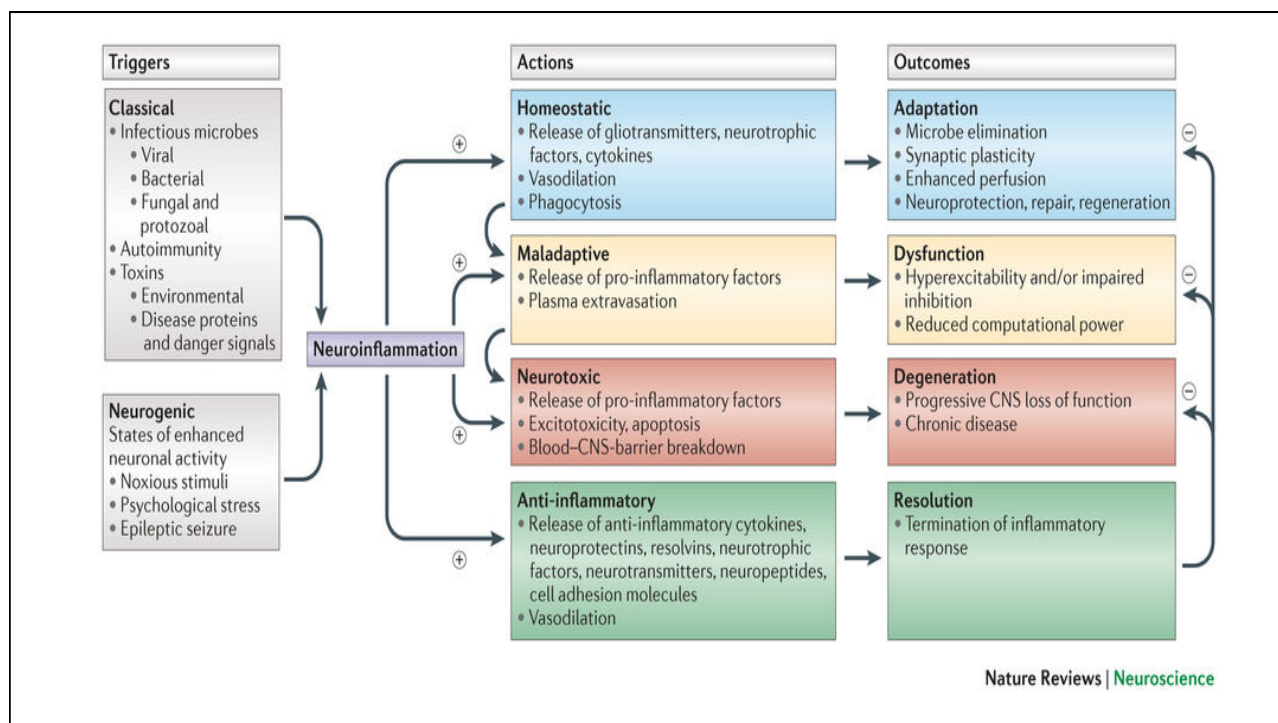


# NEUROINFLAMMATION AND AUTISM SPECTRUM DISORDERS

NAA CONFERENCE

MAY 2019

MICHAEL W ELICE, MD. FAAP. FMAPS

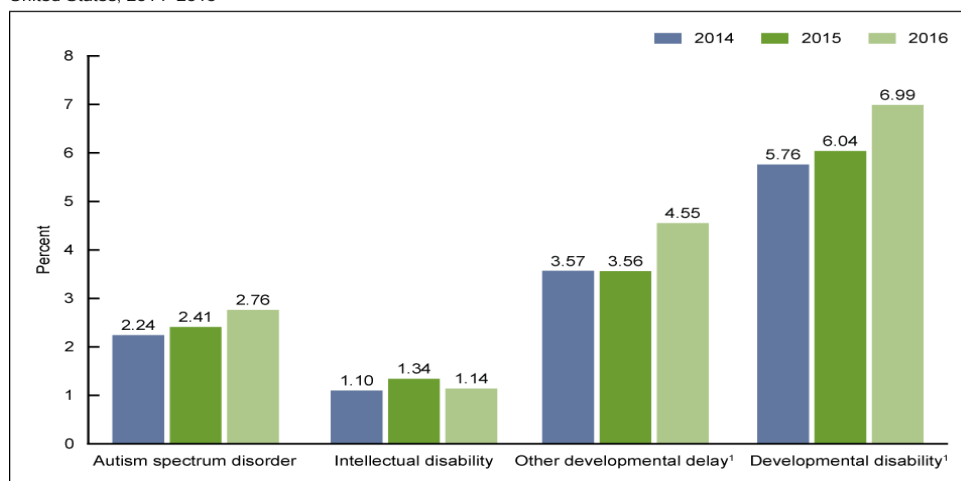


## Neuroinflammation

- Neuroinflammation is inflammation of the nervous tissue which may be initiated in response to a variety of triggers including infection, traumatic brain injury, toxic metabolites or autoimmunity
- In the CNS which includes the brain and the spinal cord, microglia are the resident innate immune cells that are activated in response to these triggers
- The CNS is typically an immunologically privileged site because the peripheral immune cells are generally blocked by the blood brain barrier, a specialized structure composed of astrocytes and endothelial cells.
- Circulating peripheral immune cells may cross a compromised BBB and encounter neurons and glial cells expressing histocompatibility complex molecules which perpetuate an immune response
- This response is designed to protect the CNS from the infectious offending agents but may be toxic and create widespread inflammation as well as further migration of leukocytes through the BBB

- Neuroinflammation was once a clearly defined term denoting pathological immune processes within the CNS.
- The four hallmarks of peripheral inflammation: CNS injuries, stroke, injury or infection
- Recently the definition of neuroinflammation has relaxed to the point that it is often now assumed to be present when even only a single classical hallmark of inflammation was measured.
- Unexpected non-classical immune actions of immune mediators and cells in the CNS in the absence of pathology have increased the likelihood that homeostatic and adaptive immune processes in the CNS will be mistaken for neuroinflammation.

Figure 1. Prevalence of children aged 3–17 years ever diagnosed with selected developmental disabilities, by year: United States, 2014–2016



<sup>1</sup>Linear increase from 2014 to 2016 is statistically significant ( $p < 0.05$ ).

NOTES: Developmental disability includes autism spectrum disorder, intellectual disability, and any other developmental delay. Access data table for Figure 1 at: [https://www.cdc.gov/nchs/data/databriefs/db291\\_table.pdf#1](https://www.cdc.gov/nchs/data/databriefs/db291_table.pdf#1)

SOURCE: NCHS, National Health Interview Survey, 2014–2016.

## What is neuroinflammation in Autism?

- Embryonic neurogenesis is the process of generating neurons, the functional units of the brain
- Dysregulation of neurogenesis due to adverse intrauterine environment such as infection, toxicant exposure, environmental and food triggers has emerged as a key mechanism underlying many neurodevelopmental disorders such as ASD
- A hallmark feature of brain disorders such as ASD is neuroinflammation which can either promote or inhibit neurogenesis depending on the context of brain microenvironment
- Brain pathology is suggestive of ongoing neuroinflammation or encephalitis in different regions of the brain\*\*
- \*Dysregulation of neurogenesis by neuroinflammation: key differences in neurodevelopmental and neurological disorders. Fan LW Neural Regen Res 2017, Mar
- \*\* Kern, JK, Geier DA, Geier MR et al. Front Cell Neurosci 2016 Jan

## Shared brain connectivity issues, symptoms and comorbidities in ASD, ADHD and Tourette Syndrome

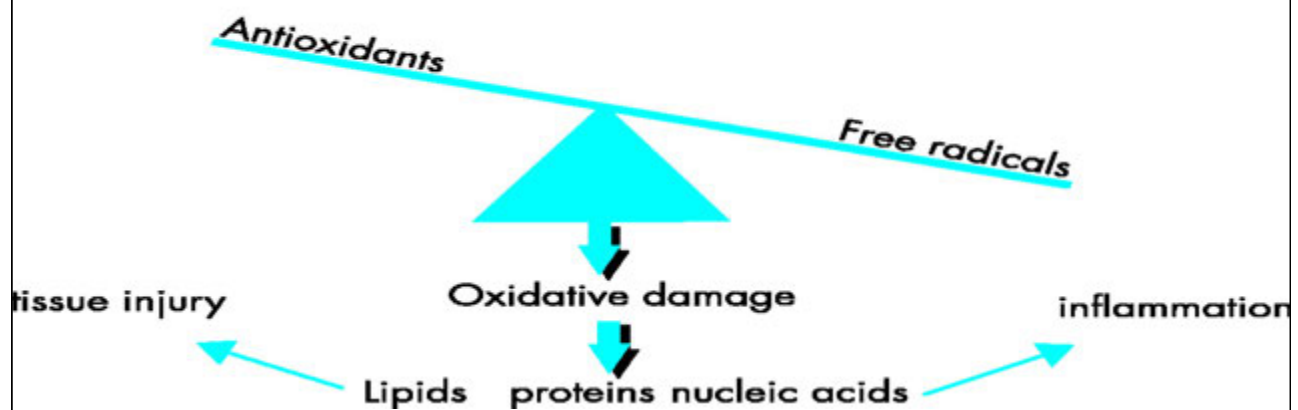
- Evidence suggests that ASD, ADHD and TS have similar neuropathology
- They may belong to a broader spectrum of neurodevelopmental illnesses based on long-range underconnectivity and short-range overconnectivity related to neuronal insult
- Neuronal insult from neurotoxicity, neuroinflammation, excitotoxicity, sustained microglial activation, proinflammatory cytokines, toxic exposure and oxidative stress.

Kern, Geier et al. Brain Connect 2015


## What is Oxidative Stress?

Injury to cells from toxic byproducts of normal cellular chemistry resulting from an insufficient response by the body's protective

**Oxidative stress**



## Health Effects of Chronic Oxidative Stress



Neurological	
ADHD	Autism
Alzheimer's Disease	Multiple Sclerosis
Anxiety & Depression	Parkinson's Disease
Asperger syndrome	Multiple Sclerosis

Cardiovascular	
Cardiovascular Disease	Hypertension
Angina Pectoris	Atherosclerosis

Multi-System Effects	
Diabetes	Chronic Fatigue Syndrome
Cancer	Metabolic Syndrome
Inflammation	Anxiety
Fibromyalgia	Hyperthyroidism
Lyme Disease	Sleep Apnea

Respiratory	
COPD	Asthma

Gastrointestinal Disorders	
Crohn's Disease	Celiac Disease
GERD	Functional Dyspepsia
Gastric Ulcers	

Joints/Skin	
Gout	Rheumatoid Arthritis
Dermatitis	Carpal Tunnel



## Causes of Neuroinflammation

- Environmental allergies
- Food allergies/intolerance
  - Infections
- Toxicant exposures



# Inflammation

- **Acute Inflammation**
  - Early response to injury/infection, lasts days
  - Swelling, redness, heat, pain at site
  - Beneficial, leads to elimination of infection and tissue healing
  - Innate cells and mediators
- **Chronic Inflammation**
  - Late or sustained response to intracellular pathogens or self antigens (autoimmunity)
  - Harmful, results in tissue destruction
  - Adaptive and innate cells and mediators
  - Often LOCAL at specific sites

Hypothesis: inflammatory events in pregnancy such as response to infection, may disrupt the normal expression of immune molecules during critical stages of neural development and thereby contribute to the risk for neurodevelopmental disorders such as ASD

- Immune molecules such as cytokines and chemokines produced by microglia in the brain are critical for normal brain development
- Single viral infection or injection of viral mimetic to pregnant mice significantly and persistently impacts offspring immune and nervous system function
- **Studies in humans and non-human animal models have supported the hypothesis that ongoing disrupted immune molecule expression and/or neuroinflammation contributes to a significant subset of ASD**
- In genetically susceptible individuals, environmental risk factors combine or synergize to create a tipping threshold point for dysfunction
- Studies showing a link between maternal immune activation and ASD-like outcomes in offspring involve diverse environmental factors beyond infection including toxin exposures, maternal stress all of which impact inflammatory or immune pathways
- \*Beyond infection-maternal immune activation by environmental factors, microglial development and relevance for ASDs  
Bilbo SD et al. Exp Neurol 2017 July 8

## Maternal Inflammation

- Environmental insults, infection or malnutrition impact brain development and is associated with neurodevelopmental disorders in children
  - Maternal viral or bacterial infections have been characterized as disruptors of brain shaping
  - Imbalance of essential fatty acids, especially polyunsaturated fatty acids (PUFAs) are observed in ASD patients and those with ADHD and schizophrenia.
  - Omega 3 PUFAs are powerful immunomodulators that exert anti-inflammatory properties affecting the fetal brain and microbiota\*
  - Maternal stress and terbutaline, used to arrest pre-term labor resulted in severe behavioral symptoms, astrogliosis and spontaneous recurrent convulsive seizures in 45% and epileptiform spikes in 100% of rats studied. This demonstrates that **not a single teratogen but a combination of teratogens may cause comorbid autism and epilepsy.** \*\*
- \*\* Bercum, etal. J Neurosci 2015 Dec
  - \*Madore C, etal. Neural Plast 2016

## Lipopolysaccharides

- Systemic lipopolysaccharide-induced neuroinflammation affects microglia and early neural developmental events in rats
  - Exposure as early as day 3 postnatally leads to a robust microglia activation both pro and anti-inflammatory phenotypes
  - Anti-inflammatory markers were upregulated in multiple white and gray matter structures associated with marked decrease in naturally occurring apoptosis, but an increase in cell proliferation in the subventricular zone and the dentate gyrus of hippocampus, increase in oligodendrocyte lineage population
  - LPS exposed rats exhibited significant impairments in communicative and cognitive functions
  - This suggests a possible role of the M2-like anti-inflammatory microglial activation in abnormal neural development in ASD behavioral impairments\*
- \*Pang, Y etal., Plos One 2016 Oct

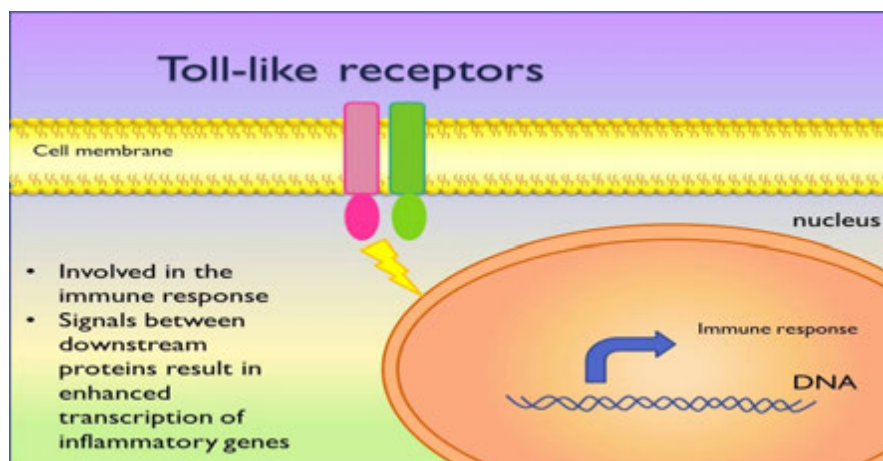


## Immune dysfunction in Neuroinflammation

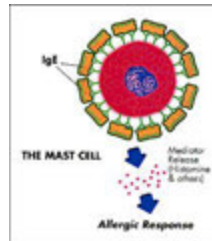
- ASD individuals have altered immune responses in T cells, B cells, monocytes, natural killer cells and dendritic cells
- ASD individuals have alterations in immunoglobulins and increased autoantibodies
- ASD individuals have elevated peripheral cytokines and chemokines associated with neuroinflammation

• \*Bjorklund, et al, Acta Neurol Exp 2016

## Potential Target of Inflammation



## MAST CELLS

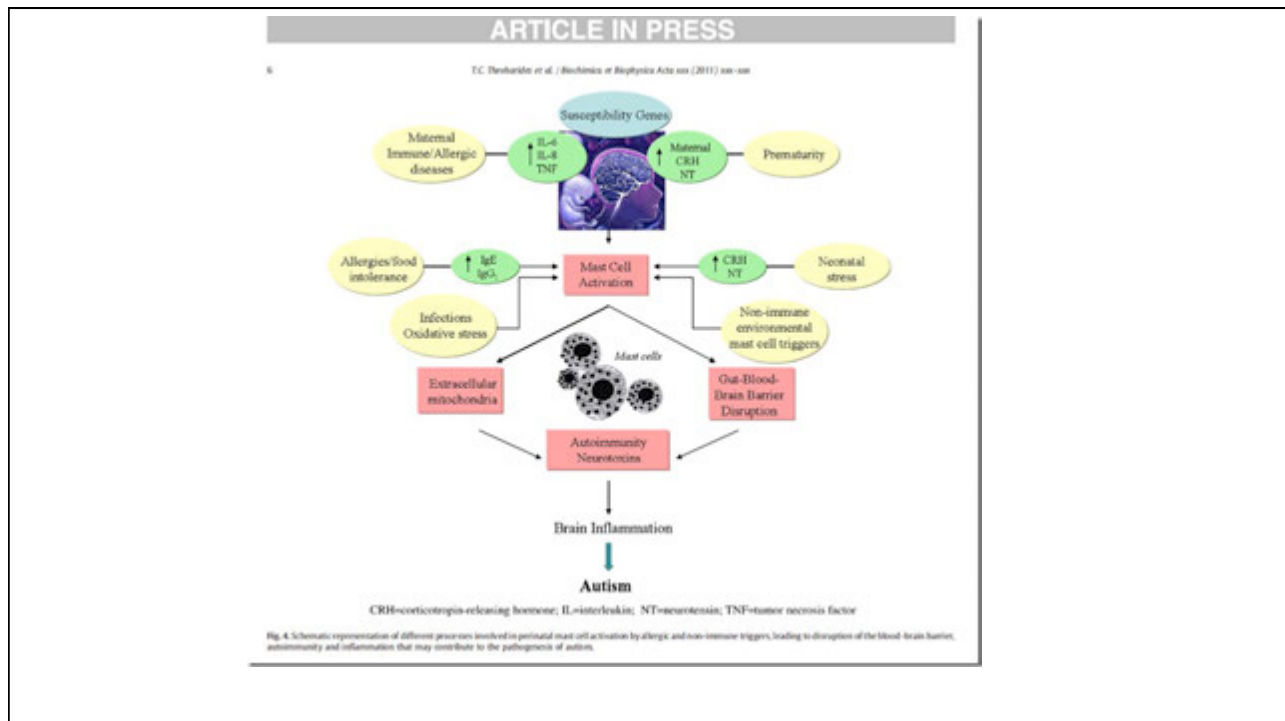


## What are Mast Cells?

- Exacerbate autoimmunity via alteration of innate and adaptive immunity that protect or damage the host
- Type I hypersensitivity responses
- Type III autoimmune diseases – Arthus reaction, SLE, RA
- Activation by IgE and inflammatory mediators
- Allergy, Atopic dermatitis and allergic rhinitis
- Inflammatory conditions – rapid release of TNF, IL-6 and mast cell proteases
- Resist bacterial infection – sepsis, lymphatic drainage
- Prevent parasite infection
- Exacerbate atherosclerosis
- Promote cancer progression

## MAST CELL ACTIVATION DISORDER AND ASD OVERLAP

- allergic symptoms that allergist say are not allergies
- intestinal inflammation
- intestinal slowdown possibly resulting in distention
- breakdown of the gut-blood barrier, i.e. leaky-gut
- high fevers and reds botchy hives
- breakdown of the blood-brain barrier
- brain inflammation
- neurotoxins in the brain
- reduced neural connectivity
- "brain fog"
- pain insensitivity
- reduced learning ability
- seizures
- and even more

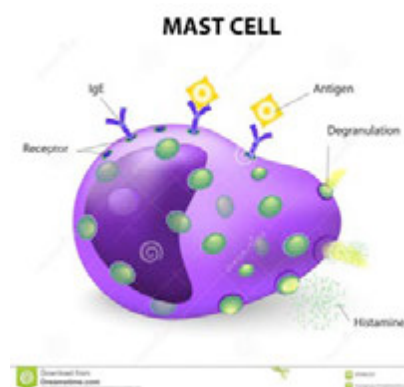


## Mast cells in neuroinflammation and brain disorders

- Microglia and astrocytes are major pathogenic components within this process which respond to proinflammatory mediators released from mast cells
- Mast cells reside in the brain and are an important source of inflammatory molecules
- Interactions with glial cells and neurons result in the release of mediators such as cytokines, proteases and reactive oxygen species
- During neuroinflammation, excessive levels of these mediators can influence neurogenesis, neurodegeneration and BBB permeability
- They have been implicated in neuronal disorders such as cerebral ischemia, TBI, neuropathic pain, multiple sclerosis, AD, migraine, autism and depression
- \*Hendriksen E et al. Neurosci Biobehav Rev 2017 Aug

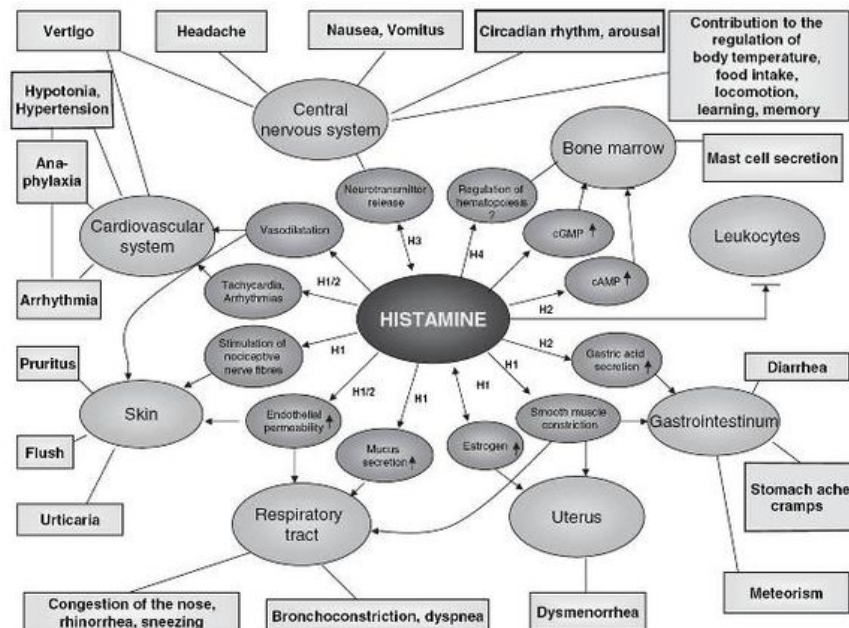
## Treatment of Mast Cell Activation

- H1 and H2 antagonists
- Mast cell stabilizers
  - Ketotifen
  - Luteolin, Quercetin
  - Epinephrine
- Steroids
- Leukotriene antagonist
  - Montelukast



## Histamine signaling in ASD

- Histaminergic system has a critical role in cognition, sleep and other behaviors
- Histamine is a neurotransmitter and an immune modulator
- Implicated in many neurological disorders which share comorbidity with ASD, including Tourette's syndrome.
- Mediates neuroinflammation, which is heightened in ASD,
- RNA sequencing focused gene set of HS genes: HDC, HNMT, HRH2, HRH3 and HRH4 which was significantly altered
- \*Altered expression of histamine signaling genes in ASD. Wright,C etal. Transl Psychiatry 2017, May



# HISTAMINE IN THE GI TRACT

HIGH LEVELS IN THE GUT  
IMMUNOREGULATOR IN:

FOOD ALLERGY  
FOOD POISONING  
HISTAMINE INTOLERANCE  
IRRITABLE BOWEL SYNDROME  
INFLAMMATORY BOWEL DISEASE

Somalinska S, Jutal M, Cramer R, O'Mahoney L, Histamine and Gut Mucosal Immune Regulation, Allergy 2014 Mar;69:pp.273-81

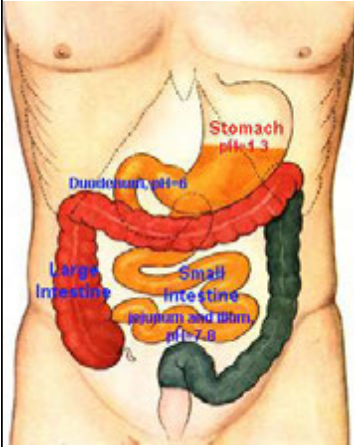
**Although no CNS disease entity has been associated directly to brain histamine dysfunction until now, the H(3) receptor is recognized as a drug target for neuropathic pain, sleep-wake disorders.... and cognitive impairment associated with attention deficit hyperactivity disorder**

[CNS Neurosci Ther.](#) 2011 Dec;17(6):620-8. doi: 10.1111/j.1755-5949.2010.00212.x. Epub 2010 Nov 22.

**Histamine pharmacology and new CNS drug targets.**

[Tiligada E<sup>1</sup>](#), [Kyriakidis K](#), [Chazot PL](#), [Passani MB](#)

## Immune Modulation in the Gut



Courtesy Patrick Hanaway, MD

- At birth - digestive tract of humans is minimally colonized.
- Colonised by microbes within the first few days of life
- At first, predominantly bifidobacteria (breast fed infants).
- With the introduction of other foods, a diverse microbial population develops in the gastrointestinal tract.
- By now, of all the cells in a human body, the overwhelming majority are non-human.

[Free Radic Biol Med.](#) 2014 Mar;68:122-33. doi: 10.1016/j.freeradbiomed.2013.11.008. Epub 2013 Nov 22.

**Role of the enteric microbiota in intestinal homeostasis and inflammation.**

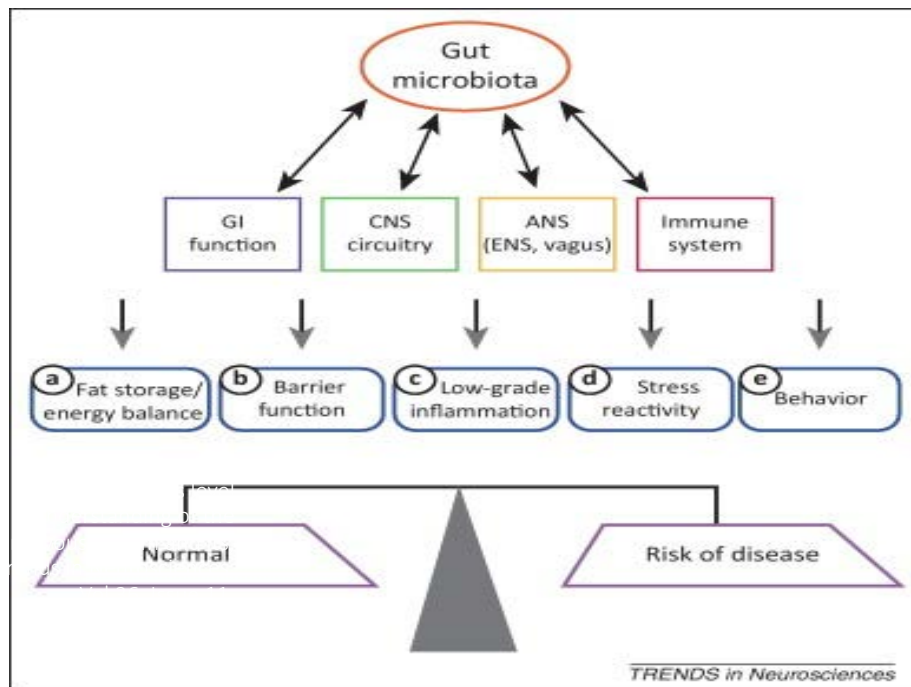
[Koboziev I](#)<sup>1</sup>, [Reinoso Webb C](#)<sup>1</sup>, [Furr KL](#)<sup>1</sup>, [Grisham MB](#)<sup>2</sup>.

- Our coexistence with the gut microbiota represents a dynamic and mutually beneficial relationship that is thought to be a major determinant of health and disease.
- Failure to properly regulate intestinal mucosal immunity is thought to be responsible for the inflammatory tissue injury observed in the inflammatory bowel diseases (IBD; Crohn disease, ulcerative colitis)

## Microbiota, immune and nervous systems

- Studies investigating the gut-brain axis demonstrate a critical role for the gut microbiota in orchestrating brain development and behavior and the immune system is an important regulator of these interactions
- Intestinal microbes modulate the maturation and function of tissue-resident immune cells in the CNS
- Microbes influence the activation of peripheral immune cells which regulate responses to neuroinflammation, brain injury, autoimmunity and neurogenesis
- Inappropriate antigen trafficking through an impaired intestinal barrier followed by passage of these antigens or immune-activated complexes through a permissive blood-brain barrier can lead to ASD
- ASD brain has altered expression of genes associated with BBB integrity coupled with increased neuroinflammation and possibly impaired gut barrier integrity\*\*
- Thus both the gut microbiota and immune system are implicated in the etiopathogenesis or manifestation of neurodevelopmental, psychiatric and neurodegenerative diseases such as ASD

• \*Fung TC et al., Nat Neurosci 2017 Feb  
 • \*\*Fiorentino, M et al. Mol Autism 2016, Nov






## Enteric short chain fatty acids

- Pre or perinatal infection, hospitalization, early antibiotic exposure which alters gut microbiota may be potential risks in ASD
- Enteric short chain fatty acids (SCFAs) present in diet and produced by opportunistic gut bacteria following fermentation of dietary carbohydrates may be environmental triggers in ASD
- Propionic acid and carnitine metabolism
- major SCFA produced by ASD-associated bacteria eg clostridia, bacteroides and also a common food preservative can produce reversible behavioral, electrographic, neuroinflammatory, metabolic and epigenetic changes closely resembling those found in ASD when administered to rodents
- Major effects of these SCFAs may be through alteration of mitochondrial function via the citric acid cycle
- Hypothesis: ASDs are produced by pre or post natal alterations in intestinal microbiota in sensitive sub-populations\*
- \*McFabe. Microb Ecol Health Dis 2015 May

## Short chain and polyunsaturated fatty acids as markers in autism

- Fatty acids are essential dietary nutrients which provide polyunsaturated fatty acids (PUFAs) for the growth and function of nervous tissue.
- SCFAs are derived from the microbiome linked to effects on the gut, brain and behavior
- Reduced levels of PUFAs, important during brain development are associated with impairments in cognitive and behavioral performance
- **Omega-3 fatty acids such as eicosapentaenoic (EPA) and docosahexaenoic acids (DHA) are involved in neurogenesis, neurotransmission and protection from oxidative stress**
- Omega-3 PUFAs antagonize omega-6 PUFA (arachidonic acid) induced prostaglandin -E2 (PGE2) formation

El-Ansary,etal. Lipids Health Dis. 2014



**Paracelsus**  
“All substances are  
poisons;  
there is none which is not  
a poison.  
The right dose  
differentiates a poison  
from a remedy.”  
Paracelsus (1493-1541)

## Toxicant exposure

- Numerous studies support an important role for heavy metal exposure, particularly mercury in the etiology of ASD
- Young children may be particularly susceptible to air pollution-induced neurotoxicity
- Prenatal exposure may cause or contribute to developmental disabilities and behavioral abnormalities
- Recent studies have found associations between exposures to traffic-related air pollution and ASD
- Higher levels of neuroinflammation and systemic inflammation
- Gene-environment interactions may play a role in determining individual susceptibility to air pollution developmental neurotoxicity

• \*Li H, et al. Biol Trace Elem Res 2017, May

## Organophosphate insecticides

- Organophosphate insecticides ie. Chlorpyrifos are widely diffused environmental toxicants associated with neurobehavioral deficits and increased risk of ASD in children
  - Oxidative stress and dysregulated immune responses are implicated in organophosphate neurodevelopmental effects
  - Gestational exposure appears to strengthen some autistic-like traits thus resulting in delayed and long-lasting alterations in specific pathways relevant to ASD\*
- 
- \*DeFelice,etal. J of Neuroinflammation 2016 Jun

## Lead

- Lead has been identified as one of the main neurotoxicants acting as environmental triggers identified with neuroinflammation and autoimmunity in ASD.
  - Levels of blood lead were measured in 60 ASD and 60 Healthy control matched children between 5 and 12 years recruited from low lead – polluted areas.
  - Blood lead levels were significantly higher in ASD (>10mcg/dl) compared to HC.
  - There were significant and positive correlations between levels of lead and values of the CARS (Childhood autism rating scale) and IQ
  - Patients with ASD showing increased lead levels had higher frequency of anti-ribosomal antibodies\*
- 
- \*Mostafa etal., Metab Brain Dis 2016 Oct

## Mercury

- Microtubule degeneration
- Long-range axon degeneration
- Dendritic overgrowth
- Neuroinflammation
- Microglial/astrocytic activation
- Brain immune response activation
- Oxidative stress and lipid peroxidation
- Decreased reduced glutathione and elevation of oxidized glutathione
- Mitochondrial dysfunction
- Disruption of calcium homeostasis
- Inhibition of glutamic acid decarboxylase (GAD) activity
- Disruption of GABAergic and glutamatergic homeostasis
- Inhibition of IGF-1, methionine synthase activity
- Impairment of methylation
- Decrease in cerebral/cerebellar blood flow
- Increase pro-inflammatory cytokine levels in the brain (TNF- $\alpha$ , IL-1B, IL-8)
- NF-kappaB activation

Kern, Geier et al. Acta Neurobiol Exp 2012

## Mercury

- Neurotoxicant that is potentially one of the triggers for ASD as it induces neuroinflammation and subsequent release of neuropeptides.
- Levels of Neurokinin A and blood mercury were measured in 84 children with ASD between 3-10 years of age and 84 matched HC
- There was a positive linear relationship between the CARS and both serum neurokinin A and blood mercury in the ASD kids compared with the HC. 78% of ASD patients had elevated mercury levels and 55% increase in serum neurokinin A\*
- \*Mostafa et al. Metab Brain Dis 2016 Jan

## Aluminum

- A known neurotoxin
- Has no place in the human body
- Is contained in almost all vaccines in extremely high levels



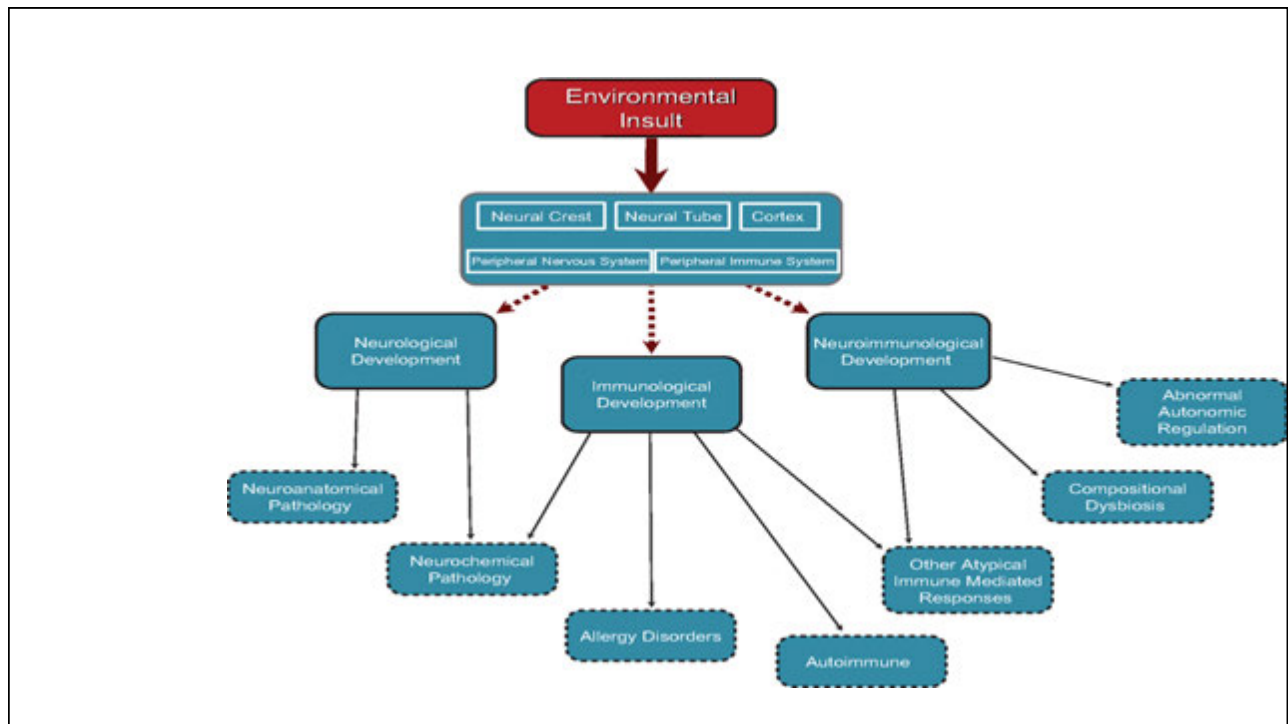
## Parabens

Butyl paraben is a preservative used in food, drugs and cosmetics

Neurotoxic and potentially estrogenic activity

Though to contribute to ASD and learning disabilities

Leads to increased oxidative stress, decreased reduced glutathione and elevated oxidized glutathione, mitochondrial dysfunction and neuroinflammation, increased pro-inflammatory cytokines in the brain (TNF- $\alpha$ , IL-6, IL1-beta)



## Electromagnetic and radiofrequency radiation exposures - EMF/RFR

- Deficiencies of antioxidants such as glutathione
- Peroxidized cell membrane lipids
- Mitochondrial dysfunction
- Immune system disturbances
- Increased oxidative stress; free radical damage compromising BBB and brain perfusion

### Clinical manifestations:

- Changes in brain and autonomic nervous system electrophysiological function
- Sensory processing disorders
- Seizures
- Sleep disturbance

Herbert, M. Pathophysiology, 2013 June

## GABAergic/glutamatergic imbalance

- Autoimmunity immune dysfunction and neuroinflammation are etiological mechanisms of ASD
- Regression is associated between reduced GABA level, neuroinflammation and glutamate excitotoxicity.
- Fever or hyperthermia may alter glutamate levels in the brain having an impact on symptoms of ASD

AUTISM



## HOW TO TREAT NEUROINFLAMMATON

- Find a physician who understands the presenting problems and who is willing to listen
- Proper patient evaluation which includes a complete history and physical exam
- Complete history includes:
  - Conception and pregnancy history
  - Delivery and birth history
  - Illnesses
  - Gastrointestinal symptoms
  - Respiratory illnesses
  - Skin problems
  - Sleep history
  - Neurological symptoms
  - Environmental history
  - Family History
- Order appropriate lab tests; blood, urine, stool, MRI of brain, EEG – 24-48 hour test, SPECT scan



- Interpretation of lab work is essential to understanding how to treat the patient
- Develop a treatment plan based on which triggers appear to be involved. Is it genetic? Environmental? Infections? Food?
- Good follow up is critical to understanding if treatment plans are effective or not.
- Remember – *EVERY CHILD IS NOT THE SAME AND ONE SIZE FITS ALL IS NOT TRUE!*

## AUTISM SPECTRUM DISORDERS ARE TREATABLE AND RECOVER IS POSSIBLE

- don't set an end point in time where you expect your child to be at a level of recovery.
- Time to see improvement in various symptoms varies from patient to patient
- It isn't a sprint, it's a marathon
- Surround yourself with supportive medical personnel, therapists, teachers and loving family members
- Don't waste time with people who tell you there is nothing you can do or those who criticize you and make you feel inadequate as a parent or caregiver
- Ask questions!!
- Don't stop until you get answers that make sense!!

**THANK YOU**