Integrated Approaches to Autism Spectrum Disorder Treatment

Nicola Antonucci MD
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Disclaimer

Information provided here are for educational purposes only. They are not intended as medical advice or as substitute for consultation with medical professionals.

Dr. Antonucci is medical director of Biomedical Center for Autism Research and Treatment – Bari (Italy) and has no relationships with pharmaceutical entities or commercial companies.

He received a grant from the Autism Research Institute (CA) for biomolecular in vitro studies on autism.
Genetic Epidemic Disease!

“...the burden of proof is upon anybody who feels that there is NOT a real increase here in the number of kids affected.”

Dr. Thomas Insel, Director of National Institute of Mental Health and head of Interagency Autism Coordinating Committee (IACC)
Genetic Epidemic Disease!

Estimated prevalence of autism in the world
Review

Epigenetic Findings in Autism: New Perspectives for Therapy

Dario Siniscalco 1,2,3,*, Alessandra Cirillo 4, James Jeffrey Bradstreet 5 and Nicola Antonucci 6

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Environmental Factors

Air pollution
Nutrition
Heavy Metals
Bisphenol A (BPA) and flame retardants
Phthalates,
Polychlorinated biphenyls (PCBs)
Pesticides (Glyphosate)
Electromagnetic waves
....
Autism Spectrum Disorder

Genes

Environment  Developmental stage
What is Autism?
Autism Clinical Phenotypes

- Toxic
- Mitochondrial
- Allergic
- Autoimmune
- Viral-Infective
- Bacterial-Infective

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Microglia Activation
Porphyrinuria in childhood autistic disorder: Implications for environmental toxicity

Robert Nataf a, Corinne Skorupka b, Lorene Amet b, Alain Lam a, Anthea Springbett c, Richard Lathe d,*

a Laboratoire Philippe Auguste, Paris, France
Urinary Porphyrin Test
n=200 ASD
median age 7.2 yo

66.2% Intoxication Porphyrin Positive

33.8% No Intoxication Porphyrin Negative
Autism Clinical Phenotypes

- Toxic
- Mitochondrial
- Allergic
- Autoimmune
- Viral-Infective
- Bacterial-Infective
- Glial Activation
Astrocytes and Microglia and Their Potential Link with Autism Spectrum Disorders

Francesco Petrelli, Luca Pucci and Paola Bezzi*

Department of Fundamental Neurosciences, University of Lausanne, Lausanne, Switzerland

Department of Fundamental Neurosciences, University of Lausanne, Lausanne, Switzerland

Physiological Conditions

Microglia

Neuron

Astrocyte

Reactive microglia

PROINFLAMMATORY MOLECULES

TNFα, IL-1β, IL-6, IL-2, IL-10

Gliotransmitters (glutamate, D-serine, growth factors)

Synaptogenic factors (thrombospondins 1-5, hevin and glypicans)

Pathological conditions

ASD

TNFα

Glutamate

Cytokines

Reactive astrocyte
Neuroglial Activation and Neuroinflammation in the brain of patients with autism

Vargas et al
Annals of Neurology
2005 Jan; 57 (1): 67-81
Microglial Activation in Young Adults With Autism Spectrum Disorder

Katsuaki Suzuki, MD, PhD; Genichi Sugihara, MD, PhD; Yasuomi Ouchi, MD, PhD; Kazuhiko Nakamura, MD, PhD; Masami Futatsubashi, BS; Kiyokazu Takebayashi, MD, PhD; Tuijro Yoshihara, MD, PhD; Kei Omata, PhD; Kaori Matsumoto, MA; Kenji J. Tsuchiya, MD, PhD; Yasuhide Iwata, MD, PhD; Masatsugu Tsujii, MA; Toshirou Sugiyama, MD, PhD; Norio Mori, MD, PhD
Excitotoxicity

*Glutamate and quinolinic acid are excitatory amino acids that can have neurotoxic effects through NMDA receptor agonism. Excess glutamate is removed by astroglial EAAT. Microglia, activated by pro-inflammatory mediators, produce quinolinic acid and inhibit EAAT expression, potentially leading to excess NMDA agonism. NMDA antagonists such as ketamine and memantine can inhibit microglial release of pro-inflammatory mediators. How this occurs is not known.

NMDA= N-methyl-D-aspartate; Th=T helper cell; IL= interleukin; EAAT = excitatory amino acid transporter; TNF = tumor necrosis factor; IFN= interferon; Rx = prescription.

Excitotoxicity
Microglial Activation & Chronic Neurodegeneration

Melinda E. Lull¹ and Michelle L. Block¹
¹ Department of Anatomy and Neurobiology, Virginia Commonwealth University Medical Campus, Richmond, VA 23298, USA

Abstract

Autism
Alzheimer's disease
Parkinson's disease
Cerebral ischemia
Multiple Sclerosis
Amyotrophic Lateral Sclerosis
Major Mepression
Excitotoxicity

*Glutamate and quinolinic acid are excitatory amino acids that can have neurotoxic effects through NMDA receptor agonism. Excess glutamate is removed by astroglial EAAT. Microglia, activated by pro-inflammatory mediators, produce quinolinic acid and inhibit EAAT expression, potentially leading to excess NMDA agonism. NMDA antagonists such as ketamine and memantine can inhibit microglial release of pro-inflammatory mediators. How this occurs is not known.*

NMDA=N-methyl-
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Mast cells, glia and neuroinflammation: partners in crime?

Summary

Figure 1. Electron microscopic image of an isolated tissue mast cell. Note the prominent appearance of numerous cytoplasmic granules.
The Mast-Cell Hypotosis

Mast-Cell Activation

Toxic

Mitochondrial

Allergic

Autoimmune

Viral-Infective

Bacterial-Infective
Novel therapeutic targets for autism

Theoharis C. Theoharides\textsuperscript{1,2}, Robert Doyle\textsuperscript{3}, Konstantinos Francis\textsuperscript{4}, Pio Conti\textsuperscript{5} and Dimitris Kalogeromitros\textsuperscript{2}

\textsuperscript{1}Laboratory of Molecular Immunopharmacology and Drug Discovery, Department of Pharmacology and Experimental Therapeutics and Departments of Internal Medicine, Biochemistry and Psychiatry, Tufts University School of Medicine, Tufts Medical Center, 136 Harrison Avenue, Boston, MA 02111, USA.

**Brain**

Central-nervous-system-originating mast-cell-activation triggers
- Adenylyl-cyclase-activating peptide, calcitonin gene-related peptide, corticotropin-releasing hormone, myelin basic protein, nerve growth factor, neurotensin, substance P

**Gut**

Intestine-originating mast cell triggers
- Butyrophilin, caselin, Clostridium difficile toxins, gliadin, gluten, interleukin-1, interleukin-33, lipopolysaccharide, neurotensin, reactive oxygen species, rotavirus, vasoactive intestinal peptide

Increased gut–blood–brain permeability

Autism spectrum disorders

Luglio 2008
Glial Activation and Excitotoxicity

*Glutamate and quinolinic acid are excitatory amino acids that can have neurotoxic effects through NMDA receptor agonism. Excess glutamate is removed by astroglial EAAT. Microglia, activated by pro-inflammatory mediators, produce quinolinic acid and inhibit EAAT expression, potentially leading to excess NMDA agonism. NMDA antagonists such as ketamine and memantine can inhibit microglial release of pro-inflammatory mediators. How this occurs is not known.*

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Mast cells, glia and neuroinflammation: partners in crime?

Summary
Gram Neg. Bacteria - LPS

1. LPS

2. TLR-4
   - Tram Trif
   - Mal
   - MyD88

3. Activated NF-kB

4. Nucleus

5. Cytokines (IL-1, IL-6, IL-8, IL-12, TNF-alpha)
A POTENTIAL ROLE FOR LPS-INDUCED INFLAMMATION IN THE INDUCTION OF ALZHEIMER’S DISEASE-RELATED PATHOLOGY AND COGNITIVE DEFICITS

Marielle Suzanne Kahn
Bachelor of Arts, 2004
The University of Texas at Austin
Austin, Texas

Figure 2. LPS-induced Aβ1-42 production and clearance. 7 days of LPS injection significantly elevates Aβ1-42 in the mouse hippocampus. 15 days following the last injection, Aβ1-42 remains significantly elevated as compared to the saline control group. * compared to saline control, p<0.05. ** compared to saline control, p<0.01.
Brain Maker

The Power of Gut Microbes to Heal and Protect Your Brain - for Life

Available Now
Step 1#: Remove Environmental Triggers

• Fix the gut:
  • Reduce permeability and inflammation with diet (Gluten, Casein, Sugar, Soy, Yeast free diet, Ketogenic Diet)
  • Balance the microbiome: remove parasite, yeast, bacteria and replenish with beneficial flora.

• Treat chronic bacterial infection/parasite: Borrelia, Bartonella, Chlamidia, Micoplasma, Babesia etc.

• Treat chronic viral infection: HHV6, CMV, HBV, HZV, HHV1-2

• Remove Oxidative Stress and Heavy Metals
N-Palmitoylethanolamine and Neuroinflammation: a Novel Therapeutic Strategy of Resolution

Stephen D. Skaper¹ · Laura Facci¹ · Massimo Barbierato¹ · Morena Zuso¹ · Giuseppe Bruschetta² · Daniela Impellizzeri² · Salvatore Cuzzocrea² · Pietro Giusti¹
Cannabinoid Receptor Type 2, but not Type 1, is Up-Regulated in Peripheral Blood Mononuclear Cells of Children Affected by Autistic Disorders

Dario Siniscalco · Anna Sapone · Catia Giordano · Alessandra Cirillo · Laura de Magistris · Francesco Rossi · Alessio Fasano · James Jeffrey Bradstreet · Sabatino Maione · Nicola Antonucci

**Fig. 1** Over-expression of CB2 receptor gene, but not of CB1 receptor and FAAH enzyme, and down-expression of NAPE-PLD gene in AD-PBMCs. The measured mRNA levels were normalized with respect to GAPDH (housekeeping gene) and gene expression values were expressed as a percentage of arbitrary units ± SEM open circle indicates significant difference versus healthy controls, p values <0.05 were considered statistically significant. CTL healthy control subjects, AD autistic patients
Case Report

Beneficial Effects of Palmitoylethanolamide on Expressive Language, Cognition, and Behaviors in Autism: A Report of Two Cases

Nicola Antonucci, Alessandra Cirillo, and Dario Siniscalco

1 Biomedical Centre for Autism Research and Treatment, 20129, Milan, Italy
2 Institute of Biosciences
3 Department of Experimental Medicine
4 Centre for Autism—La Fe
5 Cancellautismo-No Project
PEA (26 patients = 17 male/9 female)

- **No Change**: 50%
- **Better**: 38.5%
- **Worse**: 11.5%
Children with autism spectrum disorders, who improved with a luteolin-containing dietary formulation, show reduced serum levels of TNF and IL-6

I Tsilioni¹, A Taliou², K Francis³ and TC Theoharides¹,⁴,⁵,⁶

Figure 2. (a) Comparison of serum TNF levels in normal and ASDs children. (b) Serum TNF levels in children with ASDs before and after treatment with a luteolin-containing dietary formulation. Symbols represent individual data points, and the horizontal line represents the mean for each group. ASD, autism spectrum disorder; TNF, tumor necrosis factor.
Submitted to “Molecular Autism”

Beneficial effects of co-ultramicronized palmitoylethanolamide/luteolin in a mouse model of autism and in a case report of autism"

Bartolomeo Bertolino1*, Rosalia Crupi2*, Daniela Impellizzeri3, Giuseppe Bruschetta4, Marika Cordaro5, Rosalba Siracusa6, Emanuela Esposito7, Salvatore Cuzzocrea8

Correspondence: Prof. Salvatore Cuzzocrea - Department of Biological and Environmental Sciences, University of Messina, Phone: +390906765208; email: salvator@unime.it
Pealut (28 patients = 25 male/3 female)

- Better 61%
- No Change 32%
- Worse %
Our experience with the Glialia (Pealut) has been nothing short of miraculous. My daughter Ava is nine years old and was diagnosed with Autism at age three. She has suffered with sporadic grand mal seizures of unknown etiology, since 2008. She was diagnosed with epilepsy in March 2016 at the Meyer Children’s Hospital in Firenze, after several abnormal EEG results.
In February of 2016, she began to suffer with Lyme Disease after exposure to a deer tick. Her symptoms were severe and she experienced multiple absence seizures daily, behavioural disturbances, aggression and at least one grand mal seizure weekly. Ava was prescribed 900mg of Sodium Valproate and 10 mg Diazepam daily for several months. We spent the majority of our time in the emergency room, as the medication was unable to control her seizure activity.
After consultation with Dr. Antonucci, we began using Glialia twice daily. After 3 days the seizures had completely stopped and have not returned. Ava has been seizure free since July 2016. Over a period of two months, we reduced the dosage of sodium valproate and she now no longer needs this medication.
We are still working with Dr. Antonucci to combat the Lyme infection and this may take some months. However, my daughter has returned to a happy, peaceful little girl who is completely seizure free.
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Initial Observations of Elevated Alpha-N-Acetylglactosaminidase Activity Associated with Autism and Observed Reductions from GC Protein—Macrophage Activating Factor Injections

James Jeffrey Bradstreet\textsuperscript{1,2}, Emar Vogelaar\textsuperscript{3} and Lynda Thyer\textsuperscript{4}

\textsuperscript{1}Bradstreet Wellness Center, LLC, Cumming, GA, USA. \textsuperscript{2}International Child Development Resource Center, Suwanee, GA, USA. \textsuperscript{3}European Laboratory of Nutrients, Regulierenring 9, Bunnik, The Netherlands. \textsuperscript{4}Immuno Biotech Ltd., Guernsey, UK. Corresponding author email: drbradstreet@gmail.com
The *in vitro* GcMAF effects on endocannabinoid system transcriptionomics, receptor formation, and cell activity of autism-derived macrophages

Dario Siniscalco¹,²,³*, James Jeffrey Bradstreet⁴,⁵, Alessandra Cirillo⁶ and Nicola Antonucci⁷

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**Figure 2** Expression of the enzymes NACE-PLD and FAAH, and CB2R genes in blood monocyte-derived macrophages (BMDMs). The ratio NACE/FAAH was also reported. The measured mRNA levels were normalized with respect to GAPDH (housekeeping gene) and gene expression values were expressed as a percentage of arbitrary units ± SEM. * indicates significant difference versus healthy controls; ** indicates significant difference versus GcMAF untreated autistic BMDMs. P-values <0.05 were considered statistically significant. CTL, healthy control subjects; AU, autistic patients. Values were reported in percentage versus healthy control values.
Effects of Oxaliplatin and Oleic Acid Gc-Protein-Derived Macrophage Activating Factor on Murine and Human Microglia

Jacopo J.V. Branca,¹ Gabriele Morucci,¹ Francesca Malentacchi,² Stefania Gelmini,² Marco Ruggiero,²,³ and Stefania Pacini¹*

Gc-protein-derived macrophage activating factor counteracts the neuronal damage induced by oxaliplatin

Gabriele Morucci⁠¹, Jacopo J.V. Branca⁠¹, Massimo Gulisano⁠¹, Marco Ruggiero⁠²,⁴, Ferdinando Paternostro⁠¹, Alessandra Pacini⁠¹, Lorenzo Di Cesare Mannelli⁠³ and Stefania Pacini⁠¹
Is chondroitin sulfate responsible for the biological effects attributed to the GC protein-derived Macrophage Activating Factor (GcMAF)?

Marco Ruggiero, Heinz Reinwald, Stefania Pacini

*Corresponding author.
dr. reinwald healthcare gmbh + co kg, Friedrich-Luber-Straße 29, D-90592 Schwarzenbruck, Germany
Dose Management of Rerum

Rerum sq/week

Increase slowly starting from 0.01/0.02 ml
<table>
<thead>
<tr>
<th>Volume</th>
<th>Units</th>
<th>Body Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.04 ml</td>
<td>4</td>
<td>15-20 kg</td>
</tr>
<tr>
<td>0.05 ml</td>
<td>5</td>
<td>20-25 kg</td>
</tr>
<tr>
<td>0.06 ml</td>
<td>6</td>
<td>25-30 kg</td>
</tr>
<tr>
<td>0.07 ml</td>
<td>7</td>
<td>30-35 kg</td>
</tr>
</tbody>
</table>
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Marco Ruggiero, Heinz Reinwald, Stefania Pacini *

dr. reinwald healthcare gmbh + co kg, Friedrich-Luber-Straße 29, D-90392 Schwarzenbruck, Germany

Chondroitin sulfate/Vitamin D3/Oleici Acid
(40 patients = 39 male/1 female)

No Change 17,5%
Better 80 %
Worse 2,5%
GCMAF - 2013

Clinical Outcome of 100 patients
Median Age = 6,2 yo (3-23 yo)

83,1% better
12,7% unchanged
4,2% worse
Conclusion

The pathway of healing the Neuroinflammation in brain of ASD require a complex strategy of interventions aimed from one side to remove those environmental factors that chronically stimulate inflammation in all the body and from other side break the vicious circle of Mast Cell-Glial network activation.
The Italian Team:
Marco Ruggiero
Dario Siniscalco
Annalisa Brigida
Alessandra Cirillo

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