AWARD NUMBER: W81XWH-18-1-0087

TITLE:
Commensal Bacteria with High Homology to Nonmutated Tumor Antigens May Prevent Clinically Effective Vaccination in Breast Cancer

PRINCIPAL INVESTIGATOR:
Mary L. Disis, MD

CONTRACTING ORGANIZATION:
University of Washington
Seattle, WA, 98195

REPORT DATE: April 2019

TYPE OF REPORT: Annual

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

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
April 2019	Annual	15 Mar 2018 - 14 Mar 2019

4. TITLE AND SUBTITLE
Commensal Bacteria with High Homology to Nonmutated Tumor Antigens May Prevent Clinically Effective Vaccination in Breast Cancer

5a. CONTRACT NUMBER

5b. GRANT NUMBER: W81XWH-18-1-0087

5c. PROGRAM ELEMENT

6. AUTHOR(S)

Mary L. Disis

E-Mail:ndisis@uw.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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

University of Washington
Seattle, WA 98195

8. PERFORMING ORGANIZATION REPORT

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

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

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

We previously found a significant association of numerous sequence homologies to bacterial species found in the gut microbiome with IL-10-inducing Class II epitopes to tumor antigens and hypothesized that those microbial/tumor antigen-specific T-cells will prevent an anti-tumor immune response from developing. Aim 1 is 90% complete. We have demonstrated here that T-cells specific for Pseudomonas aeruginosa and the >50% homologous tumor antigen, YB1, can traffic to an TgMMTV-neu tumor implant. The bacterial and tumor antigen cross-reactive T-cells subsequently promoted tumor growth. These results speak to a potential mechanism as to why whole protein vaccines have been unsuccessful in demonstrating anti-tumor activity in Phase III clinical trials.

15. SUBJECT TERMS
Immunotherapy, Th2, IL-10, commensal bacteria

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		19b. TELEPHONE NUMBER *(include area code)*

Standard Form 298 (Rev. 8-98)

Table of Contents

Page

1. Introduction……...4

2. Keywords…….……..…….....4

3. Accomplishments……..……...…….....4

4. Impact……...…….....7

5. Changes/Problems...…..…….... .8

6. Products, Inventions, Patent Applications, and/or Licenses..…….…….....8

7. Participants & Other Collaborating Organizations…….....8

8. Special Reporting Requirements…….....9

INTRODUCTION: Type I adaptive immunity is needed for cancer eradication. We have recently identified that a major barrier in generating Type I immunity to overexpressed non-mutated tumor antigens is Class II epitopes derived from those antigens selectively eliciting only Th2 responses. We found a significant association of numerous sequence homologies to bacterial species found in the gut microbiome with the IL-10-inducing Class II epitopes. We hypothesize that Th2 specific for commensal bacteria which share significant sequence homology with non-mutated tumor antigens provide a chronically stimulated T-cell memory pool which can rapidly proliferate in response to antigen when the tumor associated self-proteins become aberrantly expressed in cancer. The dominance of IL-10 secreting Th2 will prevent the successful expansion of Th1 and cytotoxic CD8 T-cells needed for tumor eradication.

KEYWORDS: Th2, IL-10, Sequence identity

ACCOMPLISHMENTS:

Specific Aim 1: To determine whether T-cells, specific for both microbial antigens (MA) and non mutated tumor antigens (TA), can traffic to tumor, proliferate, and modify the microenvironment to enhance tumor growth.

Major Task 1: To determine whether MA-TA cross-reactive T-cells traffic to tumor and proliferate

100% COMPLETED

Pseudomonas aeruginosa-specific T-cells were generated from naïve FVB/n mouse spleen. Initially splenocytes were cultured with varying doses of P. aeruginosa lysate (10, 1, 0.1 and 0.01ug/ml) that had been treated to reduce endotoxin. On day 5 of culture, 20ng/ml recombinant murine IL-2 was added. On day 8 of culture, autologous, irradiated splenocytes loaded with P. aeruginosa lysate was added 1:1 to the original T-cell culture. On day 14 of culture, it was determined that 0.1ug/ml lysate would be used for subsequent studies as this culture condition had generated the highest number of viable cells compared to the other conditions.

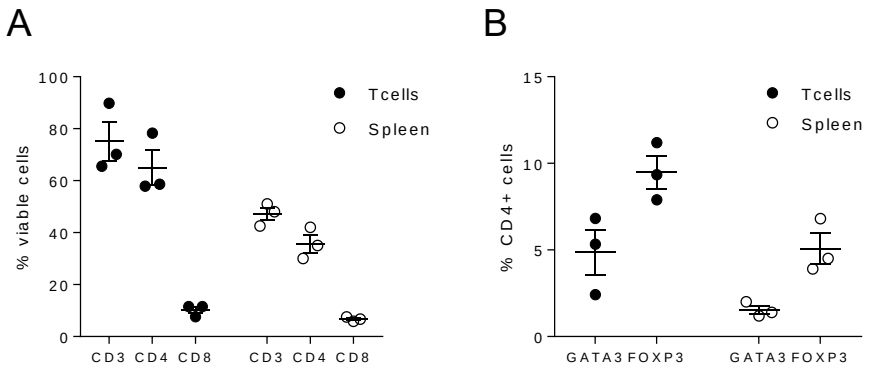

Figure 1. A consistent T-cell line can be generated to Pseudomonas aeruginosa. (A) Mean (±SEM) percentage of the viable cell population for the indictaed T-cell marker. (B) Mean (±SEM) percentage of the CD4+ cell population for the indicated Th marker. n=3 independent cultures.

We next confirmed the reproducibility of the T-cell phenotype generated by expanding three independent cultures. After 14 days in culture, the P. aeruginosa specific T-cells were a mean of 75% (range 66-90%) CD3+ T-cells, which consisted predominantly of CD4+ T-cells (mean 65%, range 58-78% of CD3+), with few CD8+ T-cells (mean 10%; range, 8-12% of CD3+; Fig 1A). Additionally, in the CD4+ T-cell compartment, there was a mean of 5% (range 2-7%) GATA3+ cells and a mean of 10% (range, 8-11% of FOXP3+; Fig 1B). Thus, we concluded that the culture conditions were consistent and reproducible.

P. aeruginosa-specific T-cells were generated as above and on day 14, an IL-10 ELISPOT was performed to asses function (Fig 2). There was a significant IL-10 response from the cells stimulated with the P. aeruginosa lysate ($p<0.0001$). Additionally, an epitope that had been previously shown to selectively secrete IL-10 in human PBMC, YB1 p82-96, and whose amino acid sequence shares >50% identity to P. aeruginosa, induced significant IL-10 secretion, compared to an irrelevant peptide HIVp52-66 ($p=0.002$). Similarly, a significant IL-10 response was observed when P. aeruginosa-specific T-cells were stimulated with recombinant YB1 protein compared to an equivalent dose of an irrelevant protein BSA ($p<0.0001$). Finally, we confirmed that the YB1 peptide and protein were being presented in the context of MHCII since significantly less IL-10 was detected from cells that had been treated with both an MHCII blocking antibody and YB1 p82-96 ($p=0.002$) or rYB1 ($p=0.002$).

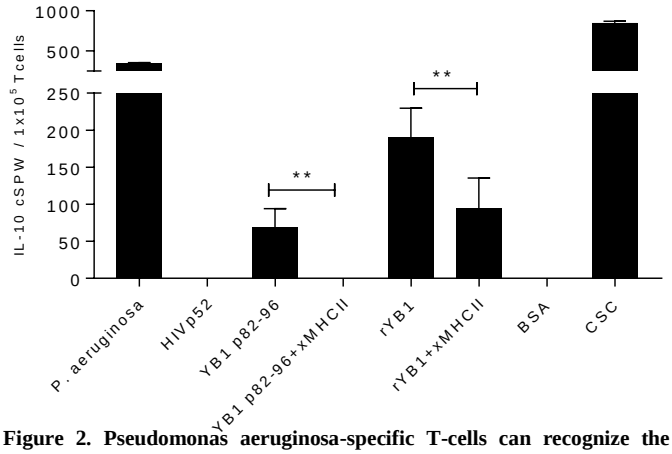

Figure 2. Pseudomonas aeruginosa-specific T-cells can recognize the tumor antigen YB1 via MHCII. Mean (±SD) corrected IL-10 spots per well (cSPW) for P. aeruginosa-specific T-cells treated with the indicated reagent. **p<0.01.

The P. aeruginosa-specific T-cells were labeled with CFSE and stimulated with P. aeruginosa for 1, 3 and 7 days. Evaluation of antigen-induced proliferation was attempted via flow cytometry. Stimulation with P. aeruginosa did not induce proliferation that could be measured with CFSE in P. aeruginosa-specific T-cells, though these cells can actively secrete IL-10 after antigen stimulation as shown in Fig.2. Thus, to address whether P. aeruginosa-specific T-cells could traffic to tumor we employed an alternative approach. The P. aeruginosa-specific T-cells were labeled with XenoLight DiR, a lipophilic, near infrared fluorescent cyanine dye that stains the cytoplasmic membrane without compromising cell function at the recommended concentration. Uptake of the dye was determined via flow cytometry. TgMMTV-neu mice were implanted with a subcutaneous syngeneic tumor cell line. This cell line has been confirmed to express the tumor associated antigen, YB1. The tumor was allowed to grow until it reached 25-75mm^3. P. aeruginosa-specific T-cells or naïve splenocytes were labeled with the XenoLight dye and 1 X 10^7 was injected into the tail vein in each of 4-5 mice tumor-bearing mice. After 72h, mice were fluorescently imaged on a Perkin Elmer IVIS imager and fluorescent output (Total flux, photons/second) was quantified on the Living Image software. P. aeruginosa-specific T-cells

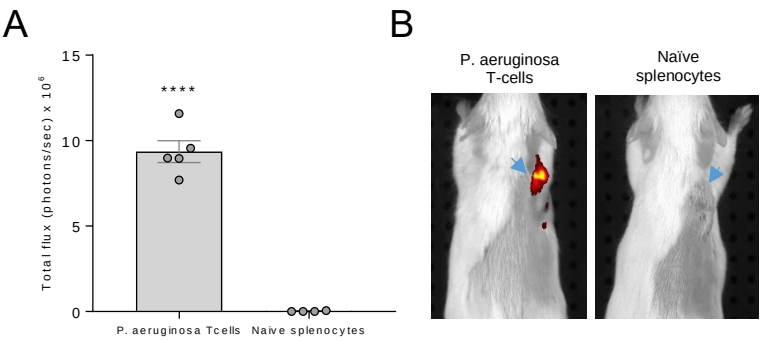

Figure 3. Pseudomonas aeruginosa-specific T-cells home to the tumor in vivo. (A) Mean (±SEM) total flux (photons per second) for the indicated treatment group. N=4-5 mice/group.****p<0.0001. (B) Representative pictures of fluorescence measured in the tumor (arrow head).

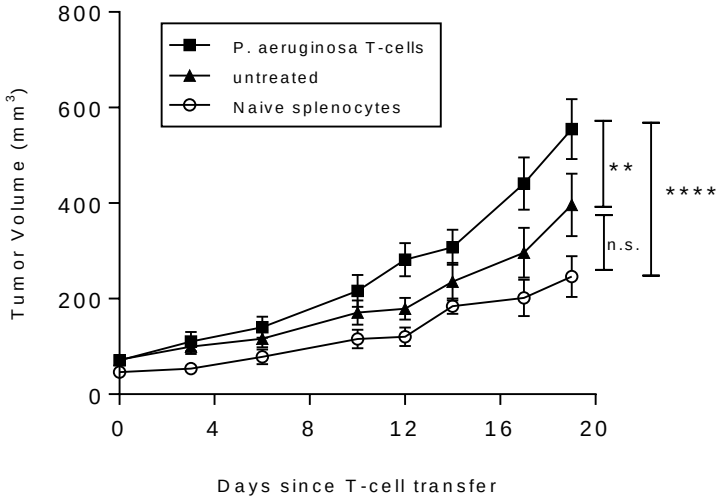

Figure 4. Pseudomonas aeruginosa-specific T-cells promote tumor growth. (A) Mean (±SEM) tumor volume (mm^3) for the indicated treatment group. n=4-5 mice/group.**p<0.01, ****p<0.0001.

were detected in the tumors of 100% of the mice that received the T-cell transfer (Fig 3A and B). Conversely, no cells were observed in the tumors from mice receiving a transfer of naïve splenocytes (Fig 3A and B). This result suggests that the P. aeruginosa-specific T-cells are recognizing tumor associated antigens in vivo.

Major Task 2: To determine whether MA-TA cross-reactive T-cells modify the microenvironment to enhance tumor growth

80% COMPLETED

P. aeruginosa-specific T-cells and naïve splenocytes generated in Task 1 that were not fluorescently labeled were transferred via tail vein into five tumor bearing TgMMTV-neu mice per group. P. aeruginosa-specific T-cells promoted tumor growth. Tumors from mice receiving P. aeruginosa-specific T-cells were significantly larger than tumors from mice receiving naïve splenocytes ($p<0.001$) or untreated mice ($p=0.005$; Fig 4). A fine needle aspiration of tumor was collected the day before and seven days after adoptive transfer. RNA will be isolated from these samples and gene signatures assessed. These data will be presented in the next reporting period.

<u>Specific Aim 2: To evaluate the level of Type I T-cells generated and anti-tumor efficacy elicited after administration of a Th1 selective vaccine or whole protein vaccine in germ free (GF) TgMMTV-neu mice as compared to specific pathogen free (SPF) controls.</u>

Major Task 1: To evaluate the level of Type I T-cells generated after administration of a Th1 selective vaccine or whole protein vaccine in GF TgMMTV-neu mice as compared to SPF controls.

20% COMPLETED

TgMMTV-neu mice to establish the germ free colony were transferred to the Gnotobiotic Animal Core

Major Task 2: To evaluate the anti-tumor efficacy of a Th1 selective vaccine or whole protein vaccine in GF TgMMTV-neu mice as compared to SPF controls

0% COMPLETED

Work on this task has not yet started.

<u>Specific Aim 3: To determine whether gut re-colonization of SPF TgMMTV-neu mice with commensal bacteria that harbor no sequence homology with non-mutated tumor antigens results in enhanced vaccine immunogenicity and efficacy as compared to unmodified mice</u>

Major Task 1: Colonize TgMMTV-neu with specific gut microbial species

0% COMPLETED

Work on this task has not yet started.

Major Task 2: Evaluate the immunogenicity and efficacy of a Th1 selective and whole protein vaccine in microbiome modified and unmodified SPF TgMMTV-neu mice

0% COMPLETED

Work on this task has not yet started.

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

Members of the group attended a one-day seminar "The Microbiome Research Initiative" sponsored by the Fred Hutch Cancer Research Center and a one day microbiome symposium sponsored by The University of Washington Center for Microbiome Sciences & Therapeutics. We were able to learn about new statistical methodologies as well as drawbacks and advantages in different mouse models.

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?

We planned to perform vaccination studies in germ free and SPF mice to evaluate the immune response generated and the impact of tumor development. In addition, we will optimize the colonization of TgMMTV-neu with specific gut microbial species and confirm dominance of the gavaged bacteria.

IMPACT:

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

Whole protein vaccines targeting tumor associated antigens have been unsuccessful in demonstrating anti-tumor activity in Phase III clinical trials. We have previously reported that a potential mechanism for this result are that sections exist in each tumor associated protein that can induce an anti-tumor response or suppress the anti-tumor response. Furthermore, the suppressive sections are more robust in stimulating immune suppressive cells and will rapidly turn off the anti-tumor responses before those responses have begun. Here we suggest that the source of the suppressive response stems from T-cells that are primed by commensal bacteria in a natural tolerance response to self. Once a tumor develops, the commensal-primed T-cells that share significant sequence homology with tumor associated antigens and home directly to the tumor suppressing any anti-tumor response.

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.

CHANGES/PROBLEMS:

Nothing to report in any category.

PRODUCTS:

Nothing to report in any category.

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: Mary L. Disis

Role: Principal Investigator

Nearest person month worked: 5% (0.6 Calendar Months)

Contribution: Dr. Disis is an expert in breast cancer immunology and immunotherapy. She will be responsible for the oversight of project, preparing and submitting manuscripts of project findings, design of experiments in collaboration with the senior scientists on the project, and assurance that the work conducted is within the context of the proposal.

Name: Denise Cecil, PhD

Role: Research Scientist

Nearest person month worked: 14.1% (1.69 Calendar Months)

Contribution: In collaboration with Dr. Disis, Dr. Cecil has been responsible for leading the project, managing the research staff and performing advanced assays. She has expertise in generating antigen-specific mouse T-cell lines for in vitro and vivo work.

Name: Lauren Corulli, MPM

Role: Research Scientist

Nearest person month worked: 2.8% (0.34 Calendar Months)

Contribution: Lauren manages the CVI mouse database, IACUC and ACURO protocol approvals and modifications. Ms. Corulli serves as the project manager of all pre-clinical work, performing project setup and tracking of project and experiment costs. She interacts extensively with the UW Gnotobiotic Facility, planning all germ free study work.

Name: Alex Paynter

Role: Bio-statistical support

Nearest person month worked: 6.8% (0.81 Calendar Months)

Contribution: Alex is responsible for the statistical analysis of all data obtained through animal work and project assays. He also verifies that group sizes are adequate to obtain statistical significance with minimal error.

Name: Noah Simon, PhD

Role: Biostatistician

Nearest person month worked: 3.2% (0.38 Calendar Months)

Contribution: Noah is responsible for overseeing all statistical analysis of all data obtained through animal work and project assays, and manages work performed by Alex Paynter. He also verifies that group sizes are adequate to obtain statistical significance with minimal error.

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.

Other.

SPECIAL REPORTING REQUIREMENTS

Nothing to report in any category.