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TITLE: Macrophage Polarization and Utility of in Vivo Therapy with a Brain-Permeable Anti-TNF Agent in Models of Autism

PRINCIPAL INVESTIGATOR: MariadeLourdes Tansey, PhD

CONTRACTING ORGANIZATION:
Emory University

Atlanta, GA 30322-4250

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6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

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

Emory University
Office of Grants and
Contracts
1599 Clifton RD NE, 4th Floor
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8. PERFORMING ORGANIZATION REPORT NUMBER

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

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Human studies and animal models suggest that maternal immune activation (MIA) can have lasting effects on the offspring's neurodevelopment, immune set points, and behavior. The overall goal of this proposal is to define relevant signaling pathways and identify molecular targets and developmental windows for intervention and treatment.
During this period, we ordered and expanded the TNFR1R2 DKO mice from Jackson labs on site. The mice are breeding and we plan to colonize them with stool of the Taconic C57Bl/6N mice so that they will be responsive to the viral mimetic we plan to use. We began work on Aim 4 because it did not involve use of transgenic mouse colonies and pharmacological studies are underway to interrogate the role of soluble TNF in the MIA model of ASD.

15. SUBJECT TERMS

MIA, MIF, TNF, Gut, Neurodevopment, neuroinflammation, macrophage, IL-17, fetal insult, ASD

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	11	19b. TELEPHONE NUMBER *(include area code)*

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1.INTRODUCTION: Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

Human studies and animal models suggest that maternal immune activation (MIA) can have lasting effects on the offspring's neurodevelopment, immune set points, and behavior. The overall goal of this proposal is to define relevant signaling pathways and identify molecular targets and developmental windows for intervention and treatment.

2.KEYWORDS: Provide a brief list of keywords (limit to 20 words).

MIA, MIF, TNF, Gut, Neurodevopment, neuroinflammation, macrophage, IL-17, fetal insult, ASD

3.ACCOMPLISHMENTS: The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.

What were the major goals of the project?

•Perform MIA model in mice deficient in TNF or MIF signaling
•Assess role of TNF and MIF signaling in neuroinflammation and behavioral dysfunction in the MIA model of ASD
•Assess role of TNF and MIF signaling in modifying expression of immune- and ASD-related genes in the MIA model of ASD
•Investigate the therapeutic efficacy of XPro1595 to attenuate neuroinflammation and ASD-like features in the MIA model of ASD.

What was accomplished under these goals?

Aim 4: As a practical matter this aim is being performed first since it does not require the expansion of transgenic colonies.
Major activities: C57B/l6 (Jackson labs) were co-house with B6 mice obtained from Taconic Farms to increase the likelihood of maternal gut bacteria to promote neurodevelopmental abnormalities in mouse offspring in MIA model. Colonization of the small intestine of mice with segmented filamentous bacterium (present in B6 Taconic mice) induces the production of IL-17 and IL-22 (Th17 cells) in the intestinal lamina propria. In pregnant mice, interleukin-17a produced induces behavioral and cortical abnormalities in the offspring exposed to MIA (Maternal immune activation).

Accomplished:
a. Taconic female mice and C57B/l6 (Jackson labs) were co-housed (2:3) in sterilized cages (4 weeks)
b. Mate: C57B/l6 (Jackson labs)
c. Poly:IC or Saline Injections pregnant dams
d. Behavior tests: Marble burying, open field, Sociability

In Progress:
d. QPCR (Hippocampus and cerebellum): TNF, MIF, CD78, LCN2, CD74, IL-6, MMP9, TNFRSF1B, PTEN, MET, GRIN2B, MECP2, CTNNB1, CUL3, CHD8, TBR1, and SCN2A.
e. Western Blot and qPCR (Small intestine and colon) – gut permeability and inflammation

Aim 4 (Subtask 1)
In progress:
MIA model in C57/Bl6 WT mice Co-housed C57/Bl6 (Jackson labs) with B6 Taconic female mice (4 weeks)
Mate: C57/Bl6 couples (N= 15 females)
Offspring injections with XPro 1595 10 mg/kg s.c. (or saline) starting at PND 2, for 6 weeks.

Specific objectives: The main objective of the aim 4 is to test the ability of XPro®1595 to interrupt pro- neuroinflammation and gut dysfunction in mouse models of ASD during the gestational period and/or in the early postnatal period and ameliorate neurological and behavioral deficits in the offspring. As a practical matter this aim is being performed first since it does not require the expansion of transgenic colonies.

Aim 1 and Aim 2
In progress:
We are establishing and expanding our TNFR1R2 double KO mouse colony to perform the maternal immune activation model in TNF deficient mice. This action will allow us to have to assess role of TNF and MIF signaling in neuroinflammation and behavioral dysfunction in the MIA model of ASD. But because these mice are on the C57Bl/6J genetic background, they need to be colonized with the Taconic

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?
We will continue working through the deliverables and milestones as stated in the original timeline.

4.IMPACT: Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

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

5.CHANGES/PROBLEMS: The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:

Changes in approach and reasons for change
There was one change in practice: we learned from Dr. Daniel Geschwind during his visit to Emory the important fact that C57Bl/6J mice from Taconic but not those from Jackson labs were responsive to the MIA model because the Jackson mice lack a Th17 T-cell response in the gut and do not make IL-17 which signals at receptors in the brain to mediate the autism-like behavior deficits. For this reason, we started with Aim 4 since it did not require the TNF-deficient mice for the studies which will first have to be colonized with the SPF bacteria in the gut of the Taconic mice (in process now).

Actual or anticipated problems or delays and actions or plans to resolve them
If the Jackson WT mice from Taconic (littermates to the TNFR1R2 DKO) mice do not respond to the MIA challenge with sociability deficits, we will not be able to interrogate the role of TNF genetically. But we will still have the pharmacological intervention with XPro1595 which is the more translational path forward**.**

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

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: List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."

- **Publications, conference papers, and presentations**

  Report only the major publication(s) resulting from the work under this award.

  Journal publications.
  Nothing to report

  Books or other non-periodical, one-time publications.
  Nothing to report

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:	**MariadeLourdes Tansey, PhD**
Project Role:	**Principal Investigator**
Researcher Identifier (eRA User ID)	**MTANSE**
Nearest person month worked:	**1**
Contribution to Project	**Dr. Tansey performed oversight of personnel, study design, wrote the report.**
Funding Support:	**N/A**
Name:	**Maria Elizabeth de Sousa Rodrigues, PhD**
Project Role:	**Post Doc**
Researcher Identifier (eRA User ID)	**IZABETEL**
Nearest person month worked:	**2**
Contribution to Project	**Dr. de Sousa Rodrigues participated in studies in Aim 4.**
Funding Support:	**N/A**
Name:	**Valerie Joers, PhD**
Project Role:	**Post Doc**
Researcher Identifier (eRA User ID)	**VLJOERS**
Nearest person month worked:	**7**
Contribution to Project	**Dr. Joers participated in studies in Aim 4.**
Funding Support:	**N/A**
Name:	**Lori Eidson, PhD**
Project Role:	**Post Doc**
Researcher Identifier (eRA User ID)	**LEIDSO2**

Nearest person month worked:	**1**
Contribution to Project	**Dr. Eidson participated in studies in Aim 4.**
Funding Support:	**N/A**
Name:	Yuan Yang
Project Role:	Research Specialist, Sr.
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	**1**
Contribution to Project	**Yuan Yang participated in studies in Aim 4.**
Funding Support:	**N/A**

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

TANSEY, MALÚ G.

Change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period.

PREVIOUS (funding complete since last reporting period)

Coins for Alzheimer's Research Trust Fund (CART) 0.0 calendar (post doc support)
Agency Contact: Bill Shillito, Exec Dir., 823-320-6410 (cell), execdir@cartfund.org
(PI: Tansey, MG/Kim, YT) 5/1/2015 – 4/30/2017 $50,000 Direct Costs
"Advanced CNS Drug Delivery via Lipoprotein-Polymer Nanocomplexes in Experimental Alzheimer's Disease"
The overall goal of this proposal is to engineer a multifunctional nanocomplex that transports a novel anti- inflammatory biologic (XPro®1595) across the BBB and examine its therapeutic properties in a mouse model of AD.
Role: Co-PI

Michael J. Fox Foundation for Parkinson's Research (PI: Tesi, RJ)
0.12 calendar Agency Contact: Allison Morris, Rsch Prog Officer, 212-509-0995, amorris@michaeljfox.org
Emory Subcontract (PI: MG Tansey) 3/1/15-2/28/17 (NCE) $277,788 Direct Costs "*Therapeutic effects of XPro1595 on motor and non-motor outcomes in a chronic progressive model of PD*" The overall aim of this proposal is to investigate the protective effects of systemic administration of the dominant negative TNF inhibitor XPro1595 against non-motor PD-like features in the low-dose chronic MPTP NHP model of PD
Role: PI of subcontract

Michael J. Fox Foundation for Parkinson's Research (PI: Tansey, MG) 1.2 calendar
Agency Contact: Allison Morris, amorris@michaeljfox.org, 212-509-0995
Target Advancement 2/1/2016-1/31/2017 $90,909 Total direct costs *"Modulation of microglia cannabinoid receptor 2 to ameliorate neuroinflammation in Parkinson's disease"* The goal of this application is to investigate the role of microglia CB2 receptors in neuroinflammation in pre-clinical models of PD and the efficacy of novel inverse agonists of CB2 receptors.
Role: PI

1R21NS084647 (PI: Tansey, MG) **0.12 calendar**
Agency Contact: Beth-Anne Sieber, PO; sieberb@ninds.nih.gov; 301-496-5680
NIH/NINDS 2/15/14-12/31/16 (NCE) $150,000 Direct Costs
"*Immunophenotyping of LRRK2 mutation carriers*"
The overall goal of this application is to explore the hypothesis that pathogenic LRRK2 mutations a) disrupt T-cell homeostasis and/or the ability of certain subsets to respond to activation signals and; b) alter monocyte responses to activation.
Role: PI

Craig Neilsen Foundation (PI: Tansey, MG) **0.6 calendar**
Agency Contact: Kim Cerise, Dir. of Grants Mgmt., kim@chnfoundation.org,
Pre-Clinical Pilot Award 7/1/14 – 8/30/16 $150,000 Direct Costs
"XPro1595 to inhibit soluble TNF and modulate inflammation-induced pathology in SCI"
The overall objective of this proposal is to test the hypothesis that soluble Tumor Necrosis Factor (solTNF) is a critical pro-inflammatory cytokine in spinal cord injury (SCI) that promotes demyelination, axonal injury, loss of locomotion, and neuropathic pain. The protective effects of a novel anti-TNF biologic (XPro1595) on injury-induced inflammation, demyelination/axonal injury, and hind limb motor function, and pain signal processing will be evaluated.
Role: PI

Parkinson Disease Foundation (PI: Tansey, MG) **0.12 calendar**
Agency Contact: Beth Vernaleo PhD, Sr. Mgr., Research Programs, 212-923-4700, info@pdf.org
Pilot PDF-IRG-1445 6/1/14-11/30/16 (NCE) $75,000 Direct Costs
"Neuroprotection by XPro1595 in a chronic MPTP monkey model of PD"
The overall goal of this application is to test the neuroprotective effects of peripherally administered XPro1595 on locus coerulus and ventral dopaminergic neurons in an MPTP NHP model of PD.
Role: PI

NEW (since last reporting period)
1R01AG054046-01 (PI: Hu, W) **0.6 calendar**
NIH/NIA 7/1/16-6/30/21 $497,000.00 Direct Costs
"CSF, MRI, and PET biomarkers of neuroinflammation in Alzheimer's disease"
The major goals of this application are to validate and expand a staging and prognostic biomarker panel consisting of CSF inflammatory proteins in early stages of Alzheimer's disease, correlate CSF inflammatory protein changes with molecular imaging characterization of neuroinflammation on PET and MRI, and confirm inflammatory dysregulation through CSF immunophenotyping.
Role: Co-Investigator

USAMRAA DoD AR150035 (Tansey) 09/30/2016-09/28/2018 **1.20 calendar**
Autism Research Program Idea Development Award $181,079 Total Direct Costs
Macrophage polarization and utility of in vivo therapy with a brain permeable anti-TNF agent in models of autism+

The overall goal of this application is to explore the role of MIF and TNF in regulation gene networks in a maternal immune activation model of autism spectrum disorder and the ability of an anti-soluble TNF-selective biologic to ameliorate deficits.
Role: PI

Hospital District of Helsinki and Uusimaa (Tansey) **0.12 calendar**
Research Agreement 02/01/2017- 01/31/2018 $22,000 Direct Costs
Microbiota and the host immune system in Parkinson's Disease
Goal: Specific Aims
1. Identifying connections between the immune response of the host and microbiota community structure in Parkinson's Disease (PD)
2. Looking for different patterns of the immune response to microbiota in healthy controls and PD patients.
3. Studying interactions between microbiota, immune response and host genotype as well as metabolomics profile. Genotype and metabolomics data will be supplied by other labs.

1R01NR014886-02 (PI: Neigh, GN) 05/12/2016-02/28/2018 **0.24 calendar**
Agency Contact: Lois Tully, PO; tullyla@mail.nih.gov; 301-594-5968
Subaward: Virginia Commonwealth Univ. NIH/NINR $38,526 Tansey Annual Direct Costs
"*Adult Implications of Chronic Adolescent Stress: Mediators and Modifiers*"
The overall goal of this application is to explore the extent to which chronic adolescent stress (CAS) alters adult regulation and function of the transcription factors: glucocorticoid receptor (GR) and nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) and how these are distinctly altered in males and females.
Role: Collaborator

1R56NS099092-01A1 (Alvarez) 09/01/2017 – 08/31/2018 **0.36 calendar**
NIH/NINDS $244,510 Total Direct Costs
Spinal cord neuroinflammation and synaptic plasticity after peripheral nerve injury
The proposed work will study the role of neuroinflammation in spinal cord synaptic plasticity after nerve injuries.
Role: Co-Investigator

1RF1AG057247-01 (MPI Tansey (contact)/Neish) 09/15/2017- 06/30/2022 **1.20 calendar**
NIH/NIA $1,025,000 Total Direct Costs (MYF)
Role of Gut Inflammation and Immunity on Proteostasis, Noradrenergic Degeneration and AD risk
The goal of this application is to investigate the role of peripheral proteostasis within the context of the gut-brain connection for noradrenergic degeneration in Alzheimer's Disease.
Role: Contact PI

What other organizations were involved as partners?

Nothing to report

8.SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to **https://ers.amedd.army.mil** for each unique award.

Dr. Bradley Pearce (partnering PI) will be submitting his Annual Technical Report separately.
AR150035P1, Award W81XWH-16-1-0721
"Macrophage Polarization and Utility of in Vivo Therapy with a Brain-Permeable Anti-TNF Agent in Models of Autism"

QUAD CHARTS: If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.
Not applicable

9.APPENDICES: Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.

Nothing to report