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13. SUPPLEMENTARY NOTES

14. ABSTRACT

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. We have previously demonstrated decreased numbers of Tregs and reduced levels of the regulatory cytokines they produce in children with ASD. We hypothesize that these immune regulatory deficits are more severe in children with ASD with GI symptoms. Local IRB has been approved for the study. Tissue processing and the technical protocol has been optimized for evaluation of functional assays of Tregs and epigenetics of lineage commitment and stability. Team development and co-ordination between clinic and laboratory has been optimized to ensure high quality samples are available for assays outlined in the proposal. Protocols for the collection and storage of detailed clinical history and assessments needed for correlative analyses have been developed.

15. SUBJECT TERMS
Autism, gastrointestinal immune mechanisms

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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. We have previously demonstrated decreased numbers of Tregs and reduced levels of the regulatory cytokines they produce in children with ASD. Our new preliminary data shows that these immune regulatory deficits are more severe in children with ASD with GI symptoms.

Hypothesis/objectives: The main objectives of this proposal are (1) to characterize defects in Tregs function and ability to suppress responses; (2) to identify epigenetic mechanisms that control Tregs lineage commitment; (3) to determine the stability of Tregs under inflammatory conditions and the Tregs- T helper (TH)17 balance, and; (4) to probe specific immune regulatory signaling pathways 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.

Keywords

Autism, Autism Spectrum disorders, gastrointestinal, colon, mucosa, T cells, immune, lymphocytes, regulation, regulatory T cells, Tregs, inflammation, stability, co-morbidities, bowel.

Accomplishments

As stated in the SOW the first goal or Major Task 1 was the recruitment of children with ASD and gastrointestinal symptoms, and TD controls with similar symptoms. Major Task 2 and 3 were to initiate functional assays for Tregs cells in the different populations and the analyses of epigenetics markers for Tregs cell lineage commitment respectively. The first aspect of Major Task 1 was to gain local IRB and DoD HRPO approvals in the first year. We successfully received local IRB approval to collect biopsy and blood samples from children with autism and typically developing controls. Even though we received approval we had to go through several rounds of questioning due to the use of invasive colonoscopy techniques and taking of pinch biopsies from children. A concern was the comfort/distress of subjects, and whether there was an increase risk of bleeding, or infections. We were able to answer these questions as taking biopsies are standard practice of care and that although we required 10-12 biopsies this was minimal compared to the size of the colon. However, we were asked to be vigilant that the numbers of biopsies taken was absolutely necessary and to consider this carefully. However, due to a slight miscommunication we have yet to receive full approval from HRPO. This miscommunication stemmed from unfamiliarity with the process. The IRB was initially reviewed at DoD by a contractor who sent us what we believed was confirmation that the IRB had been moved to the appropriate department for review and was approved. However, it transpired that it had not. Currently the IRB is being reviewed at HRPO and we await confirmation. Due to this set back we cannot officially acknowledge any participant data taken under local IRB for the study. Or at least this is our understanding from the notice of award. However, this has given us the opportunity to ensure we have the right team in place and to perform preliminary experiments. For example, we were able to work through many technical issues and create a team and lines of communications that will serve us well once approval is provided.

One major accomplishment has been the development of the team on this project. After many initial discussions between the pediatric gastroenterologist Dr. Art De Lorimier and myself we have developed a team that will help co-ordinate patient consent, sample collection and processing of tissue. There have been many years of collaboration and interactions between he laboratories of Drs Ashwood and LaSalle but we had to develop and integrate a clinical team. To do this we have added clinical coordinators to help with identifying subjects and consent. This will help the work flow enormously and decrease the burden on the scientific staff who can now concentrate solely on the experiments. In addition, we have recruited a clinical developmental pediatrician to help with understanding behavioral and developmental milestones. As IRB at the HRPO was not finalized the team members will be added within existing personnel costs for the next reporting period. The whole team meets regularly once a month in person and once every other week by teleconference to discuss patient details, observations and final diagnoses. Based on this teams work we are re-assured that we will be

able to collect enough participants to achieve the aims of the proposal. Through a series of preliminary experiments, we were able to isolate T lymphocytes and Tregs from biopsy tissues. The number of biopsies were sufficient to provide tissue. Protocols for the collection and storage of detailed clinical history and assessments needed for correlative analyses have been developed.

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 receive notification from HRPO that the IRB has been approved. To continue to recruit participants at a rate of approximately 1-2 week. To perform functional and epigenetic assays on isolated cells from blood and biopsies.

Impact

It cannot be underestimated the impact of creating the right team to be able to recruit the required number of participants for this study. The technical experiments to ensure cells can be isolated that are useable for the experiments has also a major impact on the project.

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

There were no changes in approach during the reporting period.

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 for the IRB approval from HRPO that has affected our recruitment and collection of samples we can attribute to the proposal. The IRB is still under consideration at the HRPO level, but we hope that it will be approved soon. If changes are required, we will do this in a timely and efficient manner.

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 until after the IRB is fully approved. Assembling the team will come through existing costs for personnel and be charged in the next reporting period.

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 and 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”