



2010 Research Grant Program Winning Abstract

Antibodies Present in the Plasma of Women with Autistic Progeny Alter the Neurogenic Divisions of Precursor Cells in the Prenatal Mouse Cerebral Cortex and the Social Behavior of Adult Mice

By **Veronica Martinez Cerdeno**

Objective and specific aims:

Autism spectrum disorders (ASD) are a group of heterogeneous, behaviorally defined disorders defined by a pattern of qualitative abnormalities in reciprocal social interaction, communication, and repetitive interest and behavior. The etiology of ASD has not been determined but likely results from changes in developmental programs during brain development. To the best of our knowledge very few studies have investigated whether the etiology of ASD has a prenatal component. Previous studies have reported the presence of maternal antibodies and auto-antibodies to neural proteins, which suggests that the maternal immune status during pregnancy may play a role. For example, it has been shown that when compared with age-matched typically developing controls, children with ASD exhibit a differential antibody response to specific cells located in the brain. We will determine if antibodies that are present in maternal serum can impact developmental processes in the embryonic brain, such as neurogenesis, and therefore constitute a prenatal etiology for autism spectrum disorders. Exploring the possibility that an alteration in embryonic brain development due to an immune process has a role in the etiology of autism spectrum disorders will open new lines of research that focus on the modulation of signaling factors and genes that control proliferation of neural progenitor cells, and on the immune component in the etiology of autism.

Hypothesis:

Antibodies present in the plasma of women with autistic progeny alter the neurogenic divisions of precursor cells in the prenatal mouse cerebral cortex and the social behavior of adult mice. IgGs from plasma of women with autistic progeny and from women with age-matched typically developing control progeny will be injected intraventricularly in embryonic day (E)12, E14, and E16 mouse fetuses in utero.

Specific aim 1: Determine the number, distribution, and proliferation rate of: 1a. neural precursor cells in the developing cerebral cortex in mice that were exposed to maternal serum antibodies prenatally, 1b. number and distribution of newly generated neurons in the developing cerebral cortex of mice that were exposed to maternal serum antibodies prenatally. We will analyze the number, distribution, and rate of proliferation of 4A4⁺, Pax6⁺, and Tbr2⁺ progenitor cells and the number and distribution of newly generated Tuj1⁺ neurons in the developing cerebral cortex using immunocytochemistry and stereology.

Specific aim 2: Determine changes in the behavior of adult mice that were exposed to maternal serum during gestation. Some of the injected fetuses will be allowed to be born, and when they are 6 weeks old, we will perform behavioral tests including social approach, reciprocal social interactions, and self-grooming tests.



We will perform statistical analysis to compare the results obtained from experimental and control groups using InStat (GraphPad Software).

Preliminary Data:

We have collected serum from mothers with autistic and control progeny. The human serum was obtained from the CHARGE and MARBLES Study Programs at the University of California, Davis M.I.N.D. Institute. We purified the IgGs in serum and injected them into the cerebral ventricles of E14 mice in utero. We perfused embryos two days after the injection, removed and cut the brains, and stained the tissue with antibodies against molecules that label precursor cells in the developing cerebral cortex, including 4A4, Pax6, and Tbr2. We also immunostained the tissue with the Tuj1 antibody that labels immature neurons. We are in the process of stereologically quantifying the number of cells in experimental and control animals and performing statistical comparisons between groups. We routinely perform behavioral tests in our laboratory for other projects that investigate mice social behavior.

Methods:

Serum Injection: Timed pregnant mice will be anesthetized. An abdominal incision will be made through the skin and the abdominal muscular layer. The uterine horns will be brought outside the mother's body. Injections of 0.5–1.0 microliter of solution containing the human IgGs will be made into the cerebral vesicle of each embryo by passing a 33-gauge micropipette through the uterine wall and into the cerebral ventricle. The uterine horns will be replaced, and the muscular layer and the skin will be closed.

Immunohistochemistry: Brains will be removed, post-fixed for 24 h in PFA at 4°C, cryoprotected, and frozen, and coronal 30–40- μ m slices will be prepared on a cryostat. Free-floating sections will be blocked, then incubated for 24 h at room temperature in primary antibodies. After rinsing, sections will be incubated in the secondary antibodies. Tissue will be mounted and covered.

Imaging and Stereology (Olympus spinning disk confocal microscope with stereology software): We will use the optical fractionator design and the Cavalieri principle. All cells will be counted in a microscope with a 100X oil-immersion objective. We will analyze a total of 200 cells in 10 sections per quantification experiment.

Behavioral Testing – Social Approach: Tendency to approach a novel mouse as compared with tendency to approach a novel object will be measured in 6-week-old-male mice in a 10-min test session using a three-chambered apparatus. Reciprocal social interactions: Unfamiliar adult mice will be allowed to interact for 20 min. The test session will be videotaped and scored for behavioral events including sniffing, following, mounting, allogrooming, huddling, and wrestling. Self-grooming: Mice will be scored for spontaneous grooming behaviors when placed individually in empty cages for 10 min.

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