

AWARD NUMBER: W81XWH-18-1-0681

TITLE: Regulatory Immune Mechanisms and Gastrointestinal Co-morbidity in ASD

PRINCIPAL INVESTIGATOR: Paul Ashwood

CONTRACTING ORGANIZATION: University of California Davis

REPORT DATE: OCTOBER 2020

TYPE OF REPORT: Annual progress report

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

DISTRIBUTION STATEMENT: Approved for Public Release; Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. **PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.**

1. REPORT DATE OCTOBER 2020			2. REPORT TYPE ANNUAL		3. DATES COVERED 15SEPT2019 - 14SEPT2020	
4. TITLE AND SUBTITLE Regulatory Immune Mechanisms and Gastrointestinal Co-morbidity in ASD					5a. CONTRACT NUMBER AR170145	
					5b. GRANT NUMBER W81XWH-18-1-0681	
					5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Paul Ashwood E-Mail:pashwood@ucdavis.edu					5d. PROJECT NUMBER	
					5e. TASK NUMBER	
					5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) University of California, One Shields Avenue, Davis, CA95616					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)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
13. SUPPLEMENTARY NOTES						
14. ABSTRACT Nearly half of all children with autism spectrum disorders (ASD) also have co morbid gastrointestinal (GI) symptoms that can affect behavior through a brain-immune gut feedback loop. The primary GI condition is due to uncontrolled inflammation. The main objectives of this project are (1) to characterize defects in Tregs function and ability to suppress responses; (2) to identify epigenetic mechanisms that control Tregs lineage commitment; and (3) to determine the stability of Tregs under inflammatory conditions and the Tregs- T helper (TH)17 balance, in children with ASD and GI symptoms. Our main hypothesis is that Tregs dysfunction causes a lack of immune control in children with ASD who experience GI co-morbidities.						
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	Unclassified		7	USAMRMC
Unclassified	Unclassified	Unclassified				19b. TELEPHONE NUMBER (include area code)

TABLE OF CONTENTS

	<u>Page</u>
1. Introduction	5
2. Keywords	5
3. Accomplishments	5-6
4. Impact	6-7
5. Changes/Problems	6
6. Products	7
7. Participants & Other Collaborating Organizations	8
8. Special Reporting Requirements	8
9. Appendices	8

Introduction

The most recent Interagency Autism Coordinating Committee concluded that over half of all children with autism spectrum disorders (ASD) experience gastrointestinal (GI) dysfunction, yet few receive treatment for this. GI problems occur 6-8 times more frequently in ASD than in typically developing (TD) children but the mechanisms underlying GI dysfunction in ASD remain unknown. Regulatory T cells (Tregs) are key mediators of immune tolerance that prevent inappropriate GI inflammation in response to bacteria and other luminal antigens/ components. Our main hypothesis is that Tregs dysfunction causes a lack of immune control in children with ASD who experience GI co-morbidities. The main objectives of this proposal are to characterize defects in Tregs function and ability to suppress responses; to identify epigenetic mechanisms that control Tregs lineage commitment; to determine the stability of Tregs under inflammatory conditions and the Tregs- T helper (Th)17 balance.

Specific Aims/Study Design: Specific Aim 1: To test the hypothesis that mechanisms of action of Tregs are defective in children with ASD who exhibit GI symptoms. Tregs will be isolated from the blood and intestinal mucosal tissue of ASD and TD children with GI symptoms. Tissue samples will be collected during clinically indicated colonoscopy. These Tregs will be analyzed for their ability to suppress immune responses in cocultures, to produce immunosuppressive cytokines, and express intracellular and cell surface molecules associated with regulatory function.

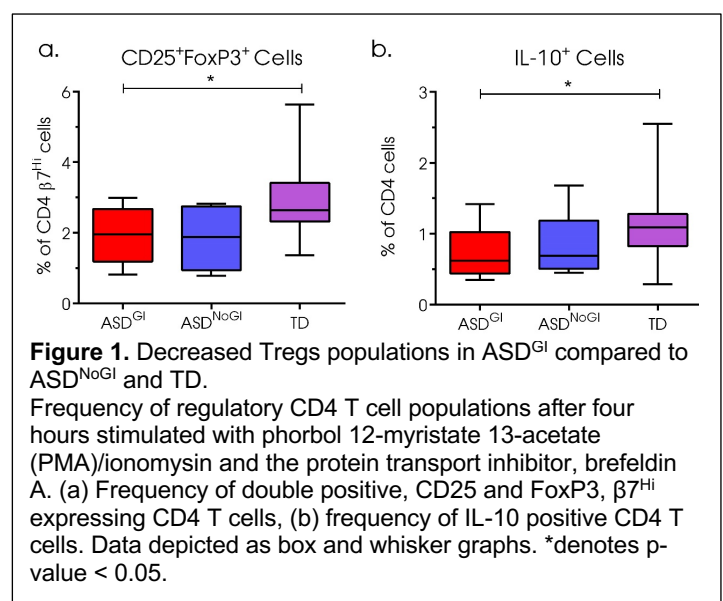
Specific Aim 2: To test the hypothesis that mechanisms determining commitment and/or stability of Tregs in children with ASD who exhibit GI symptoms are altered. Tregs lineage commitment requires the transcription factor forkhead box P3 (FoxP3) and demethylation of the Tregs-specific demethylated region (TSDR). To assess the commitment and stability of Tregs, DNA methylation will be characterized at the TSDR. Tregs lineage instability can cause Tregs plasticity leading to impaired immune tolerance and the development of TH17 cells that produce interleukin (IL)-17, a cytokine recently shown to alter neurodevelopment and lead to ASD-relevant behaviors. The plasticity of Tregs to convert to TH17 cell lineages under inflammatory conditions will be assessed.

10. Keywords

Autism spectrum disorders (ASD), Gastrointestinal (GI), co morbidities, inflammation, immunology, T cells, Lymphocytes, regulatory T cells (Tregs), regulation, immune control.

Accomplishments

Autism spectrum disorders (ASD) are behaviorally defined and affects 1 in 59 children in the United States; however, little is known about its etiology and pathophysiology. GI problems occur 6-8 times more frequently in ASD than in typically developing (TD) children and are associated with more exacerbated behavioral impairments. Although very little is known about the pathophysiological pathways underlying GI problems in ASD. Increased immune activation, pro-inflammatory cytokine production and autoantibodies directed to gut epithelium have been reported in children with ASD and GI symptoms. Regulatory T cells (T_{regs}) are key mediators of peripheral tolerance that maintain their lineage commitment and function



through epigenetic regulation. These T_{regs} are key players that help prevent inappropriate mucosal inflammation in response to bacteria and other luminal antigens/components.

Despite serious limitations to recruitment and laboratory procedures due to the Covid-19 crises we have been able to make progress in several areas. We performed flow cytometry analysis, to examine T_{regs} frequencies and intracellular production of cytokines in T cells. For characterization, isolated T cells were treated with the protein transport inhibitor Brefeldin A for 4 hours in media alone or with the addition of phorbol 12-myristate 13-acetate (PMA) to induce T cell cytokine production. A live/dead stain was also included in order to exclude dead cells. CD3 was used to select for T cells, CD8 was also included to separate out CD4⁺ vs CD8⁺ T cells. We stained for the Treg markers, CD25 and FoxP3, the gut homing integrin β 7, as well as intracellular cytokines IL-10, an immunosuppressive cytokine, and IL-17, a proinflammatory, T_H17-associated cytokine. We found children with ASD and GI symptoms (ASD^{GI}) have decreased regulatory T cell populations. Decreased frequencies of β 7⁺CD25⁺FoxP3⁺ CD4⁺ T cells (Figure 1a) and IL-10⁺CD4⁺ T cells were observed in children with ASD and GI symptoms compared to ASD with no GI issues (ASD^{NoGI}) and TD (Figure 1b; $p < 0.05$). Taken together, these data are suggestive of immune regulation dysfunction in children with ASD with GI problems both systemically as well as in the mucosal compartments. We also found increased populations of CD4⁺IL-17⁺ and CD4⁺IL17⁺IFN γ ⁺ T cells in ASD^{GI} compared to ASD^{NoGI} and TD ($p < 0.05$). IL-17 is the signature cytokine of the T_H17 lineage of T cells. In addition to IL-17, T cells that differentiate into the T_H17 lineage also produce IL-22 and are important for defense against mucosal pathogens but have also been implicated in autoimmune disorders and inflammatory bowel disease illustrating the need for balance and regulation. Taken together, these data suggest an immune imbalance, most likely centered on lack of immune regulation. Moreover, our finding of increased T_H17 T cells in the presence of reduced immune regulation may highlight the delicate balance of immune regulation and the plasticity of regulatory T cells.

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

“Nothing to Report”

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?

To continue to recruit participants which will hopefully improve during the Covid19 crisis. We intend to continue recruitment and basic characterization of T_{regs} cells as outlined in the Aims. We plan to finalize the mechanisms that T_{regs} suppression may be altered in ASD by utilizing the suppression assays and to continue to perform analyses of T_{regs}.

Impact

We seek to fill an important knowledge gap regarding how immune regulation influences GI co-morbidities in ASD. Although we don't yet know whether children with ASD who experience GI symptoms represent a unique sub-group of ASD, GI symptoms could be used for patient stratification and allow us to fast forward targeted therapeutic interventions. We will identify novel immune profiles that could aid in diagnosis of mechanisms underlying GI pathology, develop cell specific targeted therapies, and monitor and predict outcomes of those treatments. Children with ASD with co-occurring GI symptoms are more likely to experience sleep disturbance, anxiety, sudden irritability, unexplained crying, aggressive behavior and score far worse on behavioral assessments than children with ASD without GI symptoms. Our results will improve our understanding of how immune regulation overlaps with patterns of GI and behavioral symptoms. We will be able to follow up the results and determine critical upstream and downstream parameters, such as the effects of the GI microbiota, metabolites, or dietary intervention that will expand our understanding of GI dysfunction in ASD.

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

“Nothing to report”

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

One major and unexpected problem has been the Covid-19 pandemic which has hindered research on several fronts, including but not limited to: 1) limiting access to the laboratory and facilities during lockdown, 2) limited personnel allowed at any one time in the laboratory, 3) closure of core facilities, 4) institutional shut down of research laboratories, 5) limiting clinical hours for assessment and recruitment of human subjects.

Changes in approach and reasons for change

“Nothing to Report”

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

We experienced delay due to Covid19 crisis. As stated above these were in recruitment and access to facilities.

Changes that had a significant impact on expenditures

All personnel are in place for the completion of this project and we do not anticipate any problems with recruiting enough subjects. No changes have occurred that have significantly affected the expenditures. However, we did defray some personnel costs during the crisis. Costs will be charged in the next reporting period from existing funds.

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”

Products

“Nothing to Report”

Participants & Other Collaborating Organizations

Name: Paul Ashwood (principal investigator)

“No change”

Name Janine LaSalle

“No Change”

Name: Daniel Tancredi

“No change”

Name: Arthur de Lorimier

“No Change”

Post doctoral scholar

“No Change”

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

“Nothing to Report”

What other organizations were involved as partners?

“Nothing to Report”

Special Reporting requirements

“Nothing to Report”

Appendices

“Nothing to Report”