCII in MelJuSo cells expressing MARCH1 together with CD83 wild type (WT) or mutants.

in transmembrane domains, we tested whether mutating tyrosine alone could ablate CD83 ability to inhibit MARCH1. The Y>A mutant where the tyrosine (Y) was replaced with alanine (A) behaved similarly to the mutant #5 (Fig. 3C). To test whether the aromatic ring, the hydroxyl group, or both in the tyrosine residue is essential, we made and tested two additional mutants where the tyrosine was replaced with either serine (Y>S) or phenylalanine (Y>F). Both of these mutants behaved similarly to the mutant Y>A (Fig. 3C). Therefore, **we found that the tyrosine localized in the center of the CD83 transmembrane domain plays a crucial role in conferring CD83 with the ability to inhibit MARCH1 and that both the aromatic ring and the hydroxyl group in the tyrosine contribute to this ability.**

What opportunities for training and professional development has the project provided?
This project has provided an opportunity to train the graduate student Carlos Castellanos. Carlos has learned various experimental skills including the development of a mouse model of allergic asthma and immune phenotyping of mice using flow cytometry. Carlos also attended the Annual SACNAS conference and gave an oral presentation about this project.

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?
Nothing to report

4. **IMPACT:**

What was the impact on the development of the principal discipline(s) of the project?
We found that MARCH1 plays a significant role in evoking type 2 T helper cell-driven inflammation in asthmatic airways. We also found that MARCH1 activity can be inhibited by the membrane trans-passing domain of CD83 involving the tyrosine-containing helical face. These findings suggest that one could develop a small molecule inhibitor of MARCH1 by exploiting the CD83 transmembrane domain and utilize this inhibitor to control Th2 cell inflammation associated with allergic asthma.

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
Nothing to report

Actual or anticipated problems or delays and actions or plans to resolve them
Nothing to report

Changes that had a significant impact on expenditures
Nothing to report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Nothing to report

Significant changes in use or care of human subjects
Nothing to report

Significant changes in use or care of vertebrate animals
Nothing to report

Significant changes in use of biohazards and/or select agents
Nothing to report

6. PRODUCTS:

- **Publications, conference papers, and presentations**
  Journal publications.
  Books or other non-periodical, one-time publications.
  Other publications, conference papers and presentations.
  Nothing to report

- **Website(s) or other Internet site(s)**
  Nothing to report

- **Technologies or techniques**
  Nothing to report

- **Inventions, patent applications, and/or licenses**
  Nothing to report

- **Other Products**
  Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	Jeoung-Sook Shin
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	0000-0002-0711-8234
Nearest person month worked:	4.2
Contribution to Project:	Ms. Shin has performed work for the specific Aim 2 and supervised Mr. Castellanos who has worked on the specific Aim 1.
Funding Support:	National Health of Institute

Name:	Carlos Castellanos
Project Role:	Graduate Student
Researcher Identifier (e.g. ORCID ID):	0000-0003-3615-2009
Nearest person month worked:	2
Contribution to Project:	Mr. Castellanos has performed work related to the specific Aim 1.
Funding Support:	The American Association of Immunologists

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

COLLABORATIVE AWARDS:
QUAD CHARTS:
Not applicable

9. APPENDICES:,
Nothing