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 2020

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 2020	Annual	03/15/2019-03/14/2020

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

5a. CONTRACT NUMBER
BC170516

5b. GRANT NUMBER

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)*

Table of Contents

Page

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, reported in the last period.

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

100% COMPLETED

In the last reporting period, we demonstrated that P. aeruginosa-specific T-cells promoted tumor growth in an implant model. We next questioned if P. aeruginosa-specific T-cells could be detected de novo in spontaneous tumors. We allowed tumors to develop in the C3Tag basal breast cancer model to about 500mm^3. Tumor infiltrating lymphocytes (TIL) from dissociated tumors and splenocytes from matched animals were stimulated with P. aeruginosa lysate. We also stimulated with the lysate of two other bacteria (E.coli and M. aurum) which also share a high sequences homology with tumor associated antigens and three bacteria (L. Lactis, L. acidophilus and R. Albus) which do not share any sequence homology with tumor associated antigens. Significantly more IL-10 secreting T-cells were observed after TIL stimulation with the lysate from P. aeruginosa ($p<0.0001$) and E.coli ($p=0.003$) as compared to the levels observed in normal circulation (spleen) (Fig. 1). There was no IL-10 detected in TIL or spleen when the cells were stimulated with L. lactis, L. acidophilus or R. Albus.

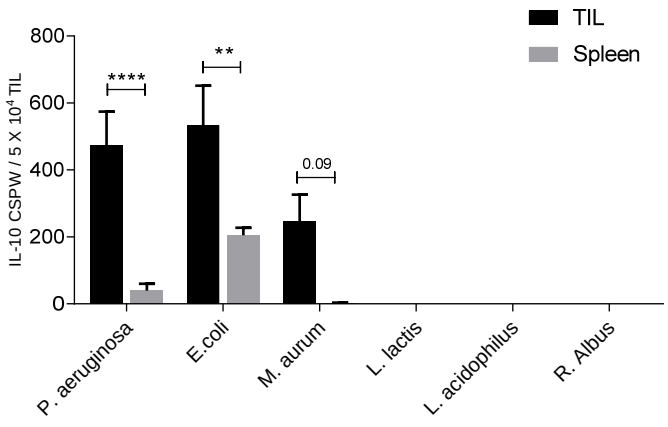

Figure 1. Mean (± SEM) IL-10 CSPW from TIL (black bar) or spleen (gray bar) from C3T mice with spontaneous tumors treated with the indicated bacterial lysate.

We next questioned the Th subtype that that represented the TIL. We hypothesize that the TIL are enriched for gut intraepithelial lymphocytes (IELs) that have migrated to antigen. We have developed a gene signature panel to identify these cells. This gene expression of TIL compared to match IELs and spleen will be reported in the next report.

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.

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.

50% COMPLETED

There is nothing yet to report on this task for this reporting period as the generation of the GF mice took longer than expected. The mice have now been immunized and the results will be reported in the next period.

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

50% COMPLETED

There are no results to report here as stated above.

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

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

50% COMPLETED

SPF TgMMTV-neu mice were treated for one week with vancomycin and metronidazole (0.5mg/kg) in the drinking water. This protocol reduces high frequency species in the gut >90% as assayed via 16s rRNA sequencing. The mice received weekly (for 6 weeks) oral treatments of 1e9 CFU of bacteria. We included the original species as proposed (P. aeruginosa and R. Albus), but based on the result observed in Fig 1, we also combined L. acidophilus with the R. Albus and E.coli with the P. aeruginosa. Stool was collected after the antibiotic and 1 week after the last dose of bacteria and sent for sequencing. Results are pending.

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 is waiting on the pending result as described above.

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

Members of the group have had the opportunity to attend the journal club The University of Washington Center for Microbiome Sciences & Therapeutics.

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 plan 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 evaluate immunogenicity and tumor growth after re-colonization of TgMMTV-neu with specific gut microbial species. A publication of these data is now in preparation.

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: Principle Investigator

Nearest person month worked: 5.3% (0.635 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: 31.3% (3.75 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 in vivo work.

Name: Nicholas Drovetto, MS
Role: Research Scientist
Nearest person month worked: 46.3% (5.55 Calendar Months)
Contribution: In collaboration with Dr. Cecil, Mr. Drovetto has been responsible for performing advanced assays. He has expertise in flow cytometry and cell culture for the in vitro and in vivo work.

Name: Lauren Corulli, MPM
Role: Research Scientist
Nearest person month worked: 7.8% (0.94 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 (GNAC), planning all germ free study work.

Name: Erin Rodmaker
Role: Research Scientist
Nearest person month worked: 11.8% (1.41 Calendar Months)
Contribution: Erin oversees the activity of all animal work. Ms. Rodmaker performs all vaccinations and tumor measurements, oversees weights/cage-side observation activities performed by animal core staff, and schedules all activities and reservations required for animal work within the GNAC.

Name: Ekram Gad, PhD
Role: Research Scientist

Nearest person month worked: 1.7% (0.2 Calendar Months)

Contribution: Ekram performed all vaccinations of SPF animals within the animal core team.

Name: Carissa Pityer

Role: Research Scientist

Nearest person month worked: 11.7% (1.405 Calendar Months)

Contribution: Carissa performs weights/cage-side observation activities, gavages, and fecal sample collections within the animal core team. She directly assists Ms. Rodmaker in all GNAC animal activities.

Name: Catherine Gard

Role: Research Scientist

Nearest person month worked: 1.5% (0.175 Calendar Months)

Contribution: Catherine performed weights/cage-side observation activities and gavages within the animal core team. She directly assisted Ms. Rodmaker in all GNAC animal activities.

Name: Alex Paynter

Role: Bio-statistical support

Nearest person month worked: 3.125% (0.375 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.

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.