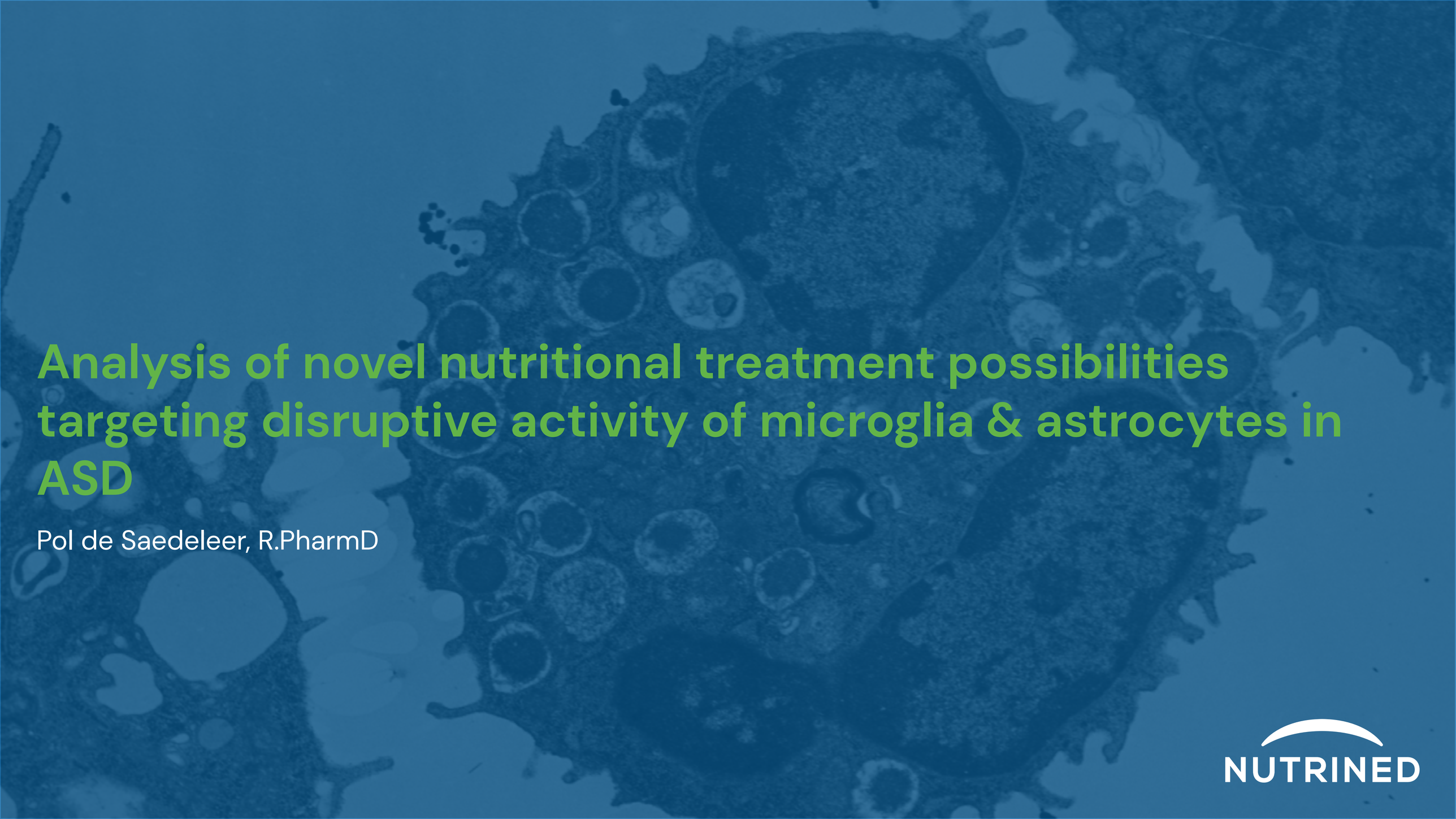


Analysis of novel nutritional treatment possibilities targeting disruptive activity of microglia & astrocytes in ASD

PoI de Saedeleer, R.PharmD

NUTRINED

Autism Spectrum Disorder: a multifactorial disease

From the “refrigerator mother theory” to a developmental disorder with associated biological dysfunctions

Refrigerator mother theory (Kanner 1956):
traumatized unloved children develop autism

Common symptoms in AD even if the presentation is very heterogeneous

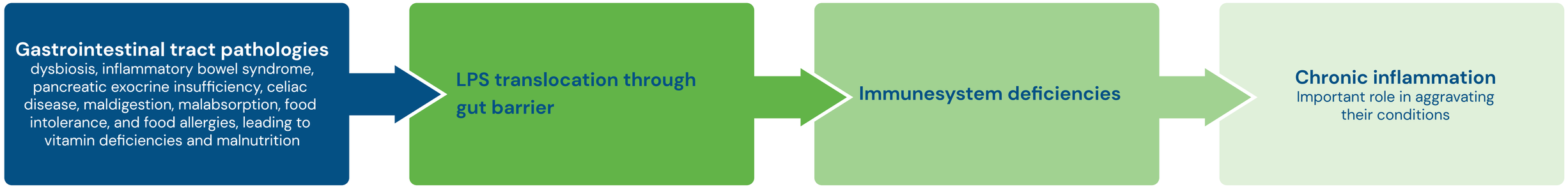
- poor social interactions, poor social communication sometimes non-verbal
- repetitive behavior
- sometimes aggression, anxiety & depression
- epilepsy, comorbidity

Symptoms manifest early – it's basically a development disorder
Incidence 1 on 150

Multi-factorial, multi-systemic disease with different biological abnormalities

1. Gastrointestinal tract pathologies

Horvath, Karoly, and Jay A. Perman. “Autism and gastrointestinal symptoms.” Current gastroenterology reports 4.3 (2002): 251-258.



2. Immune deficiencies

Poor innate immunity, disrupted adaptive immunity, autoimmunity

3. Disrupted biochemical pathways → impaired ability to detoxify

- methylation
- transsulphation

4. Neuroinflammation

Increasing evidence exists that neuroinflammation caused by immune activation, exacerbated with reactive gliosis, contributes to the pathogenesis of ASD.

Cubala-Kucharska, Magdalena. “The review of most frequently occurring medical disorders related to aetiology of autism and the methods of treatment.” Acta Neurobiol Exp (Wars) 70.2 (2010): 141-146.

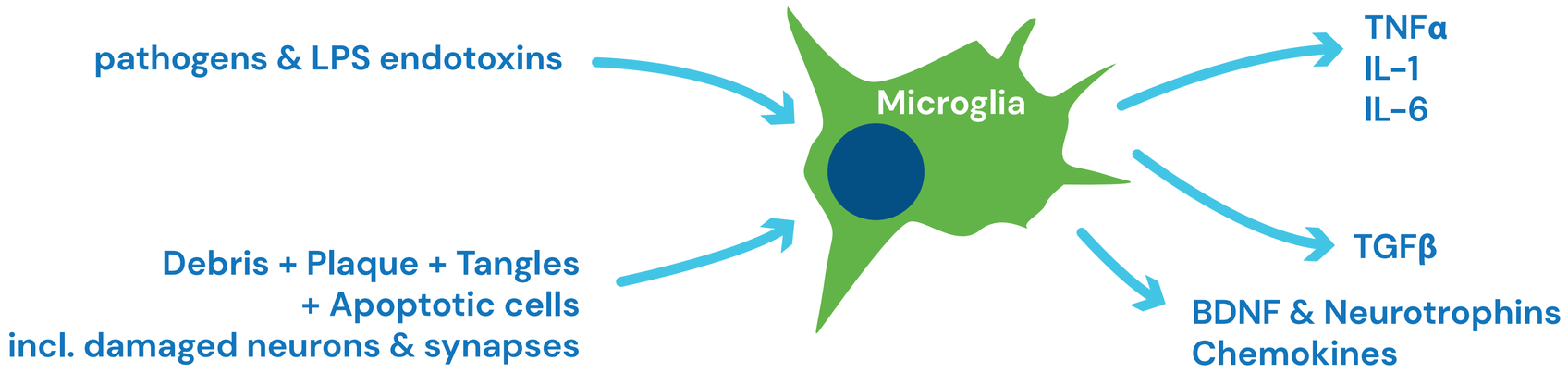
Petrelli, Francesco, Luca Pucci, and Paola Bezzi. “Astrocytes and microglia and their potential link with autism spectrum disorders.” Frontiers in cellular neuroscience 10 (2016): 21.

Neuroinflammation in ASD

Microglial cells are the resident macrophages of the brain that form the innate immune defense system

Microglia are in constant surveillance

After conversion from a resting state to an activated form, they detect and remove pathogens, endotoxins and self-antigens like amyloid plaque, myelin debris, apoptotic cells

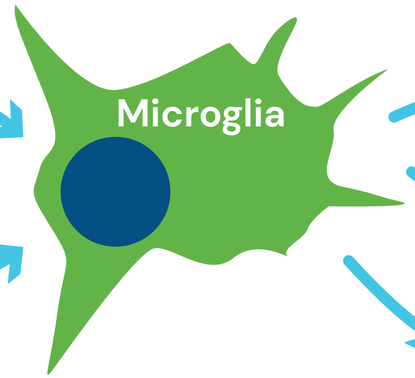


Simultaneously a wide variety of soluble factors are released : inflammatory mediators like TNFα, IL-1, IL-6, Prostaglandins, Neuroptrophic factors like BDNF
Goal = reorganization and recruitment of more immune cells like Astrocytes

pathogens & LPS endotoxins

Incomplete clearance / poor sleep

Debris + Plaque + Tangles
+ Apoptotic cells
incl. damaged neurons & synapses

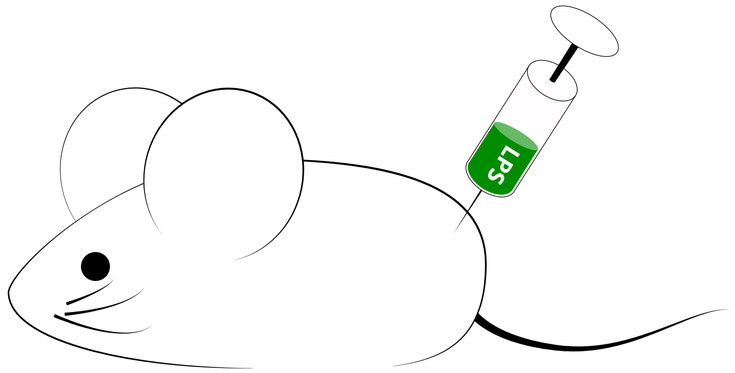


Excessive release of inflammatory mediators
= neuroinflammation

TNFα ▲
IL-1 ▲
IL-6 ▲

TGFβ

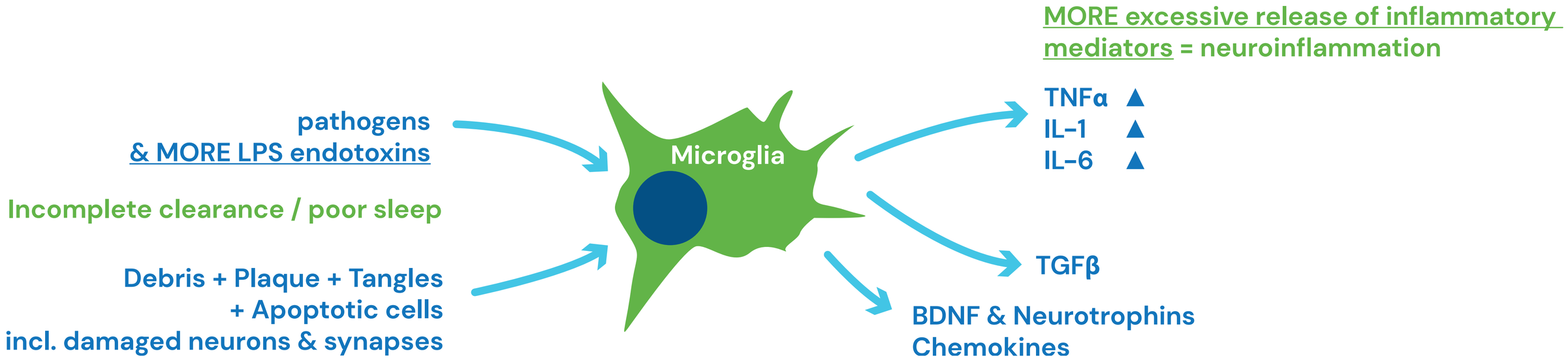
BDNF & Neurotrophins
Chemokines



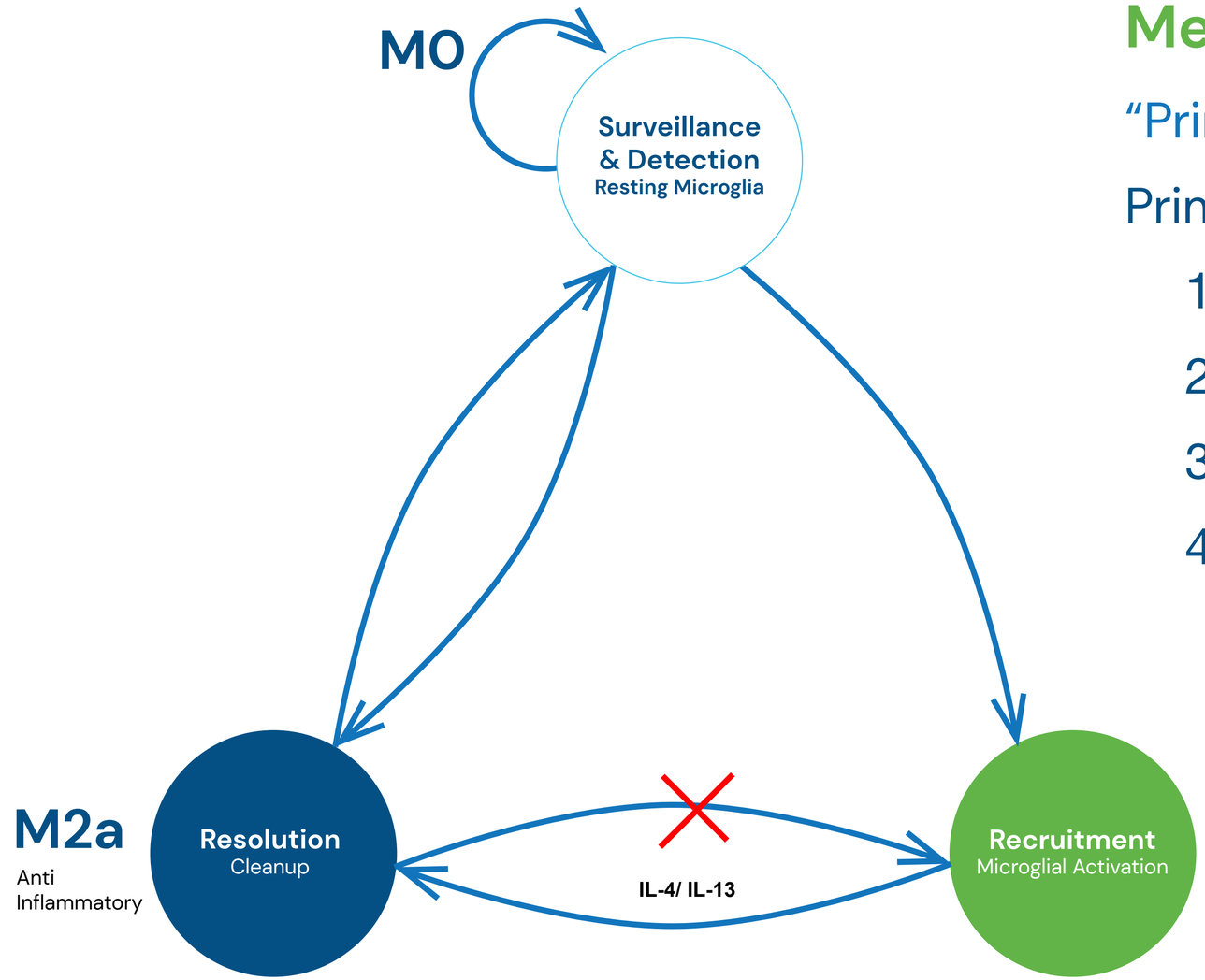
Repeated exposure to systemic immune challenge increased brain IL-1β

LPS challenge on a regular basis

+ higher levels inflammatory mediators cause cognitive issues, behavior issues



Hoogland, Inge, et al. "Microglial activation after systemic stimulation with lipopolysaccharide and Escherichia coli." Frontiers in cellular neuroscience 12 (2018): 110.

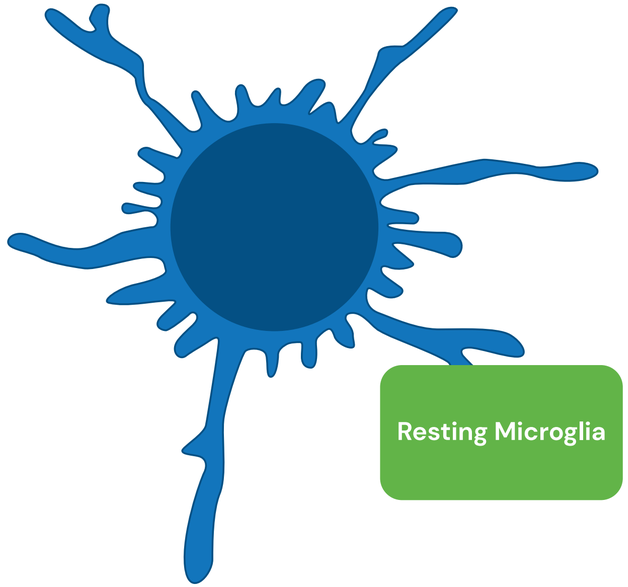


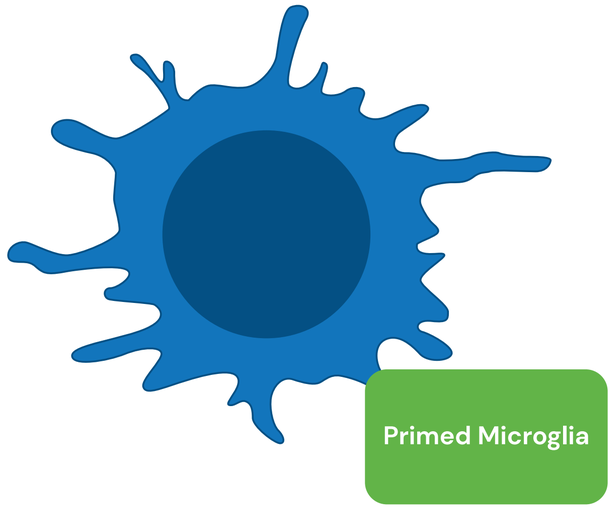
Medical history causing primed microglia

"Priming is a stage with no way back"

Priming results from

1. Traumatic Brain Injury (TBI)
2. Stress
3. Aging
4. Pre-existing inflammation





What are primed microglia?

Primed microglia have changed morphology:

- Less branches or no branches
- Amoeboid structure
- Exaggerated and uncontrolled inflammatory response to any kind of secondary challenge
  = More reactive to secondary insults
- Microglia are in a dominant M1 shift and stay chronically inflamed

Hanisch, Uwe-Karsten, and Helmut Kettenmann. “Microglia: active sensor and versatile effector cells in the normal and pathologic brain.” Nature neuroscience 10.11 (2007): 1387-1394.

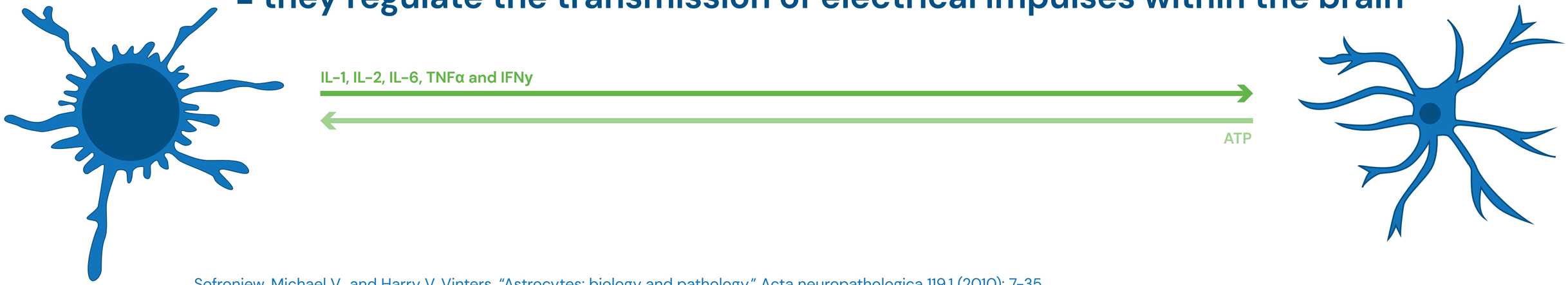
Matta, Samantha M., Elisa L. Hill-Yardin, and Peter J. Crack. “The influence of neuroinflammation in Autism Spectrum Disorder.” Brain, behavior, and immunity 79 (2019): 75-90.

Microglial-astrocytic interactions: dual talk

Astrocytes are star-shaped glial cells
Astrocytes perform many functions in brain

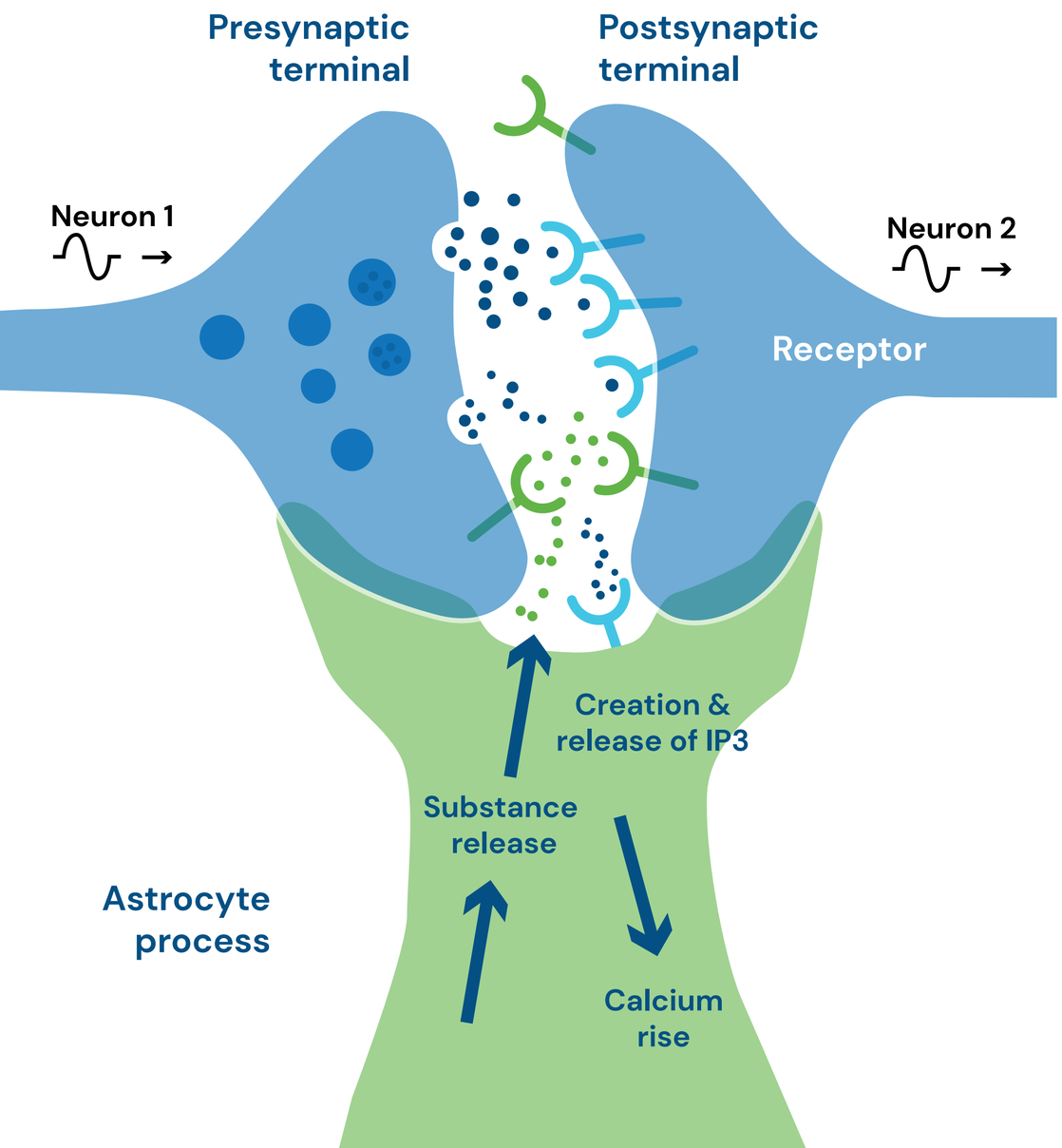
- biochemical support of endothelial cells that form the blood-brain barrier
  Contributes to the maintenance of BBB integrity
- repair and scar formation of the brain and the spinal cord following traumatic injuries
- propagation of neurotransmission , similar to neurons they release transmitters called gliotransmitters

= they regulate the transmission of electrical impulses within the brain



Sofroniew, Michael V., and Harry V. Vinters. "Astrocytes: biology and pathology." Acta neuropathologica 119.1 (2010): 7-35.

Michinaga, Shotaro, and Yutaka Koyama. "Dual roles of astrocyte-derived factors in regulation of blood-brain barrier function after brain damage." International journal of molecular sciences 20.3 (2019): 571.



Concept of tripartite synapse has been proposed

Tripartite synapse

- Presynaptic membrance, postsynaptic membrane and intimate association with surrounding astrocytes
- Astrocytes are integrated in bidirectional synaptic communication

Other functions of astrocytes

- **Fuel:** Astrocytes contain Glycogen and are capable of Gluconeogenesis; the astrocytes next to the neurons in the hippocampus and the frontal cortex can fuel neurons during periods where high rates of glucose are needed
- **Vasomodulation:** Astrocytes regulate blood flow
- Promotion of the myelinating activity of oligodendrocytes

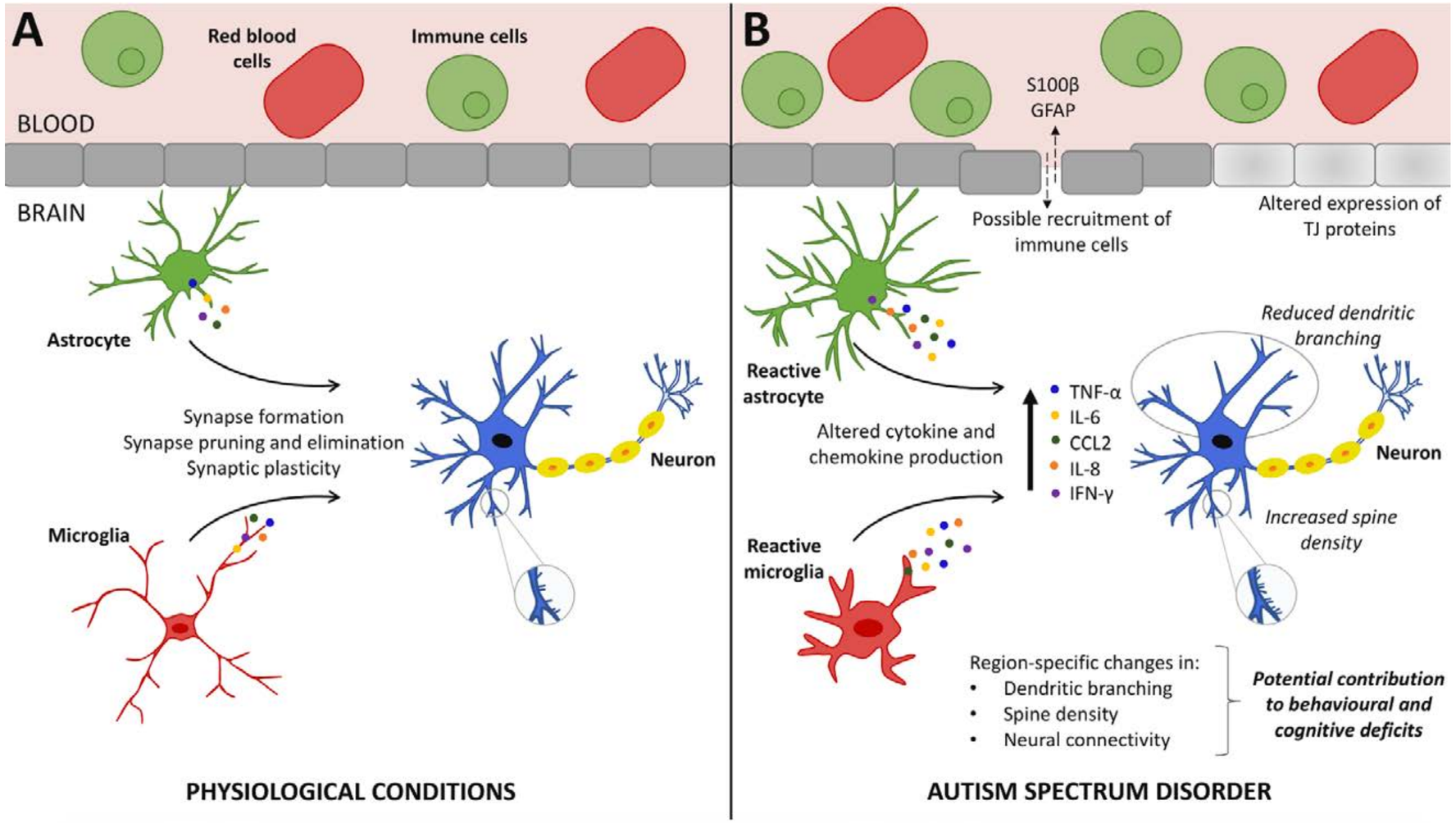
Increasing evidence shows that astrocytes and microglial cells play a major role in synapse maturation and function

Clarke, Laura E., and Ben A. Barres. “Emerging roles of astrocytes in neural circuit development.” Nature Reviews Neuroscience 14.5 (2013): 311-321.

Sahlender, Daniela A., Iaroslav Savtchouk, and Andrea Volterra. “What do we know about gliotransmitter release from astrocytes?.” Philosophical Transactions of the Royal Society B: Biological Sciences 369.1654 (2014): 20130592.

Increasing evidence exists that neuroinflammation caused by excessive immune activation, exacerbated with reactive gliosis, contributes to the pathogenesis of ASD.

Matta SM, Hill-Yardin EL, Crack PJ. The influence of neuroinflammation in Autism Spectrum Disorder [Internet]. Vol. 79, Brain, Behavior, and Immunity. 2019 [cited 2020 Sep 1]. p. 75–90.



Microglial (over-)activation and astrocyte dysfunction both contribute to improper neural circuitry which underlies certain disorders like asd

Proof for the association reactive microglia and astrocytes in ASD

- Cerebellum of postmortem ASD patient:
  - Extensive microglial activity: increased microglial soma size, retraction/thickening of filopodia and increased microglial density
    - Visualized by immunocytochemical staining for human leukocyte antigen-DR (HLA-DR)
  - Increased astrocyte immunoreactivity: western analysis of GFAP
- ASD is linked with immune dysfunction and synaptic deficits
  - Maternal immune activation (MIA) is mouse models
    - LPS or Polyinosinic:polycytidylic: altered microglial receptor function, deficits in synaptic pruning in the hippocampus, immune dysregulation and ASD relevant behaviour
    - Inhibiting the activity of interleukin-6 (IL-6) via administration of anti-inflammatory reagents: alleviation of the autism-like behaviors

Vargas, D.L., Nascimbene, C., Krishnan, C., Zimmerman, A.W., Pardo, C.A., 2005. Neuroglial activation and neuroinflammation in the brain of patients with autism. Ann. Neurol. 57, 67-81.

Tetreault, N.A., Hakeem, A.Y., Jiang, S., Williams, B.A., Allman, E., Wold, B.J., et al., 2012. Microglia in the cerebral cortex in autism. J. Autism Dev. Disord. 42, 2569–2584.

Parker-Athill, E., Luo, D., Bailey, A., Giunta, B., Tian, J., Shytle, R.D., et al., 2009. Flavonoids, a prenatal prophylaxis via targeting JAK2/STAT3 signaling to oppose IL- 6/MIA associated autism. J.Neuroimmunol. 217, 20–27.

Voineagu, Irina, et al. “Transcriptomic analysis of autistic brain reveals convergent molecular pathology.” Nature 474.7351 (2011): 380-384.

Surveillance mechanisms are mainly controlled by microglia & astrocytes, ensuring that inflammatory processes efficiently remove invading pathogens and self-antigens and contribute to tissue repair.

Any dysfunction in these mechanisms leads to chronic inflammation

Chronic inflammation plays a role in the pathogenesis of ASD

As a result chronic inflammatory conditions (autoimmune disorders or infections) are often observed in the mothers of children with ASD – as a causative contributing factor

Altered levels of maternal cytokines contribute to ASD-like behavior in the offspring.

Atladóttir, Hjördís Ó., et al. "Association of family history of autoimmune diseases and autism spectrum disorders." Pediatrics 124.2 (2009): 687-694.

Keil, Alexander, et al. "Parental autoimmune diseases associated with autism spectrum disorders in offspring." Epidemiology (Cambridge, Mass.) 21.6 (2010): 805.

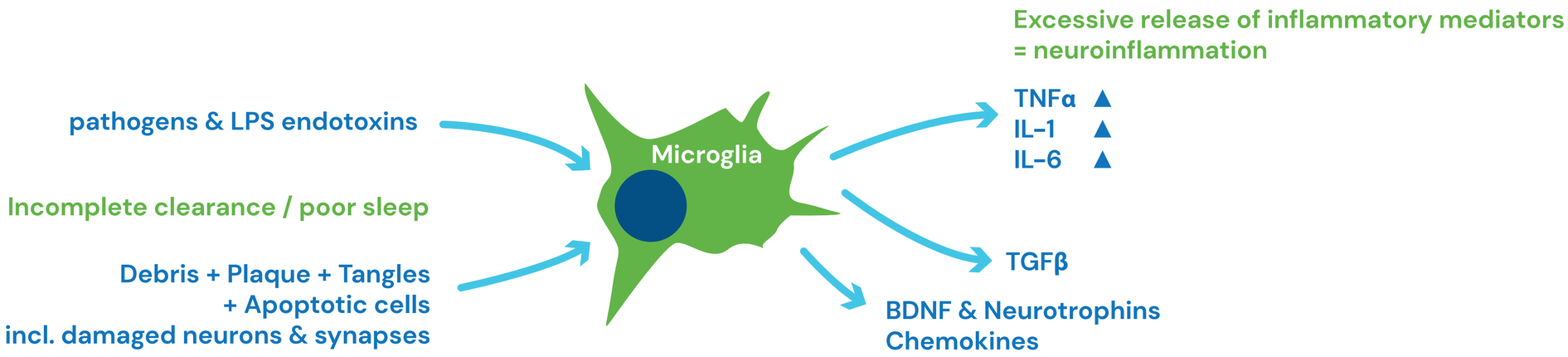
Estes, Myka L., and A. Kimberley McAllister. "Immune mediators in the brain and peripheral tissues in autism spectrum disorder." Nature Reviews Neuroscience 16.8 (2015): 469-486.

Ponzio, Nicholas M., et al. "Cytokine levels during pregnancy influence immunological profiles and neurobehavioral patterns of the offspring." Annals of the New York Academy of Sciences 1107.1 (2007): 118-128.

Smith, Stephen EP, et al. "Maternal immune activation alters fetal brain development through interleukin-6." Journal of Neuroscience 27.40 (2007): 10695-10702.

Other mechanisms serve as potential therapeutic targets in treatment of ASD

1. Oxidative stress
2. Excitotoxicity
3. Cerebral hypoperfusion
4. Immune dysfunction
5. Mast cells



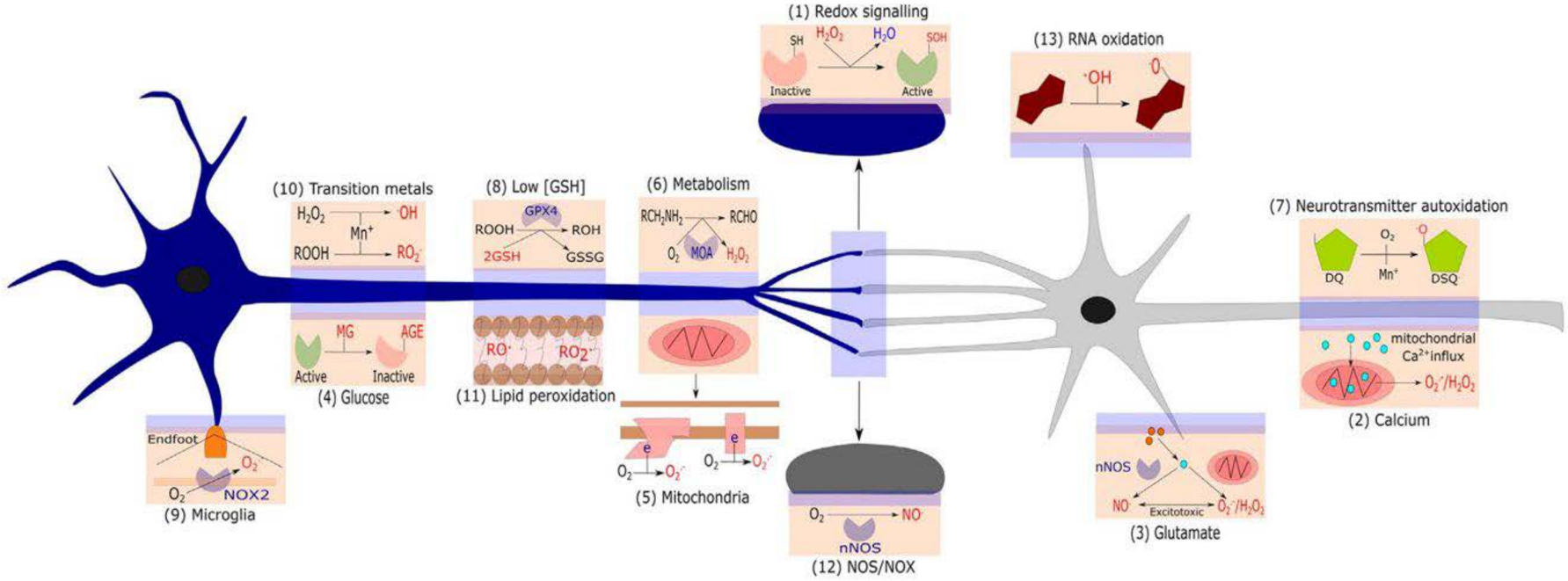
1. Oxidative stress: High levels of reactive oxygen species (ROS), which cause oxidative stress are present in ASD patients

The brain is susceptible to oxidative stress because it harnesses chemically diverse reactive species to perform signalling functions.

14 targets where O_2 interferes with the normal functioning of the brain are present in ASD patients

Human brain consumes 20% of the total basal oxygen budget

The brain is susceptible to oxidative stress because…



Oxidative stress:
imbalance between generation and elimination of ROS and RNS

Origin of ROS – related to neuroinflammation

- **Activated microglia:**
  - **production of ROS:**
    activated microglia use NADPH oxidase to generate reactive superoxide to destroy pathogens, can damage neurons if not properly balanced with antioxidants
  - **Production of RSN:**
    IL1β by activated microglia, induces the expression of iNOS gene,
    responsible for triggering NO-release
- **Mitochondrial dysfunction**

BUT dual talk:
oxidative stress induces inflammation via activation of NF-κB:
transcriptional activator of inflammatory response that can also induce the production of more free radicals

Consequences of oxidative stress

- Damage by free radicals: lipid peroxidation, oxidative modification of proteins…
- Oligodendrocytes damaged: myelination problems
- Central role in oxidative stress-induced neuronal cell death

Ljubisavljevic, Srdjan. "Oxidative stress and neurobiology of demyelination." Molecular Neurobiology 53.1 (2016): 744-758.

Leo, EE Martínez, and MR Segura Campos. "Systemic oxidative stress: a key point in neurodegeneration—a review." The journal of nutrition, health & aging 23.8 (2019): 694-699.

Block, Michelle L., Luigi Zecca, and Jau-Shyong Hong. "Microglia-mediated neurotoxicity: uncovering the molecular mechanisms." Nature Reviews Neuroscience 8.1 (2007): 57-69.

ASD patients show major alterations in the expression of genes coding for enzymes involved in the ROS scavenging system

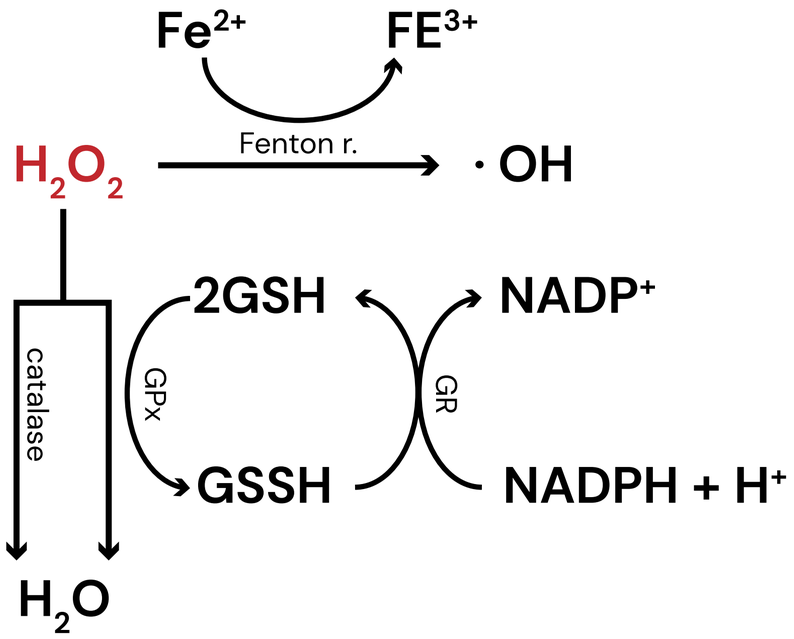
Neutralizing ROS by our own Antioxidant systems

Step 1 $O_2^- + O_2^- + 2H^+ \xrightarrow{SOD} O_2 + H_2O_2$

Step 2 $H_2O_2 + H_2O_2 \xrightarrow{CAT} 2H_2O + O_2$

Step 3 $H_2O_2 + 2GSH \xrightarrow{GPx} 2H_2O + GSHG$

$GSHG \xrightarrow{GR} 2GSH$



Glutathione plays a major role in scavenging ROS

- **Impairments in the metabolism of Glutathione have been documented=** Increased levels oxidized Glutathione, low levels reduced Glutathione in cerebella of ASD patients
- **SOD activity was higher in ASD children in comparison with controls**

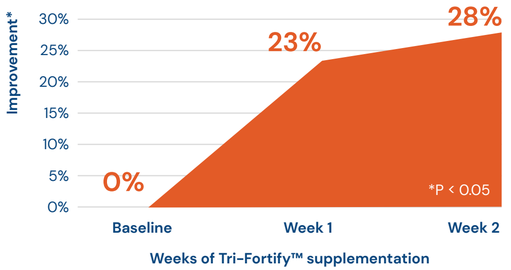
Tri-Fortify Watermelon® or Orange®

indication	Detoxification with glutathione in high bioavailable formulation, powerful antioxidant, Natural Killer Cell support	
dosage	1 teaspoon (1 pack) per day, away from food	
packaging	236 ml per tube or 20 packs per box	
composition (amount per 1 teaspoon)	Glutathione Liposomal Vitamin C	450 mg 50 mg

Please find our referenced version on the professional section of our website.
All information is exclusively aimed at and released to an audience of health care professionals.

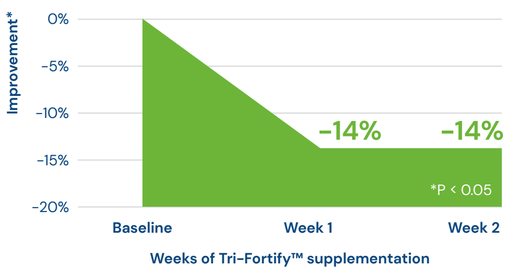
Glutathione levels

Increase in red blood cell levels (Erythrocytes)



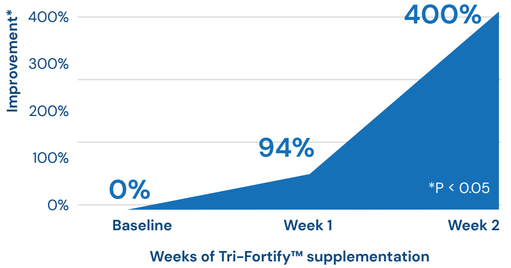
Oxidative stress markers

Oxidized / Reduced GSH

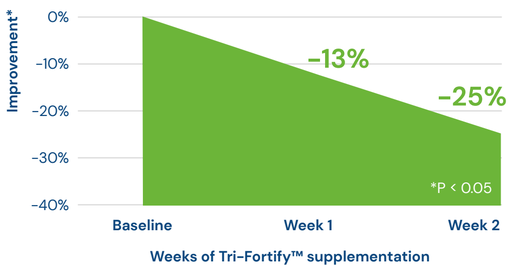


Immune function

Natural Killer Cell activity



Lipid Peroxidation (Reduced Cellular Membrane Oxidation)



Published research : Sinha, R., Sinha, I., Calcagnotto, A., Trushin, N. Oral supplementation with liposomal glutathione elevates body stores of glutathione and markers of immune function. Eur J Clin Nutr. 2018 Jan;72(1):105-111.

In addition levels of endogenous antioxidant molecules are known to be reduced in ASD in comparison with healthy controls:

- **Coenzyme Q10 is a mitochondrial antioxidant cofactor crossing BBB**
- **Administration of Q10 led to an improvement in communication**

Rose, S., et al. “Evidence of oxidative damage and inflammation associated with low glutathione redox status in the autism brain.” Translational psychiatry 2.7 (2012): e134-e134.

James, S. Jill, et al. “Metabolic endophenotype and related genotypes are associated with oxidative stress in children with autism.” American Journal of Medical Genetics Part B: Neuropsychiatric Genetics 141.8 (2006): 947-956.

Al-Gadani, Y1, et al. “Metabolic biomarkers related to oxidative stress and antioxidant status in Saudi autistic children.” Clinical Biochemistry 42.10-11 (2009): 1032-1040.

James, S. Jill, et al. “A functional polymorphism in the reduced folate carrier gene and DNA hypomethylation in mothers of children with autism.” American Journal of Medical Genetics Part B: Neuropsychiatric Genetics 153.6 (2010): 1209-1220.

Manivasagam, Thamilarasan, et al. “Role of Oxidative Stress and Antioxidants in Autism.” Personalized Food Intervention and Therapy for Autism Spectrum Disorder Management. Springer, Cham, 2020. 193-206.

Gvozdjáková, Anna, et al. “Ubiquinol improves symptoms in children with autism.” Oxidative Medicine and Cellular Longevity 2014 (2014).

Mousavinejad, Elham, et al. “Coenzyme Q10 supplementation reduces oxidative stress and decreases antioxidant enzyme activity in children with autism spectrum disorders.” Psychiatry research 265 (2018): 62-69.

2. Excitotoxicity : GLUTAMATergic /GABAergic imbalance

Glutamate: excitatory NT

Increased probability of seizures in ASD patients: enhanced glutamatergic signalling, increased expression of mRNA's encoding the AMPA1 receptor (iGlu)

GABA: inhibitory NT

GABA-mediated calcium signalling: control developmental processes which include, cell proliferation, differentiation, synapse maturation, and cell death

→ dysfunction of the GABAergic signalling early in development: severe excitatory/inhibitory imbalance in neuronal circuits, leading to behavioural deficits, as observed in ASD patients

Shinohe A, Hashimoto K, Nakamura K, Tsujii M, Iwata Y, Tsuchiya KJ, Sekine Y, Suda S, Suzuki K, Sugihara G, Matsuzaki H, Minabe Y, Sugiyama T, Kawai M, Iyo M, Takei N, MoriN: 'Increased serum levels of glutamate in adult patients with autism'. Prog Neuropsychopharmacol Biol Psychiatry 2006, 30(8):1472–1477.

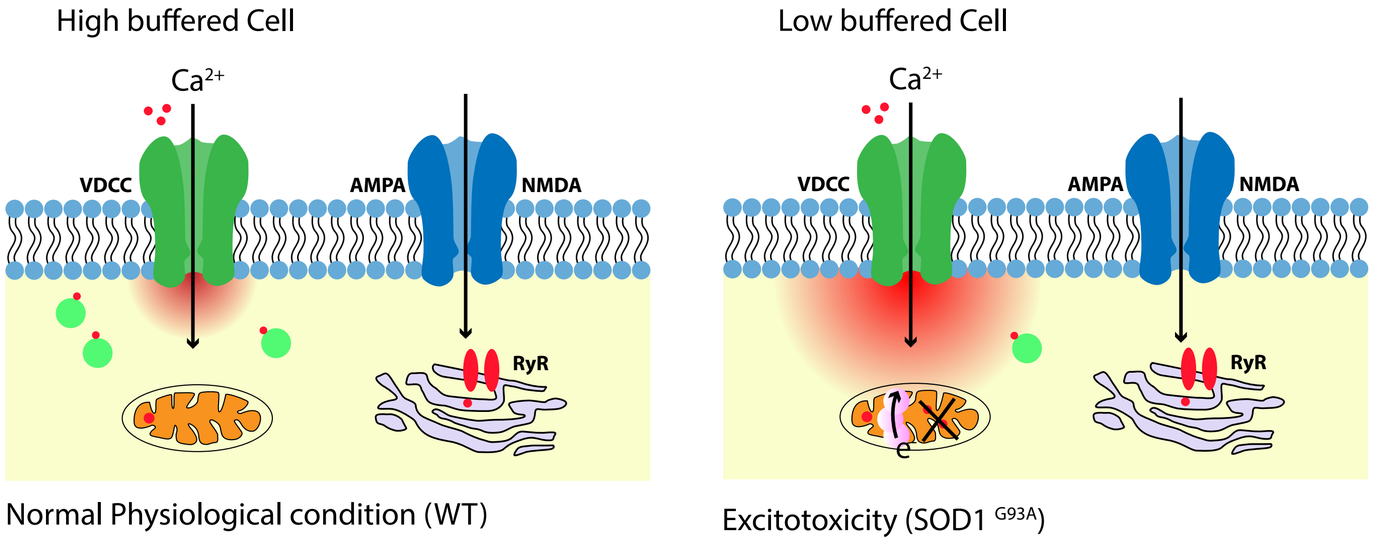
Pizzarelli R, Cherubini E: Alterations of GABAergic signaling in autism spectrum disorders. Hindawi Publishing Corporation. Neural Plast, 2011:297153. 12 pages doi:10.1155/2011/297153.

Glutamate is the primary excitatory neurotransmitter produced in CNS, an overactivity of Glutamate and it receptors leads to excitotoxicity

Excitotoxicity = excessive overactivation of NMDA & AMPA receptors triggered by extracellular accumulation of the excitatory neurotransmitter Glutamate

NMDARs & AMPAs mediate Calcium entry into the cells to regulate physiological processes such as synaptic plasticity and memory

Excessive stimulation causes a pathological increase in calcium, which activates enzymes causing damage



Normal Physiological condition (WT)

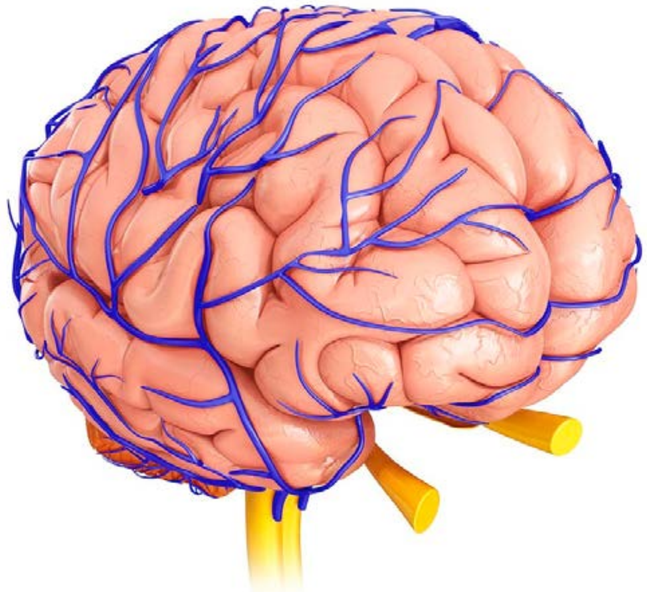
Excitotoxicity (SOD1 ^{G93A})

Levels of glutamate, GABA, glutamate/GABA, TNF-α, IL- 6, differed significantly between ASD patients and controls (n=40)

- IL-6 elevation stimulated excitatory synapse formation and impair the development of inhibitory synapses
- TNF-α was association with an impaired glutamate/ GABA ratio
- IFN-γ increasement was associated with the low glutamate/GABA ratio

Essa, M. M., et al. "Excitotoxicity in the pathogenesis of autism." Neurotoxicity research 23.4 (2013): 393-400.

El-Ansary, Afaf, and Laila Al-Ayadhi. "GABAergic/glutamatergic imbalance relative to excessive neuroinflammation in autism spectrum disorders." Journal of neuroinflammation 11.1 (2014): 189.



3. Cerebral hypoperfusion

Cerebral hypoperfusion, or insufficient blood flow in the brain, occurs in many areas of the brain in patients diagnosed with autism spectrum disorder (ASD)

Hypoperfusion detected in nearly 75% of the ASD children

10 studies detected hypoperfusion using SPECT
4 studies detected hypoperfusion using PET

Hypothesis of cause

- **Neuroinflammation:**
  - **Inflammation or swollen tissues increase pressure**
  - **Inflammation has an important role in ischemic brain damage**
    IL-1β, IL-6 and TNF-α are the primary inflammatory factors
- **Epigenetic factors**
- **Vascular Inflammation**

Zlibovicius, M., N. Boddaert, and P. Belin. "Temporal lobe dysfunction in childhood autism: a PET study. Positron emission tomography." Am J Psychiatry 152.12 (2000): 1988-1993.

Licata, G., et al. "Immunoinflammatory activation during the acute phase of lacunar and non-lacunar ischemic stroke: association with time of onset and diabetic state." International journal of immunopathology and pharmacology 19.3 (2006): 639-646.

Cerebral hypoperfusion and ASD: symptoms

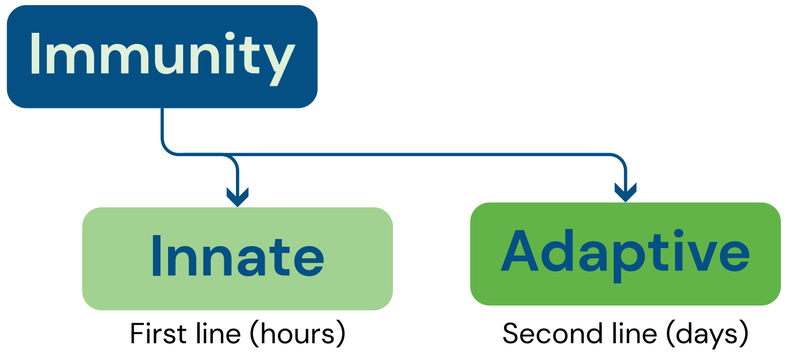
Decreased rCBF in...

- **Thalamus:** repetitive, selfstimulatory, unusual behaviours, a negative attitude towards changes in routine and environment and unusual sensory interests
- **Temporal and frontal lobes:** decreased IQ
- **Temporal lobes and amygdala:** difficulties in processing facial expressions and emotions
- **Fusiform gyrus:** difficulties in recognizing familiar faces
- **Wernicke's and Brodmann's areas:** impairments in language development and auditory processing
- **Left superior temporal gyrus:** negative correlation with the Autism Diagnostic Interview-Revised (ADI-R) scores

Bjørklund, Geir, et al. "Cerebral hypoperfusion in autism spectrum disorder." (2018).

Gallagher, Carolyn M., and Melody S. Goodman. "Hepatitis B vaccination of male neonates and autism diagnosis, NHIS 1997–2002." Journal of Toxicology and Environmental Health, Part A 73.24 (2010): 1665-1677.

Fatemi, S. Hossein, and Amy R. Halt. "Altered levels of Bcl2 and p53 proteins in parietal cortex reflect deranged apoptotic regulation in autism." Synapse 42.4 (2001): 281-284.

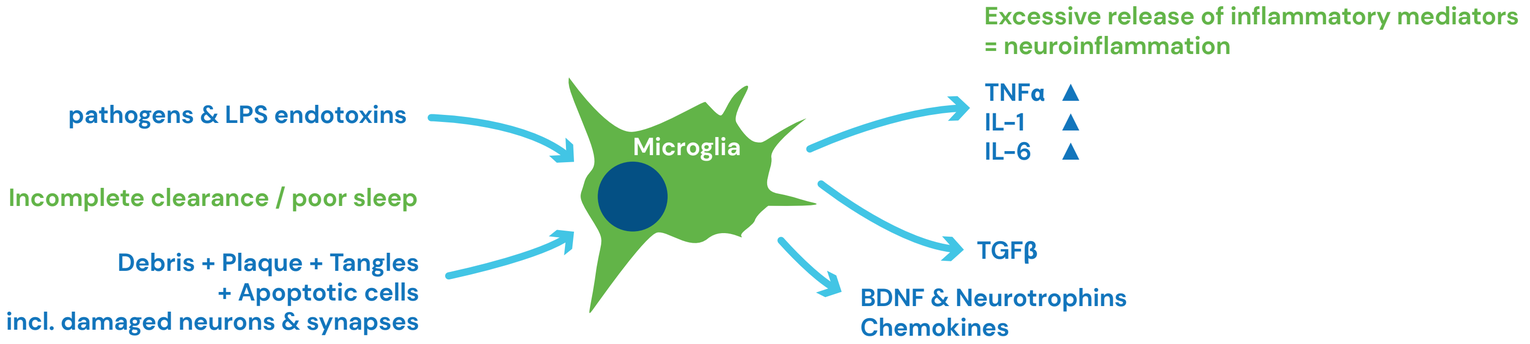


4. Immune dysfunction

Microglia, the resident macrophages, belong to the innate immune system

Microglia represent 80% of the overall of brain immune cells

Impairments in both innate and adaptive immune system support the onset of pro-inflammatory conditions



Korin, Ben, et al. "High-dimensional, single-cell characterization of the brain's immune compartment." Nature neuroscience 20.9 (2017): 1300.

Lawson, L. J., V. H. Perry, and S. Gordon. "Turnover of resident microglia in the normal adult mouse brain." Neuroscience 48.2 (1992): 405-415.

Pflieger, Fabian Johannes, et al. "The role of neutrophil granulocytes in immune-to-brain communication." Temperature 5.4 (2018): 296-307.

Autism & autoimmunity

Evidence of abnormal immune activation is well know in ASD patients:

- **Genetic explanation:**
  genetic associated studies implicate the occurrence of genes related to innate immune
- **Irregular cytokine production:**
  Post mortem studies, elevated IL-6 and TNFα and decreased production of regulatory cytokines as IL-1
- **Brain autoantibodies:**
  Antibody reactivity to several regions of the brain (rat study)
- **Maternal brain-reactive antibodies and models of neurodevelopment:**
  Purified IgG (brain-reactive antibodies) from the mothers of children with ASD can induce abnormalities in behavioral symptoms in offspring

Enstrom, Amanda M., Judy A. Van de Water, and Paul Ashwood. "Autoimmunity in autism." Current opinion in investigational drugs (London, England: 2000) 10.5 (2009): 463.

Martin, Loren A., et al. "Stereotypies and hyperactivity in rhesus monkeys exposed to IgG from mothers of children with autism." Brain, behavior, and immunity 22.6 (2008): 806-816.

Stereotypies and hyperactivity in rhesus monkeys exposed to IgG from mothers of children with autism

Hypothesis:
a possible cause of ASD is exposure of the foetal brain to maternal autoantibodies during pregnancy

4 rhesus monkeys: prenatally exposed to human IgG collected from mothers of multiple children diagnosed with ASD

4 control rhesus monkeys were exposed to human IgG collected from mothers of multiple typically developing children

5 untreated controls

Method:

- Observation in a variety of behavioural paradigms (for ex unique social situations)
- Overall activity was monitored with actimeters

Martin, Loren A., et al. “Stereotypies and hyperactivity in rhesus monkeys exposed to IgG from mothers of children with autism.” Brain, behavior, and immunity 22.6 (2008): 806-816.

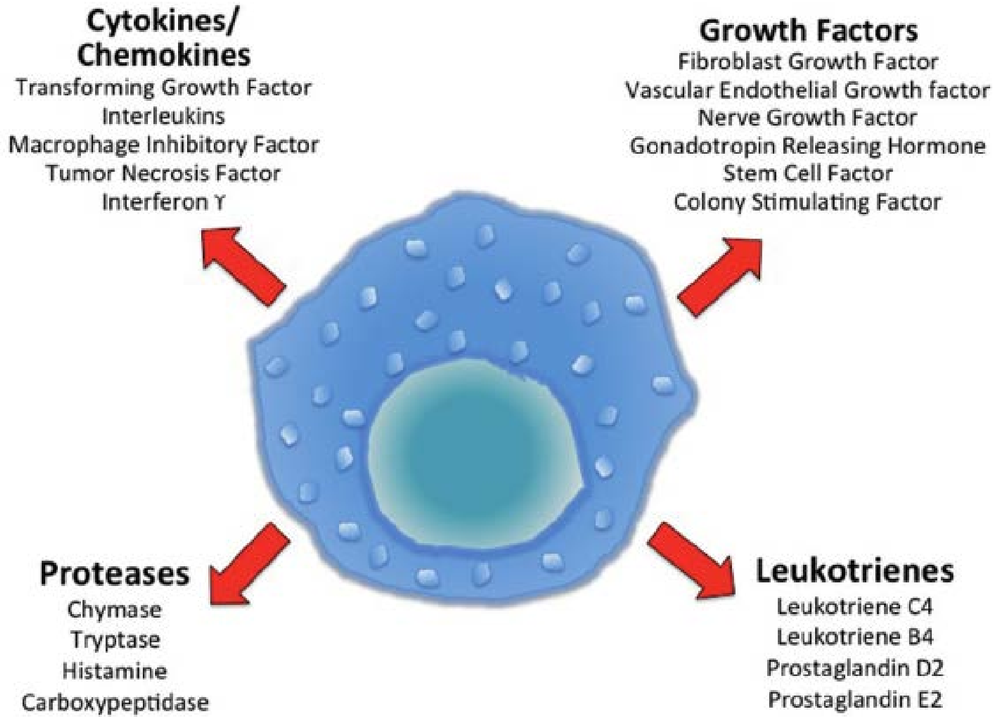
Results:

- **Prenatally IgG exposed monkeys demonstrated significantly more stereotypies than control groups**
  - 6 different testing paradigms: mother preference testing, solo observations, familiar dyadic interactions, unfamiliar dyadic interactions and during solo activity monitoring
  - 1.47 fold increase actimetters
  - not related with the present of stereotypies

Singh, Vijendra K., et al. “Antibodies to myelin basic protein in children with autistic behavior.” Brain, behavior, and immunity 7.1 (1993): 97-103

Abstract

Based on a possible pathological relationship of autoimmunity to autism, antibodies reactive with myelin basic protein (anti-MBP) were investigated in the sera of autistic children. Using a screening serum dilution of 1:400 in the protein-immunoblotting technique, approximately 58% (19 of 33) sera of autistic children (< or = 10 years of age) were found to be positive for anti-MBP. This result in autistics was significantly (p < or = .0001) different from the controls (8 of 88 or only 9% positive), which included age-matched children with normal health, idiopathic mental retardation (MR) and Down syndrome (DS), and normal adults of 20 to 40 years of age. Since autism is a syndrome of unknown etiology, it is possible that anti-MBP antibodies are associated with the development of autistic behavior.



5. Mast cells and Neuroinflammation

The Mastcell is a tissue resident granulocyte, active in the allergic response but also playing a vital role in immune tolerance.

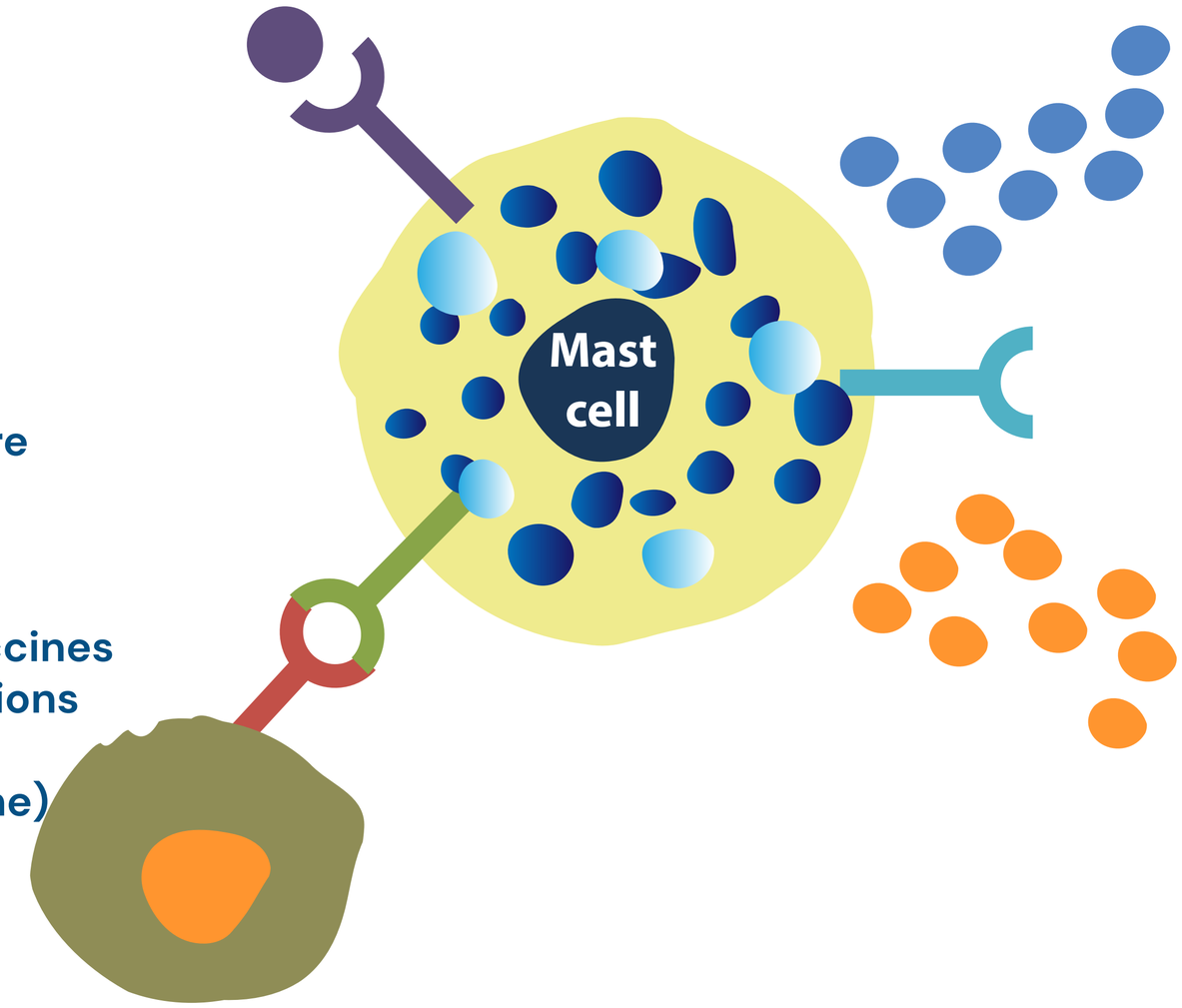
Mastcells are activated by the binding of allergens to receptor-bound IgE or by multiple other non-specific stimuli.

After activation Mastcells release histamine and many other mediators

Mast Cell Activators

Receptor-binding agonists
IgE + Antigen or IgE alone
Ig light chain
Complement
Neuropeptides
Microbial products
Cytokines
Chemokines
Physical activators
Heat, changes in temperature
Pressure

CRH / stress, anxiety
NSAID's, Opioids, Anesthetics, **Vaccines**
Insect bites: Lyme & Co-infections
Viral infections
Electromagnetic waves (phone)
Mold / Mycotoxins
Parasites, Fungal infections
Heavy Metals, Pesticides
Methylation issues
Estrogen Dominance
Genetic Predisposition
Glyphosate



Mast Cell Molecules

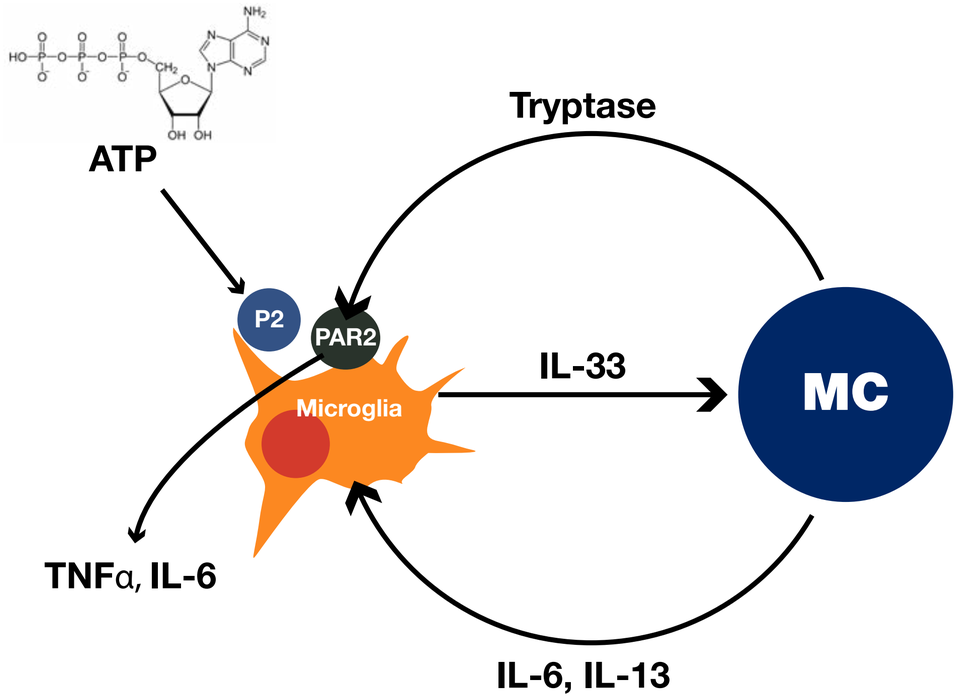
Preformed mediators
Histamine
Proteases
Serotonin
Heparin
IL-4, TNF, GM-CSF

T and B cell ligands
PD-L1, OX40L, CD30L,
CD40L, CCL19, 4-1BB

Newly synthesized mediators
Lipid derived: Prostaglandins
Leukotrienes
PAF
Cytokines
Growth Factors
Chemokines
Free radicals
others: Substance P

Mast cells:

- **Reside on the brain side of the BBB**
- **Can penetrate the BBB and break its integrity**
  By secretion of vasoactive and matrix degrading components such as histamine, NO, VEGF, TNF-α



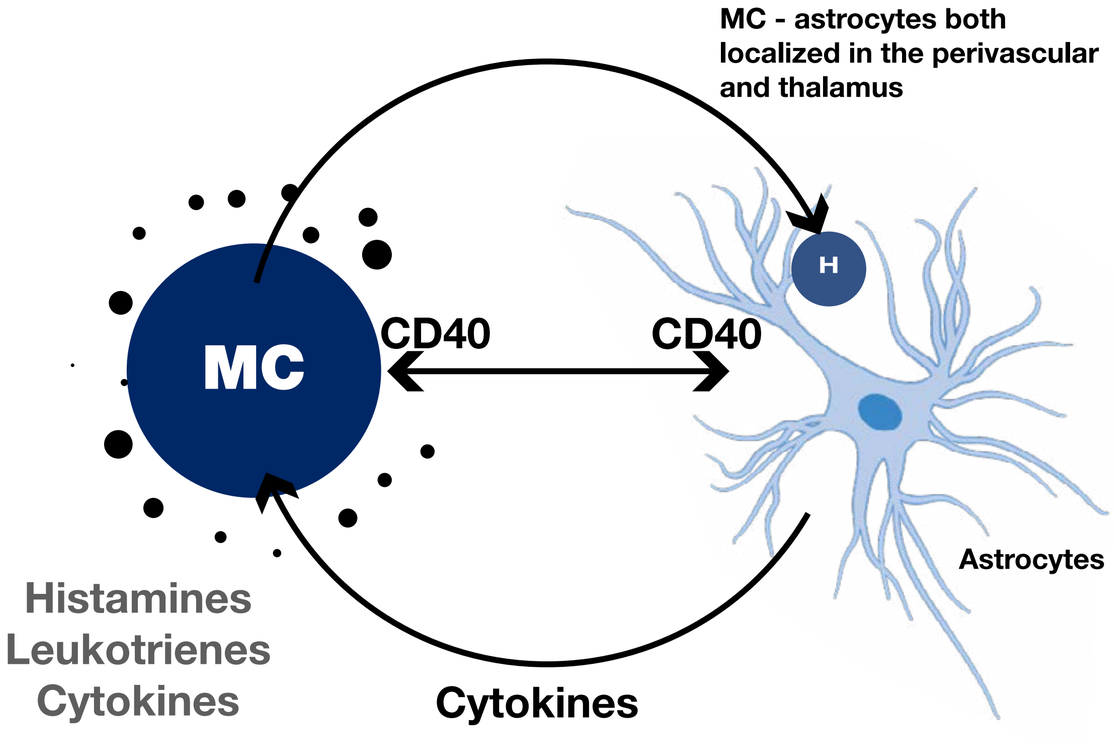
Dual talk between Microglia & Mastcells

= Partners in crime?

= Recognition that there is extensive communication between the immune system and the central nervous system

- ATP released from damaged cells is activating microglial P2 receptors to release IL-33
- IL-33 binds to MC receptors
- This event triggers the release of IL-6 & IL-13, which actually activates microglia
- Similarly Tryptase from MC's turns on PAR2 receptors on microglia to release TNF-alpha & IL-6

+ **Neuropeptides directly activate Mastcells**



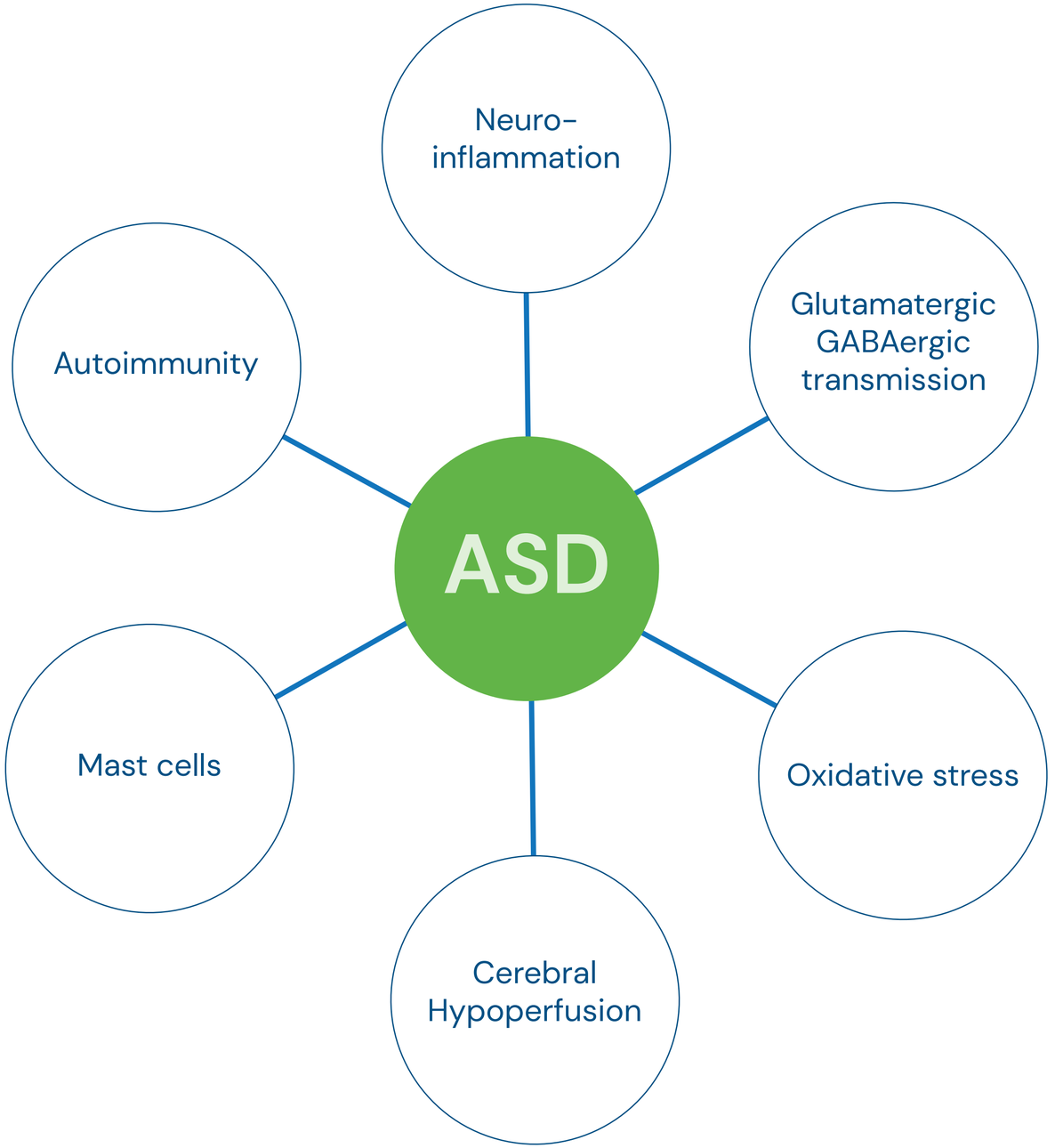
Dual talk between Mast cells & astrocytes

- CD40-CD40 ligand interactions
- MC's are stimulated to release Histamine, Leukotrienes and Cytokines
- Astrocytes have Histamine-receptors and in turn cytokines released by Astrocytes induce further MC degranulation
- **Children with macrocytosis have tenfold higher prevalence of ASD than the general population**

Dong, Hongquan, Xiang Zhang, and Yanning Qian. "Mast cells and neuroinflammation." Medical science monitor basic research 20 (2014): 200.

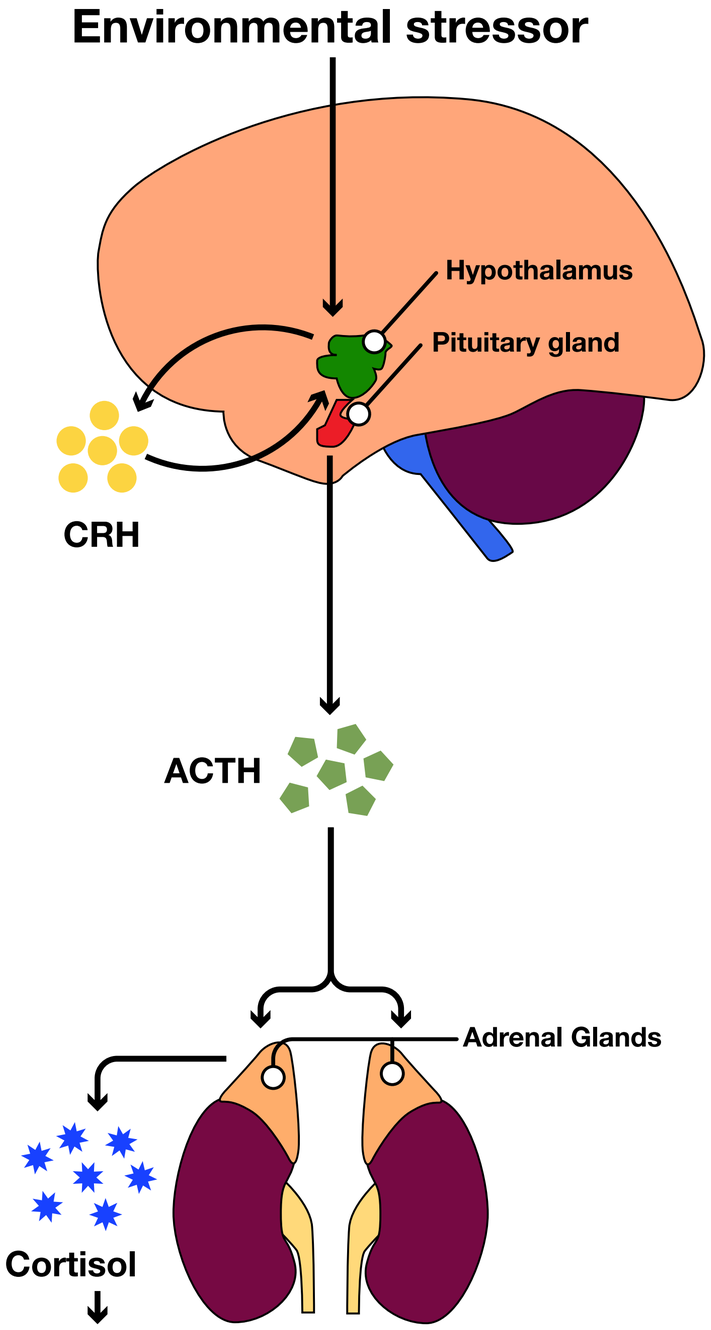
Dong, Hongquan, et al. "Stabilization of brain mast cells alleviates LPS-induced neuroinflammation by inhibiting microglia activation." Frontiers in cellular neuroscience 13 (2019): 191.

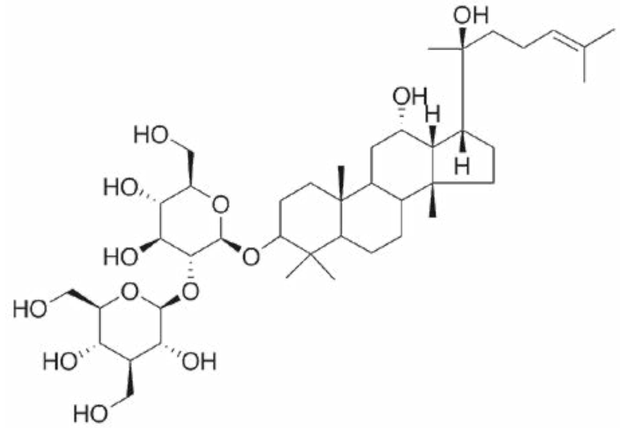
Theoharides, Theoharis C. "Autism spectrum disorders and mastocytosis." (2009): 859-865.


Neuro-
inflammation
Autoimmunity
Glutamatergic
GABAergic
transmission
ASD
Mast cells
Oxidative stress
Cerebral
Hypoperfusion

Evaluation of 3 novel compounds aimed to counteract the interplay between neuroinflammation, oxidative stress, excitotoxicity, cerebral hypoperfusion, immune dysfunction and mast cells

- **RG3 nasal application**
- **Butyrate coated**
- **PEA**





1. RG3 nasal application

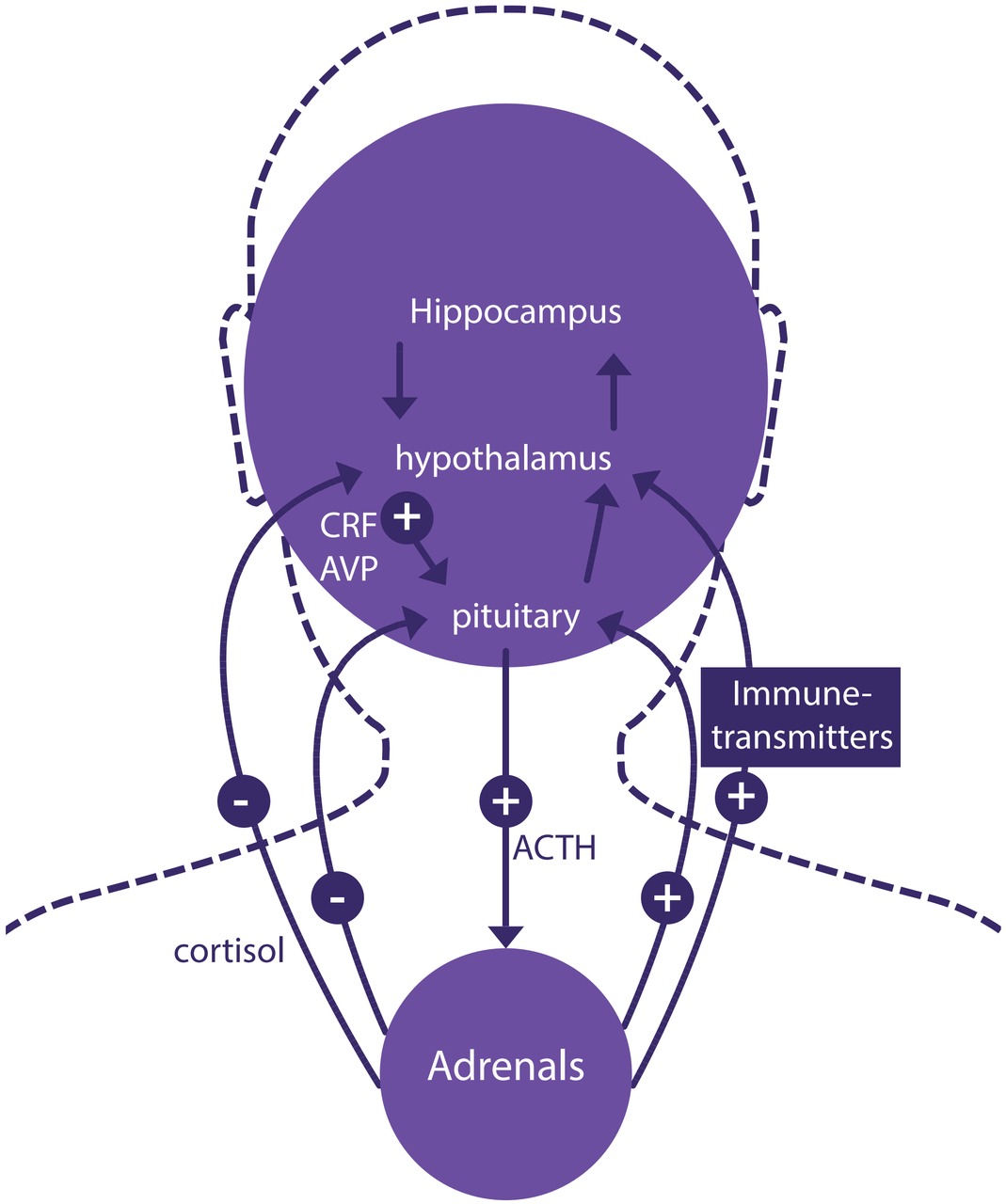
Ginsenoside Rg3 is a steroidal saponin that is highly enriched in Korean Red Ginseng. It exhibits a broad range of pharmacological activities

RG3 was first introduced for its neuroprotective activity on neuronal cells in adrenal fatigue:

The hypothalamus-puititary-adrenal axis (HPA-axis) is our major neuroendocrine axis

In stressful situations Corticoid hormones are secreted from the adrenals = our response to stress

Oxidative stress



The hippocampus is the regulator our stress response + mediator of the activity of the hypothalamus

If left unchecked... Cortisol gets catabolic

= Breakdown of epithelial lining
= Breakdown of blood-brain barrier
= Breakdown of the vascular lining
= Breakdown of skeletal muscles

The hippocampus can't sustain this overactivated state and will get damaged

Hippocampus will go from swollen to shrunken state

= **BRAIN DAMAGED THROUGH STRESS**

Jacobson, Lauren, and Robert Sapolsky. "The role of the hippocampus in feedback regulation of the hypothalamic-pituitary-adrenocortical axis." Endocrine reviews 12.2 (1991): 118-134.

Gjerstad, Julia K., Stafford L. Lightman, and Francesca Spiga. "Role of glucocorticoid negative feedback in the regulation of HPA axis pulsatility." Stress 21.5 (2018): 403-416.

Oxidative stress

The nasal delivery system guarantees biodisponibility through the blood-brain barrier.

usual dose

2x 2 sprays per nostril

Neuronal Cells + Dexamethasone + RG3
= Neuroprotection

Neuronal Cells + Dexamethasone
= Neurotoxicity / Neuronal Death

Sung-Ok K, Jung-Man Y, et al. Ginsenoside Rb1 and Rg3 attenuate glucocorticoid-induced neurotoxicity. Cell mol neurobiol. 2010,857-862

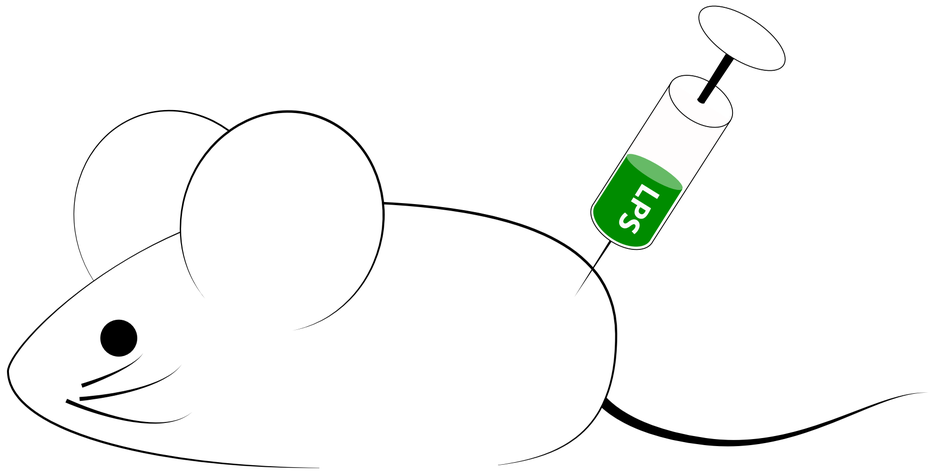
Seong SJ, Yeong MY, et al. Prevention of inflammation-Mediated Neurotoxicity by Rg3 and it's role in microglial activation. Research Institute of veterinary medicine. 2008,156-756

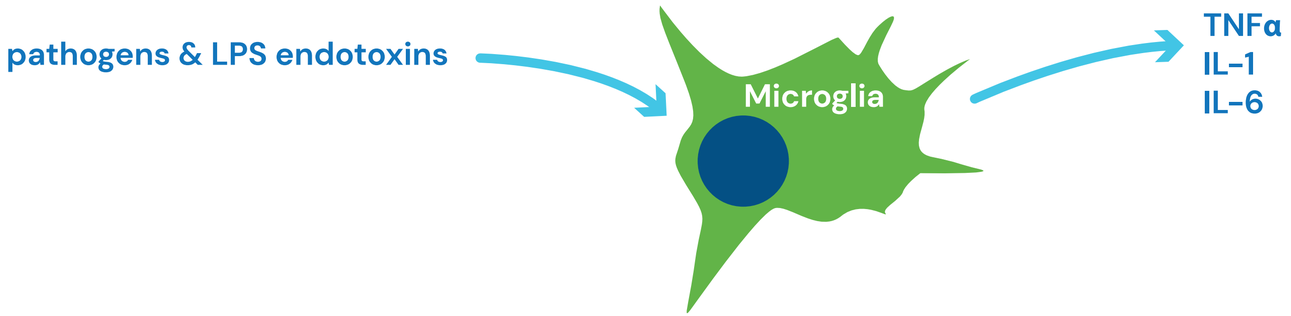
Hyeongming Kim, Jong Hyuk Lee, et al. Micro-/nano-sized delivery systems of ginsenosides for improved systemic bioavailability. J Ginseng Res. 2018, 19

Oxidative stress

Evaluation of properties RG3 beneficial in treatment ASD:

Modulation of neuroinflammation : peripheral injection of LPS in mice





Neuro-inflammation

Rg3 and the expression of inflammatory mediators in the hippocampus:

Induction of neuroinflammation: peripheral LPS injection

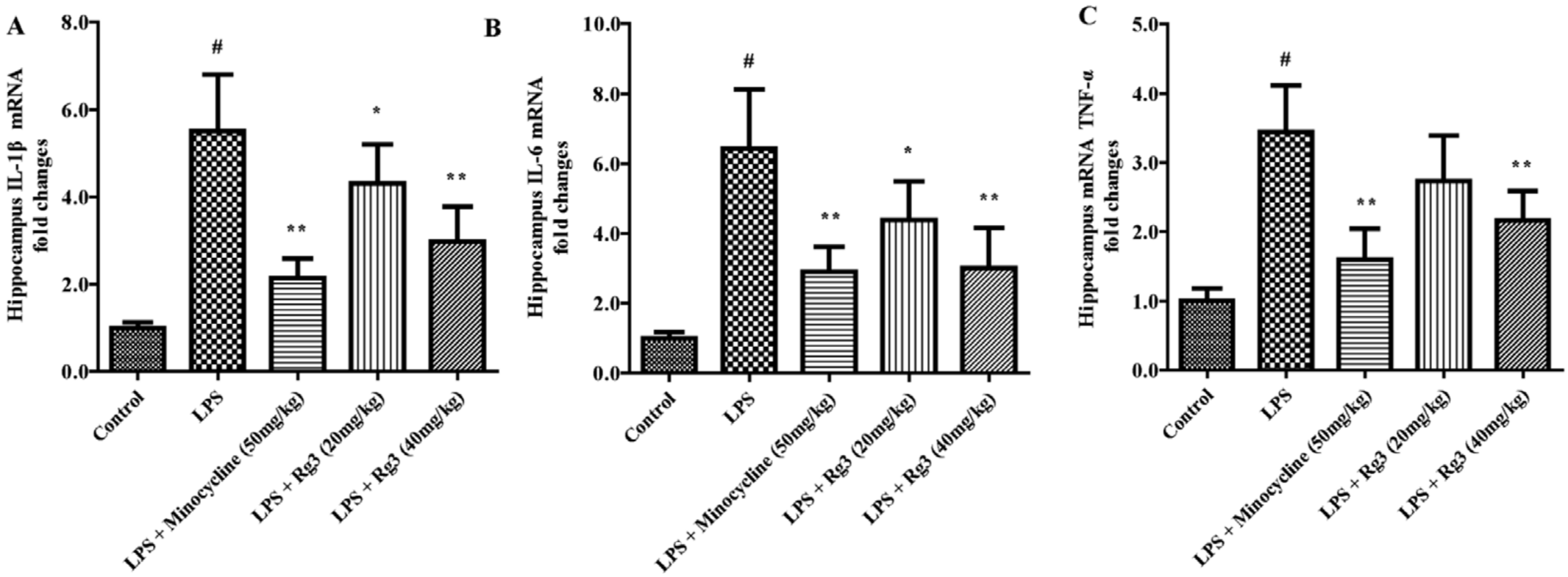
→ markedly increased mRNA expression of IL-1β, IL-6, TNF-α
(mRNA concentrations analysed with Quantitative Real-Time PCR)

→ Pre-treated with Rg3 **20 mg/kg**: significantly attenuated expression of IL-1β and IL-6

→ Pre-treated with Rg3 **20 mg/kg**: significantly attenuated expression of IL-1β, IL-6 and TNF-α

→ Minocycline used as positive control

Kang, An, et al. "Suppressive effect of ginsenoside Rg3 against lipopolysaccharide-induced depression-like behavior and neuroinflammation in mice." Journal of agricultural and food chemistry 65.32 (2017): 6861-6869.



Rg3 treatment decreases expression of inflammatory mediators

Neuro-inflammation

Kang, An, et al. "Suppressive effect of ginsenoside Rg3 against lipopolysaccharide-induced depression-like behavior and neuroinflammation in mice." Journal of agricultural and food chemistry 65.32 (2017): 6861-6869.

Cytokine profile of ASD

	Findings	Sample
TNF-α	↑	CSF and brain tissue
IL-6	↑	Brain tissue
IL-1β	=	Brain tissue

Li, X., Chauhan, A., Sheikh, A.M., Patil, S., Chauhan, V., Li, X.M., et al., 2009. Elevated immune response in the brain of autistic patients. J. Neuroimmunol. 207, 111–116.

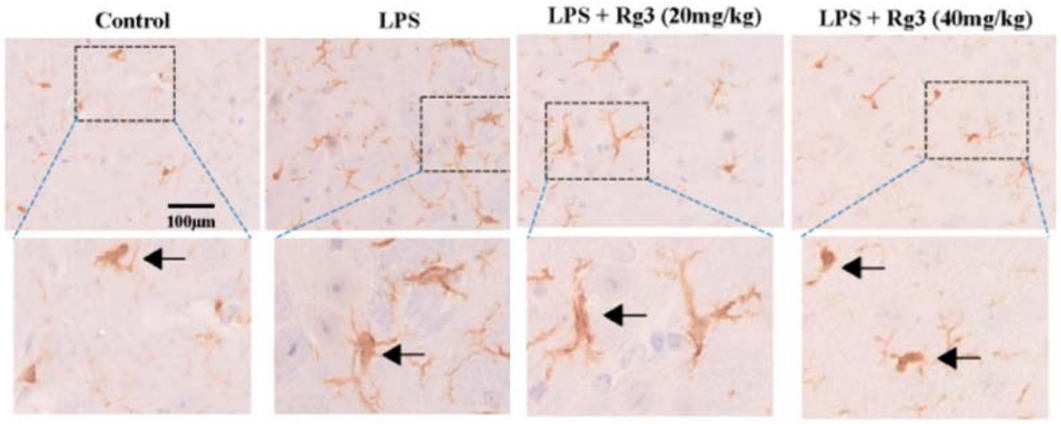
Chez, M.G., Dowling, T., Patel, P.B., Khanna, P., Kominsky, M., 2007. Elevation of tumor necrosis factor-alpha in cerebrospinal fluid of autistic children. Pediatr. Neurol. 36, 361–365.

Wei, H., Zou, H., Sheikh, A.M., Malik, M., Dobkin, C., Brown, W.T., et al., 2011. IL-6 is increased in the cerebellum of autistic brain and alters neural cell adhesion, migra- tion and synaptic formation. J. Neuroinflammation 8, 52. 5Vargas, D.L., Nascimbene, C.,

Krishnan, C., Zimmerman, A.W., Pardo, C.A., 2005. Neuroglial activation and neuroinflammation in the brain of patients with autism. Ann. Neurol. 57, 67–81.

Rg3 supresses microglia activation:

Immunohistochemical images of Iba-1 in the brain:



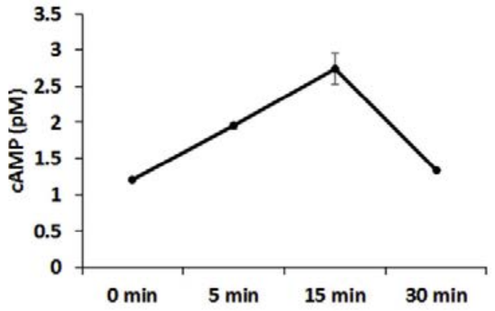
Rg3 treatment significantly reduced numbers of overactivated microglia (compared to the LPS treated group)

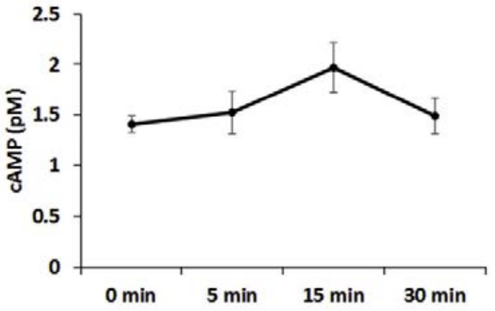
Kang, An, et al. "Suppressive effect of ginsenoside Rg3 against lipopolysaccharide-induced depression-like behavior and neuroinflammation in mice." Journal of agricultural and food chemistry 65.32 (2017): 6861-6869.

Neuro-inflammation

Rg3 as mast cell stabiliser:

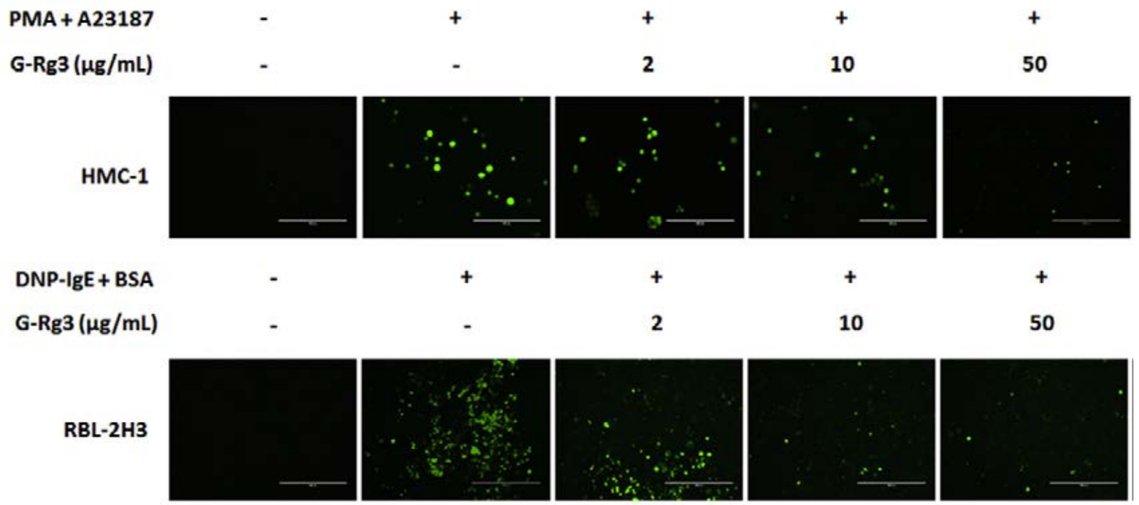
In vitro test on HMC-1 and RBL-2H3 mast cell, respectively activated by PMA + A23187 or DNP-BSA





↓cAMP concentration = ↑degranulation of mast cells
cAMP measured with ELISA

→ **Rg3 increases cAMP concentration**

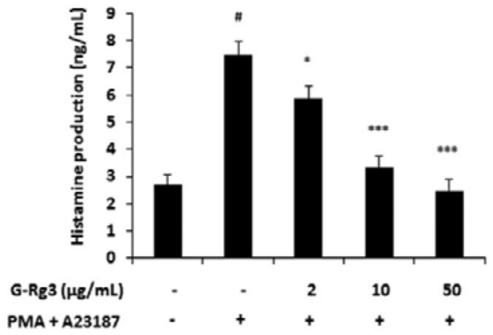


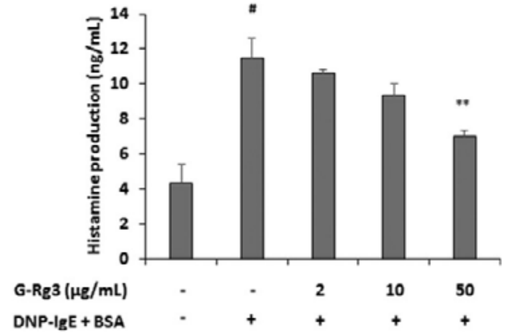
↑Ca^{2+} influx = ↑mast cell degranulation
Ca2+ measured with Fluo-4 AM
Ca^{2+} influx was elevated by PMA + A23187 or DNP-BSA stimulation

→ **Rg3 reduced intracellular Ca^{2+} concentrations**

Mast cells

Kee, Ji-Ye, and Seung-Heon Hong. "Ginsenoside Rg3 suppresses mast cell–mediated allergic inflammation via mitogen-activated protein kinase signaling pathway." Journal of Ginseng Research 43.2 (2019): 282-290.



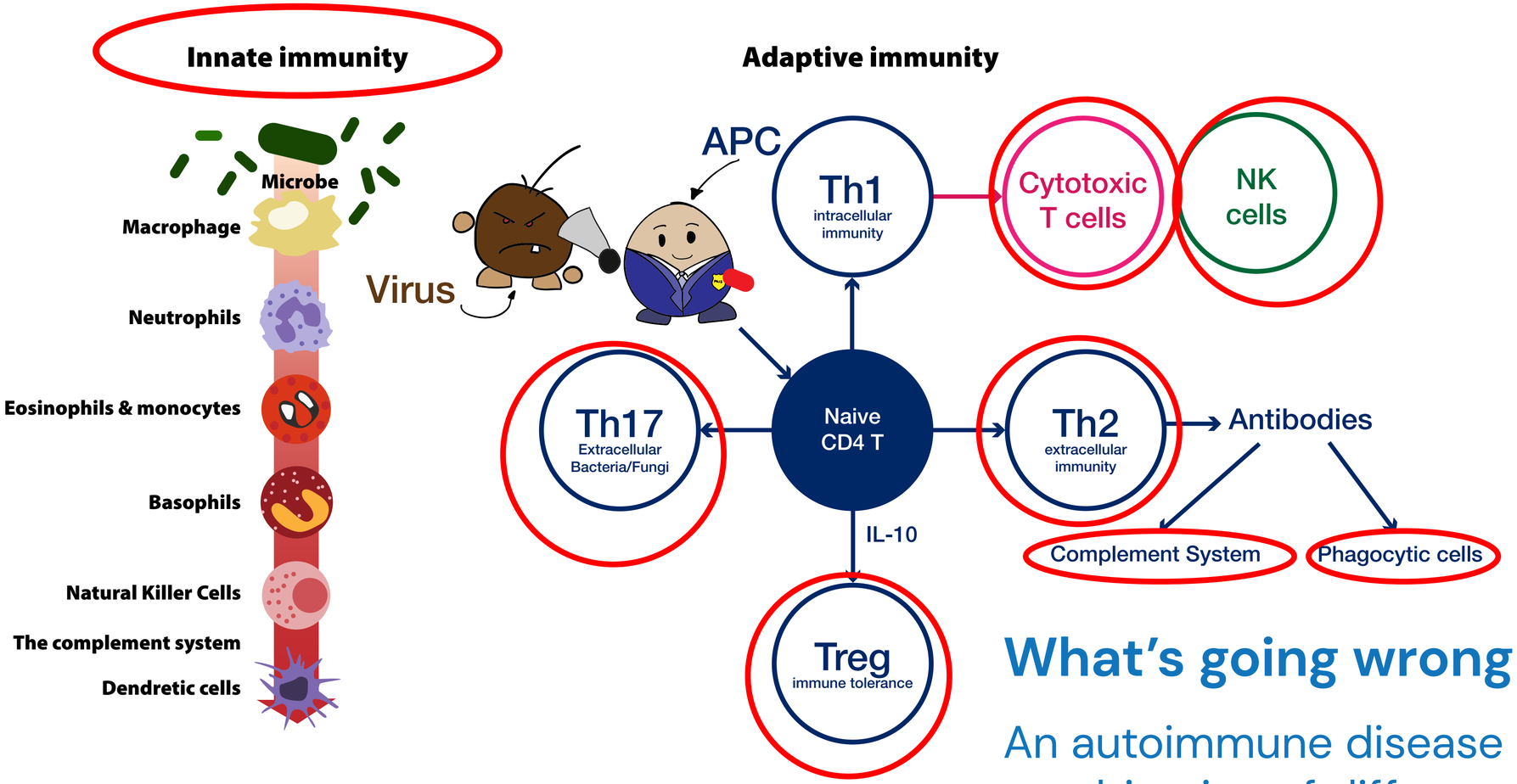


→ Rg3 supresses the release of histamin by mast cells, dose dependently

\+ Rg3 decreases the release of inflammatory mediators

→ decrease in dual talk between microglia and mast cells

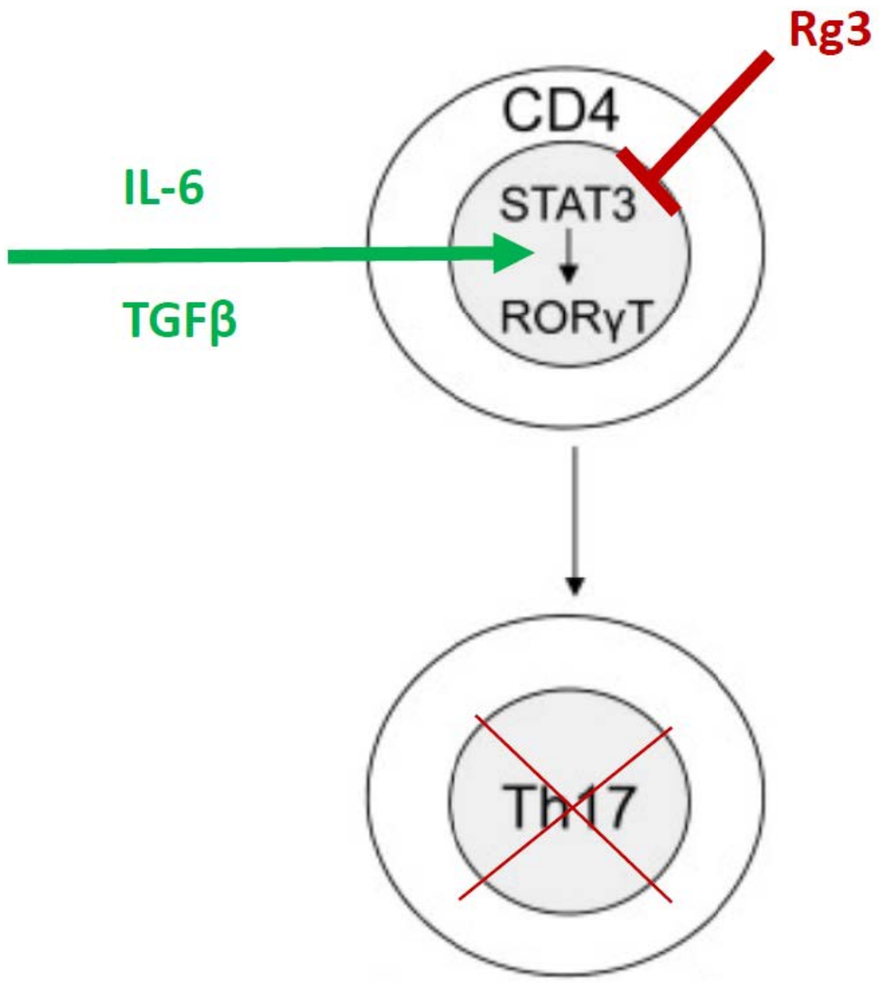
Mast cells



What's going wrong in autoimmunity?

An autoimmune disease is a personalized combination of different immune dysfunction patterns

The most characteristic is the loss of self-tolerance, T reg



Rg3 and autoimmunity

Type of immune response generated: dependent upon nature of the immune stimulation

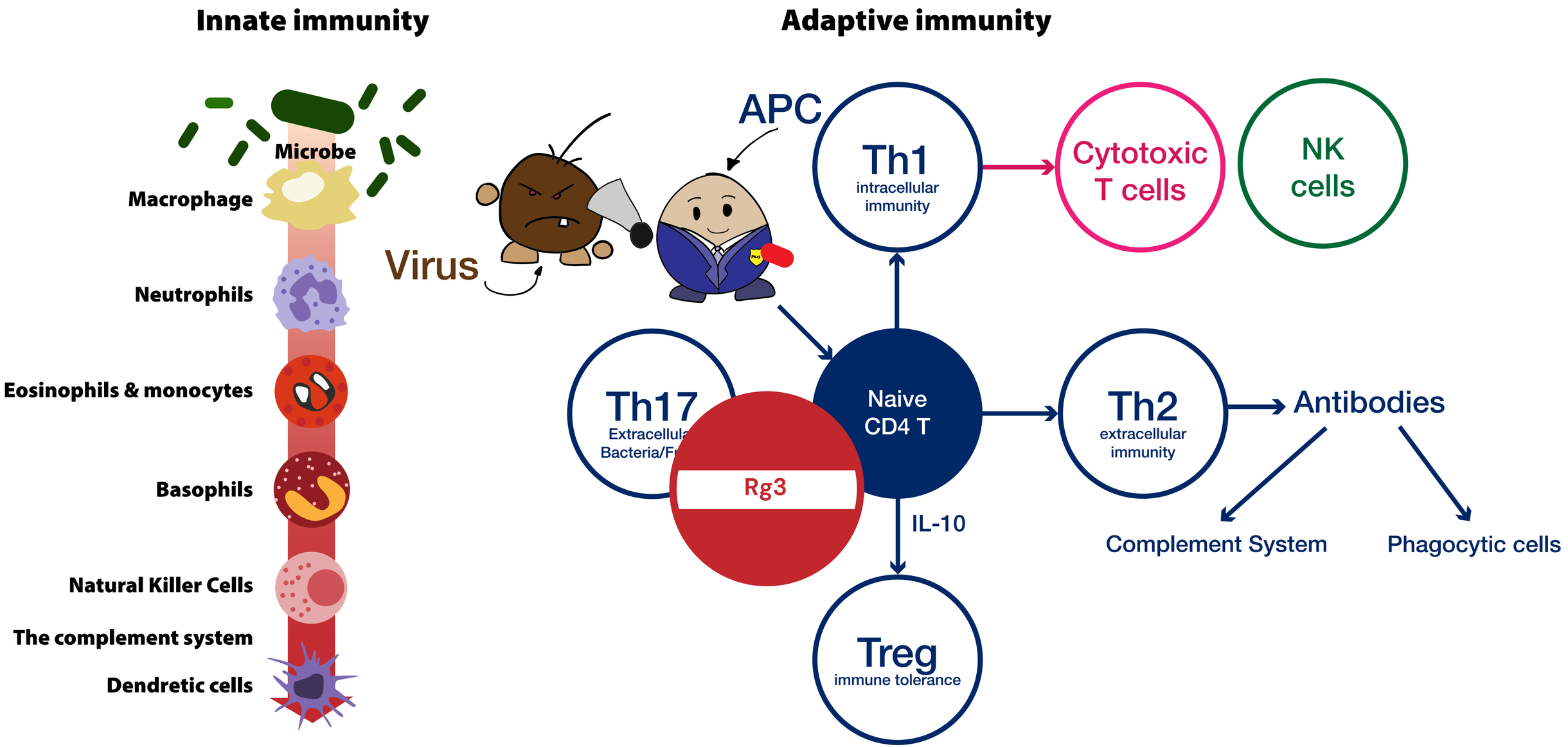
→ CD4 T cell is one of the most important players in autoimmunity

→ **Rg3 can suppress the differentiation of Th17 cells from naïve precursor, acting on the RORγt expression in CD4+ T cells**

Rg3 is a potential candidate for Th17 driven autoimmune disorders

Autoimmunity

Martin, Loren A., et al. "Stereotypies and hyperactivity in rhesus monkeys exposed to IgG from mothers of children with autism." Brain, behavior, and immunity 22.6 (2008): 806-816.


Innate immunity
Adaptive immunity
Microbe
Macrophage
Neutrophils
Eosinophils & monocytes
Basophils
Natural Killer Cells
The complement system
Dendretic cells
Virus
APC
Th1
intracellular
immunity
Cytotoxic
T cells
NK
cells
Th17
Extracellula
Bacteria/F
Rg3
Naive
CD4 T
Th2
extracellular
immunity
Antibodies
IL-10
Complement System
Phagocytic cells
Treg
immune tolerance

Rg3 and cerebral ischemia

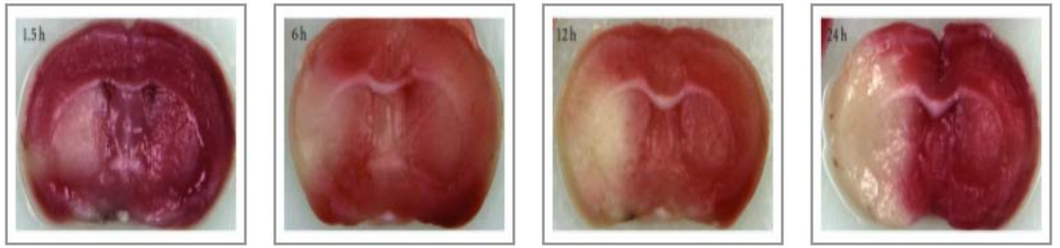
Systematic review: much evidence for ginseng providing functional as well as neuroanatomical protection from ischemic stroke

36 articles acknowledge

→ **Rg3 can reduce cerebral ischemic injury**

Method:

- CoCl2-induced PC12 cells: in vitro model for hypoxia injury
- MCAO model: in vivo model to measure the cerebral ischemic area and neurological score



Kim HJ, Kim P, Shin CY. A comprehensive review of the therapeutic and pharmacological effects of ginseng and ginsenosides in central nervous system [Internet]. Vol. 37, Journal of Ginseng Research. Elsevier B.V.; 2013. p. 8–29

Cheng Z, Zhang M, Ling C, Zhu Y, Ren H, Hong C, et al. Neuroprotective effects of ginsenosides against cerebral ischemia. Molecules[Internet]. 2019;24(6)

results:

- **MCAO model:**
  Rg3 could significantly improve the **neurological score** and reduce **cerebral infarction area**
- **PC12 cells:**
  Rg3 significantly reduced **cell apoptosis**, intracellular **ROS** content and improved the protective effect of **mitochondrial membrane potential** (≈ integrity of mitochondrial function)

Neuroprotective mechanism: associated with TLR4/MyD88 and SIRT1 pathways

→ **Inhibiting NF-κB transcriptional activity and the expression of proinflammatory cytokines IL-1β, TNF-α and IL-6**

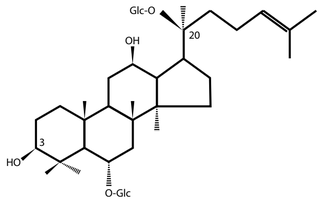
Cerebral Hypoperfusion

Cheng Z, Zhang M, Ling C, Zhu Y, Ren H, Hong C, et al. Neuroprotective effects of ginsenosides against cerebral ischemia. Molecules[Internet]. 2019;24(6)

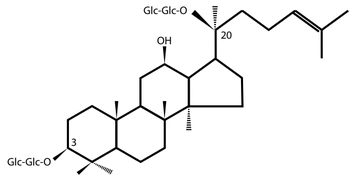
RG3 Nasal Spray

indication	Modulates microglial activity and downregulates neuroinflammation. Scientically confirmed activity in oxidative stress, hypoperfusion, auto-immunity and mast cell activation in Central Nervous System.	
dosage	2 sprays in each nostril twice daily.	
packaging	30 ml per bottle	
composition (amount per 2 sprays)	RG3 (Ginseng extract)	60 mg

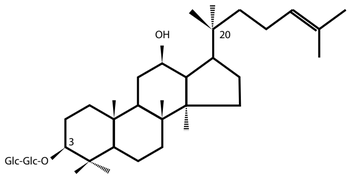
Please find our referenced version on the professional section of our website.
All information is exclusively aimed at and released to an audience of health care professionals.



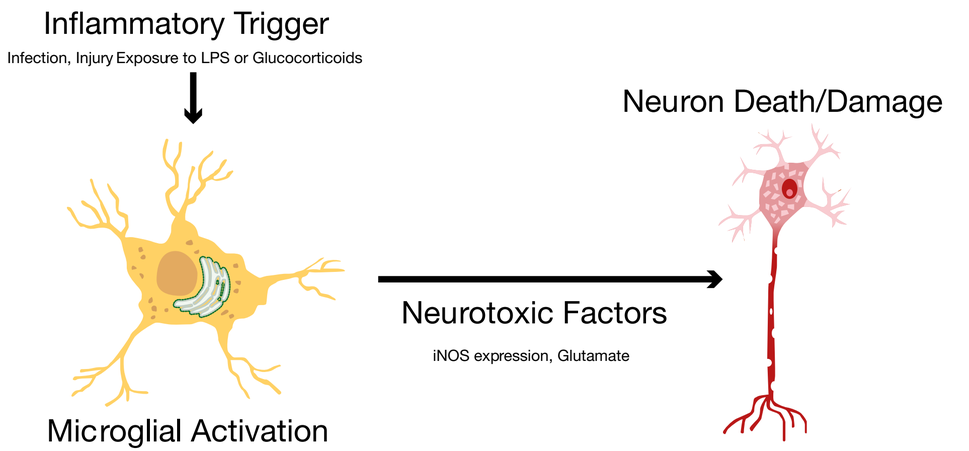
Rg1



Rb1



Rg3



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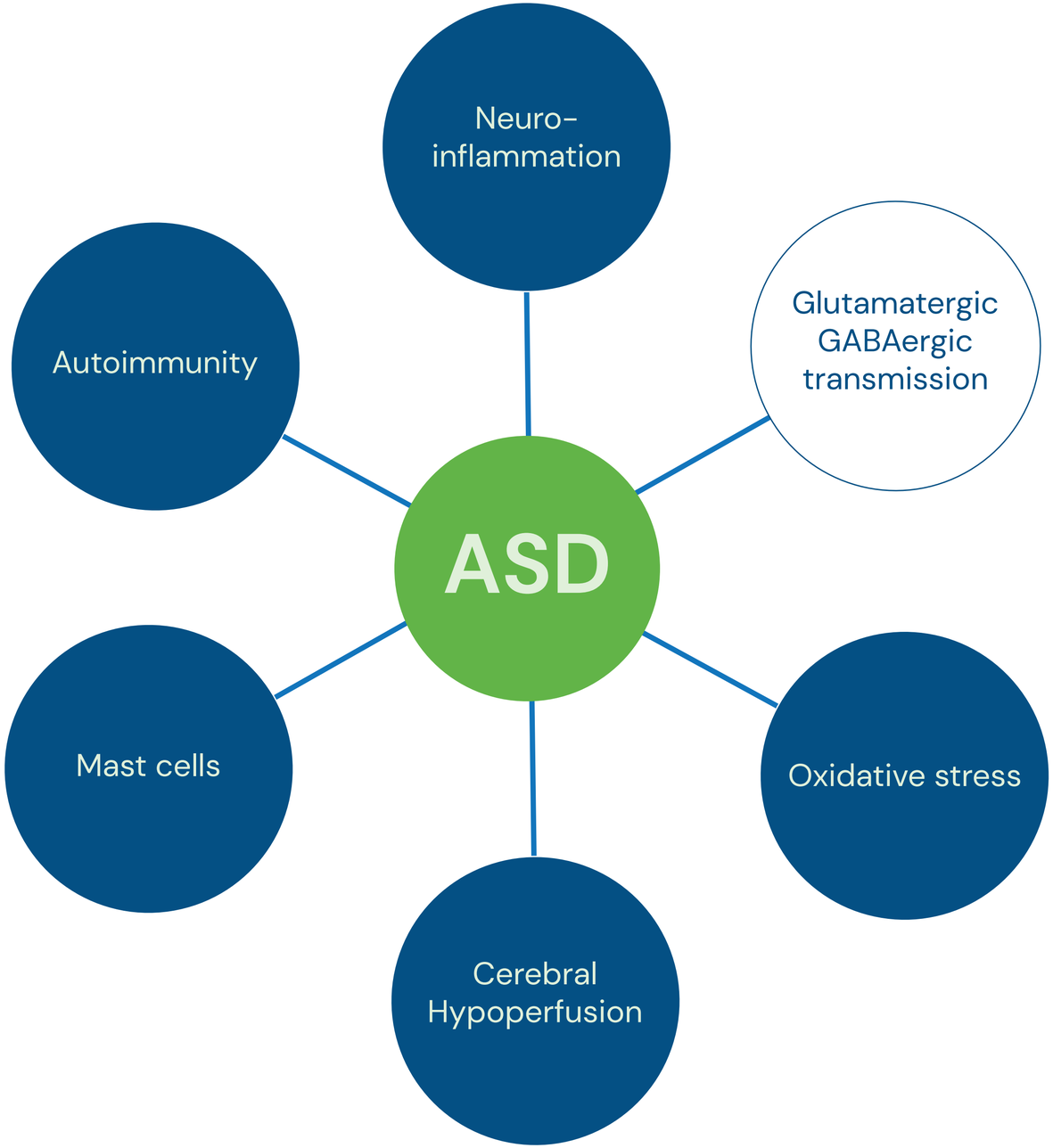
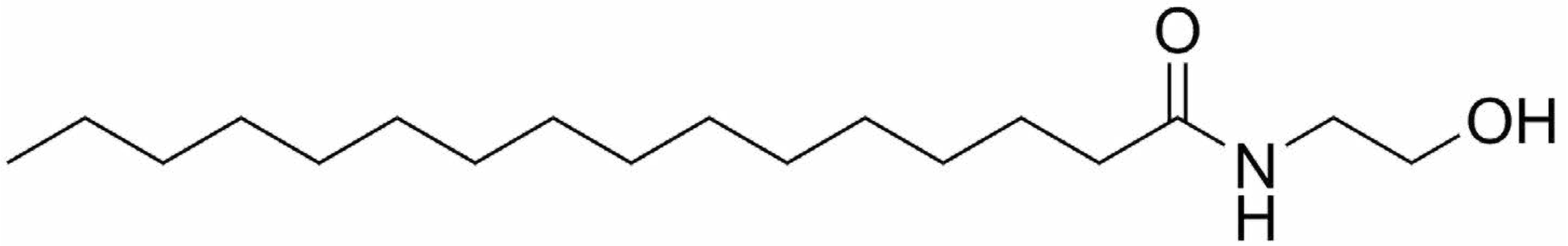
Cerebral Hypoperfusion

RG3 nasal application 5/6

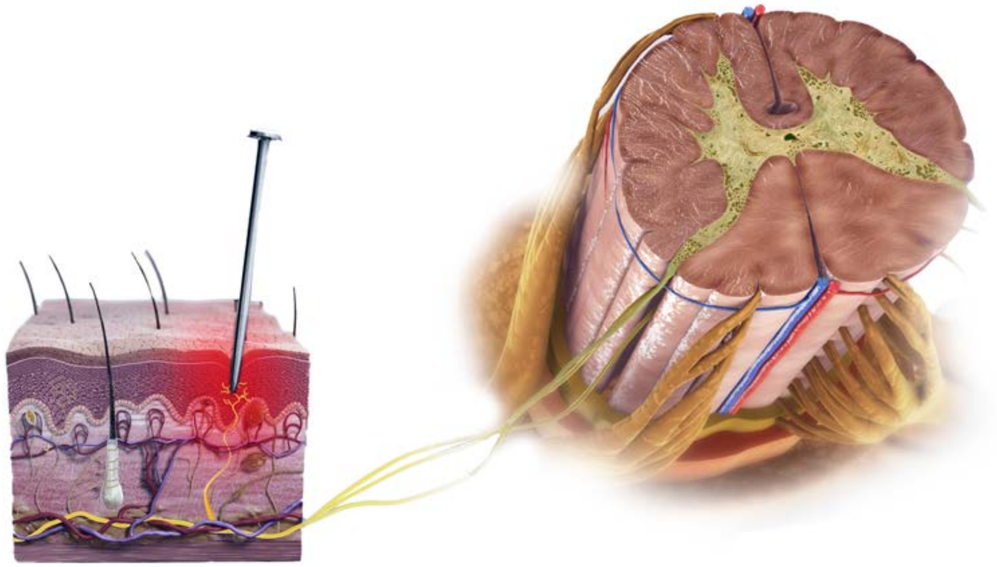




PEA palmitoylethanolamide

indirect interaction with the endocannabinoid system

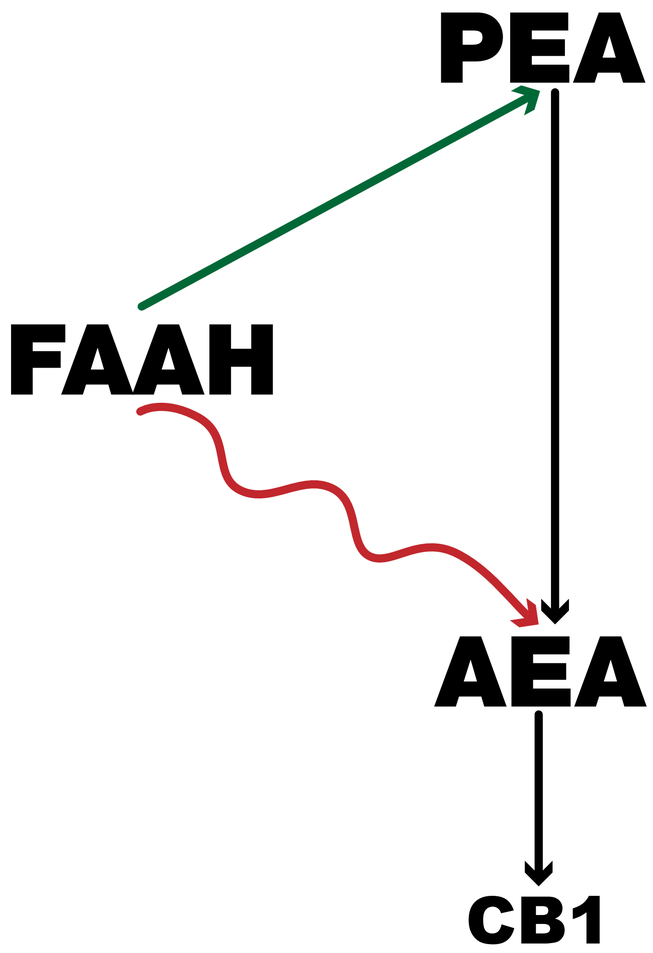
- naturally occurring fatty acid amide in plants, invertebrates & mammalian tissue
- PEA is produced on demand and accumulates locally during severe inflammatory and painful disorders
- PEA counteracts pain and inflammation
- PEA acts as an endogenous regulator of nociception



Nociception

= Stimulation of sensory nerves, called nociceptors, produces a signal that travels along a chain of nerve fibers via the spinal cord to the brain

Activation of nociceptors finally results in perception of pain.



PEA multiple mechanisms of action

1. Effect on Cannabinoid receptors

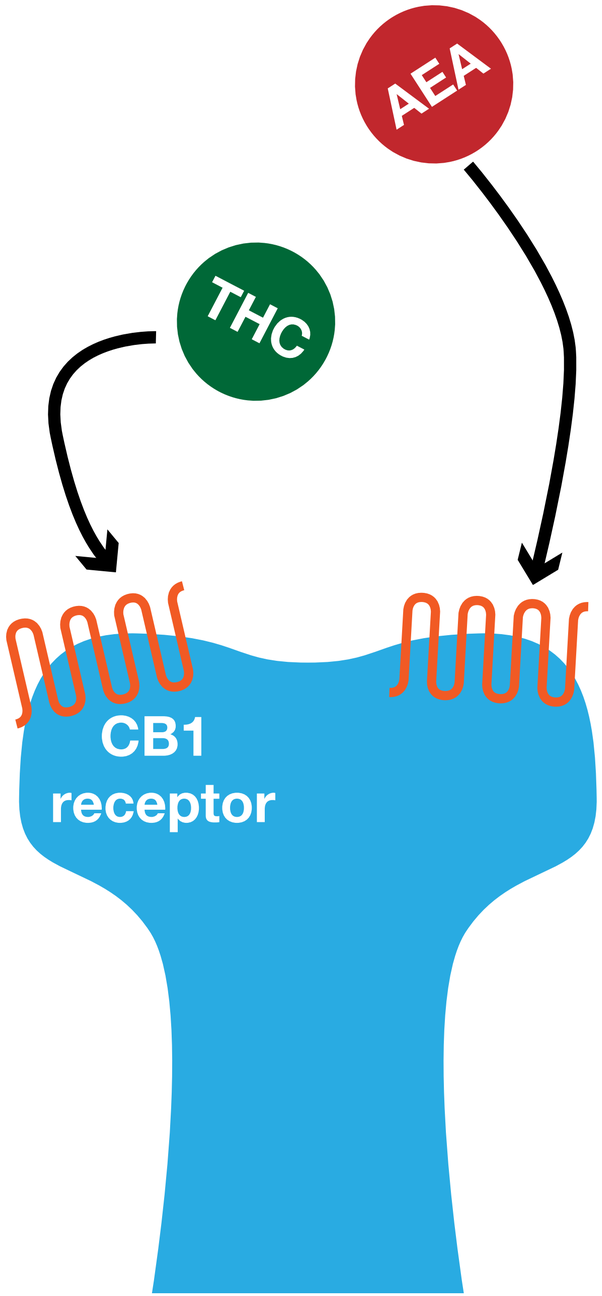
PEA is cannabimimetic compound or indirect endocannabinoid

- poor affinity for CB1 & CB2 receptors but receptor antagonists prevent the antinociceptive effects

PEA acts as an enhancer of the pain-reducing activities exerted by AEA.

= PEA inhibits the metabolic degradation of AEA due to the ability to compete with AEA for the catalytic activity of FAAH

Neuro-inflammation



AEA & THC are the main agonists on CB1

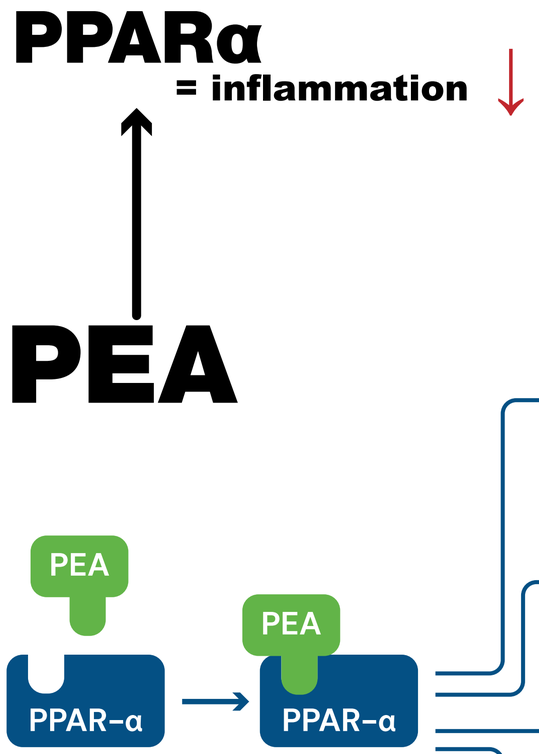
AEA = anandamide, endogenous

THC = tetra-hydro-cannabidiol, active component of Cannabis sativa, exogenous

Cannabis Sativa
more than 500 cannabinoids:

THC = relief of pain + euphoric effect

CBD = poor pain killing effect, relaxing effect, anti-inflammatory, limiting seizures



2. PEA = PPAR alpha ligand = anti-inflammatory activity

= decrease in output of inflammatory mediators like TNF alpha and interleukins

= Immune modulation

Switching off NF-κB: ↓transcription of genes coding for proinflammatory mediators

PPARα in CD4+ T cells: decreased IFNg and IL-17 expression (a.o. suppression of p38 MAPK activation)

Epithelial cells: PPARα ligands repress TNF-elicited chemokine production

PPARα ligands inhibit recruitment of leukocytes to the site of inflammation: ↓chemokines by NF-KB inhibition, ↓TNF-α secretion leads to ↓ expression of VCAM-1 (adhasion molecule)

Autoimmunity

Neuro-inflammation

Mattace Raso G, Esposito E, Vitiello S, Iacono A, Santoro A, D'Agostino G, et al. Palmitoylethanolamide stimulation induces allopregnanolone synthesis in C6 Cells and primary astrocytes: involvement of peroxisome-proliferator activated receptor-. J Neuroendocrinol 2011;23:591–600.

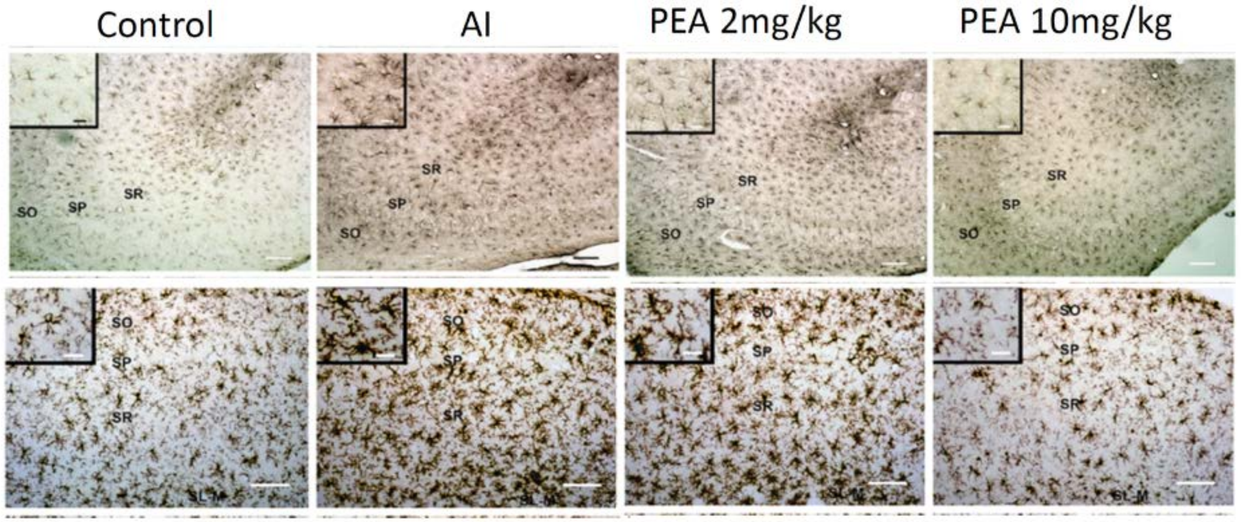
D'Agostino G, La Rana G, Russo R, Sasso O, Iacono A, Esposito E, et al. Centraladministration of palmitoylethanolamide reduces hyperalgesia in mice via inhibition of NF-kB nuclear signalling in dorsal root ganglia. Eur J Pharmacol 2009;613, 54-9.

Straus DS, Glass CK.Anti-inflammatory actions of PPAR ligands: new insights on cellular and molecular mechanisms. Vol. 28, Trends in Immunology. 2007. p. 551–8.

PEA and neurinflammation:

Mechanisms of action:

Microglia and astrocytes



PEA inhibits AI induced astrogliosis

→ Activated astrocytes visualised with GFAP immunostaining

PEA inhibits AI induced microglia activation

→ Activated microglia visualised with Iba-1 expression immunostaining

AI anoxia –ischemia in 7-days old rats
Animals were subjected to behavioral tests followed by immunohistochemical studies.

Results: Neonatal AI was associated with decreased locomotion, as well as recognition and memory impairments. Furthermore, these deficits were accompanied with enhanced neuroinflammation and astrogliosis, as well as a decreased PPARα expression. PEA treatment was able to prevent neuroinflammation, reduce astrogliosis and preserve cognitive functions.

Cerebral Hypoperfusion

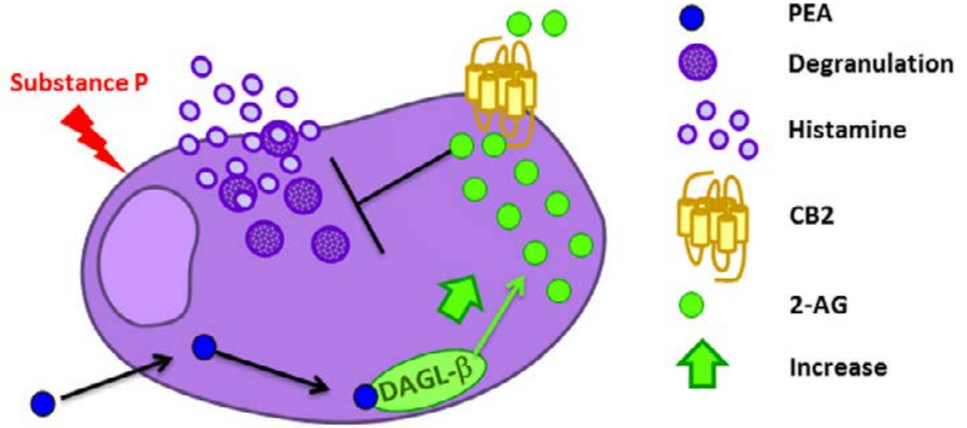
Neuro-inflammation

Holubiec MI, Romero JI, Suárez J, Portavella M, Fernández-Espejo E, Blanco E, et al. Palmitoylethanolamide prevents neuroinflammation, reduces astrogliosis and preserves recognition and spatial memory following induction of neonatal anoxia-ischemia. Psychopharmacology (Berl). 2018;235(10):2929–45.

Mast cell stabilization:

Mechanisms of action:

Mast cells: PEA inhibits mast cell degranulation by enhancing 2-AG levels



PEA stimulates (dose dependant) DAGL activity, which enhances 2-AG (endocannabinoid)

↓

2-AG interact with CB2 receptor

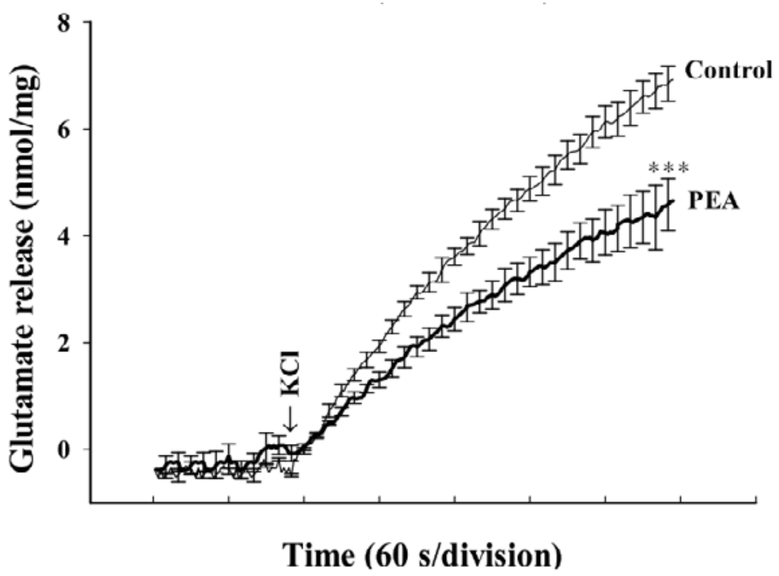
↓

CD2 activation inhibits mast cell degranulation

+ PEA acts via “autacoid local injury antagonism” to downregulate mast cell activation

Petrosino S, Schiano Moriello A, Verde R, Allarà M, Imperatore R, Ligresti A, et al. Palmitoylethanolamide counteracts substance P-induced mast cell activation in vitro by stimulating diacylglycerol lipase activity. J Neuroinflammation. 2019;16(1).

Levi-Montalcini R, Skaper SD, Dal Toso R, Petrelli L, Leon A (1996). Nerve growth factor: from neurotrophin to neurokine. Trends Neurosci 19: 514–520



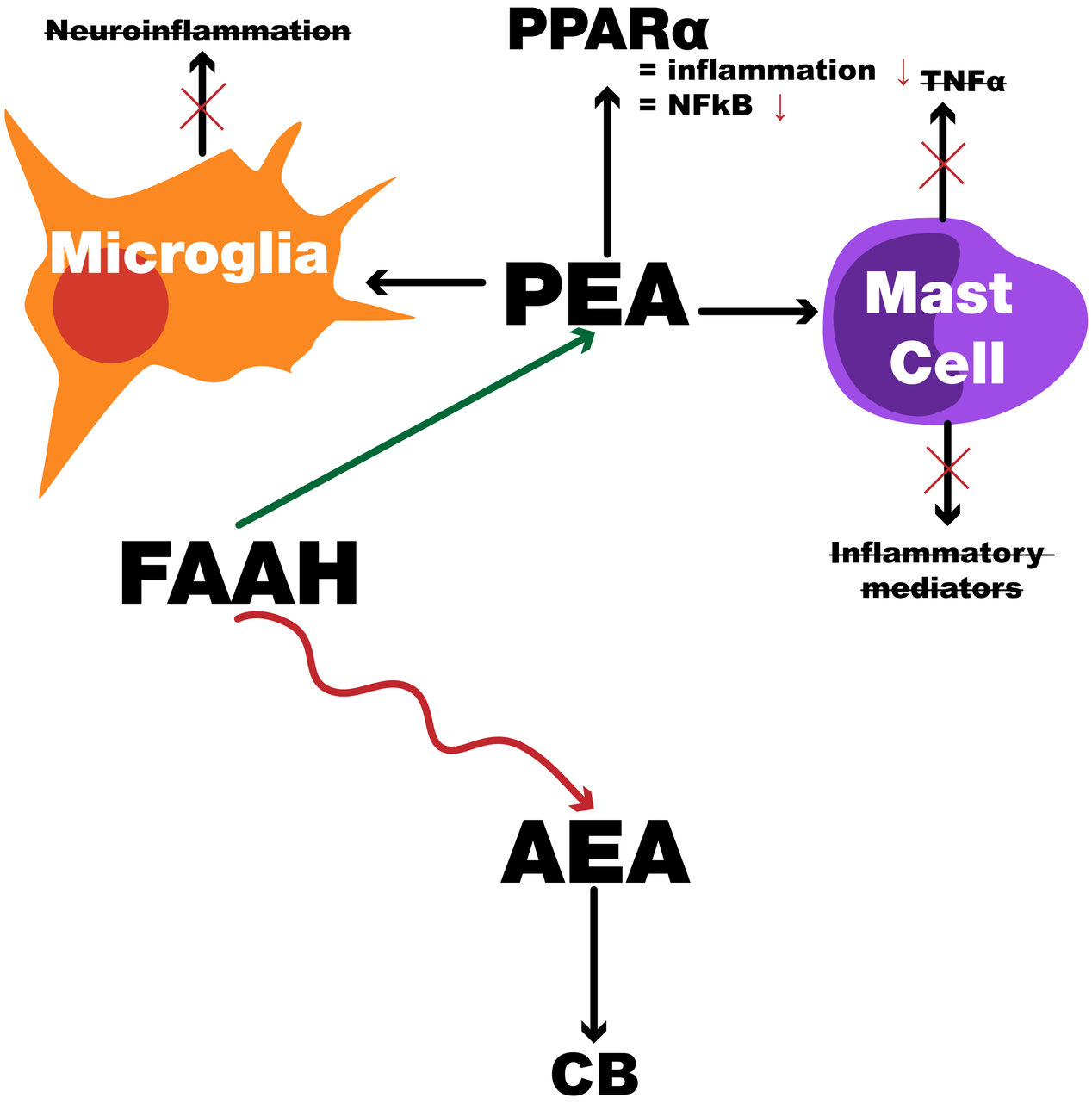
Glutamate/GABA:

Mechanisms of action:

- **Depress glutamate release**
  Excessive glutamate release → excitotoxicity
  - Inhibition of glutamate release by PEA:
    reduction in Ca2+ influx mediated by **Cav2.1 channels**
  - PEA-inhibited glutamate release involves a **PKA pathway:**
    PKA is known to phosphorylate voltage-dependent Ca2+ channels and several synaptic proteins, subsequently increasing glutamate release
    → **Glutamate release measured in the presence of H89 (PKA inhibitor)**
    and PEA was similar to that obtained in the presence of H89 alone

Glutamatergic GABAergic transmission

Lin TY, Lu CW, Wu CC, Huang SK, Wang SJ. Palmitoylethanolamide inhibits glutamate release in rat cerebrocortical nerve terminals. Int J Mol Sci. 2015 Mar 11;16(3):5555–71.



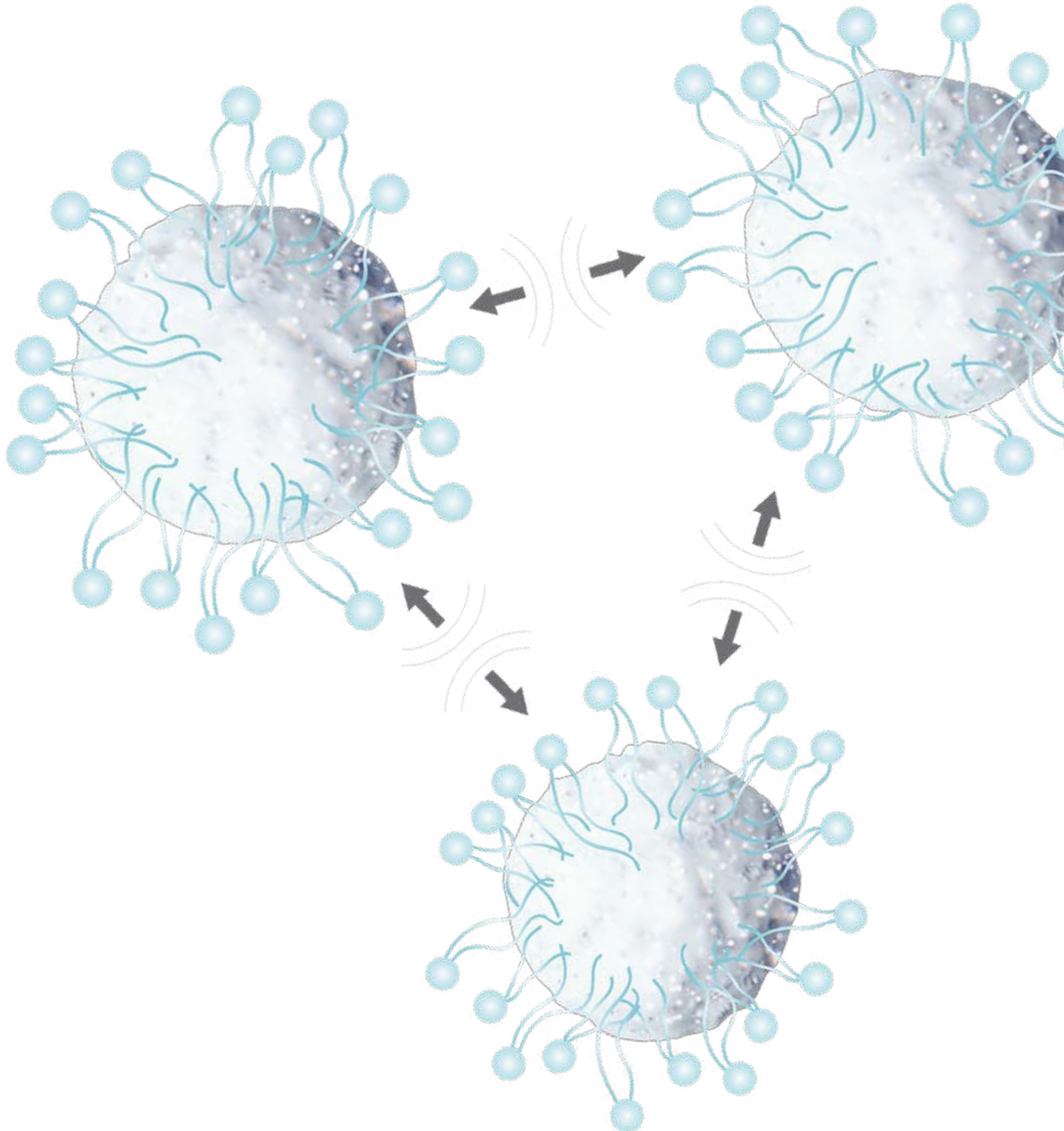
Mast cells

Neuro-inflammation

How would you react if things were suddenly almost 2x better ?

Published pharmacokinetic studies have demonstrated superiority of PEA with Lipisperse coating ® with an increased bioavailability of PEA by times 1.75 compared to standard PEA.

PEA X 1.75



LipiSperse® coats the surface of the PEA molecule, reducing the hydrophobic nature of PEA and acting as a dispersing agentand likely responsible for the increase in gastrointestinal absorption

By increasing the absorption of PEA, there is the potential for increasing the efficacy of PEA in conditions where
PEA Lipisperse ® acts at a number ofreceptors
and exerts its various effects.

Recent studies show additional clinical possibilities

PEA Lipisperse improves sleep & relaxation

- **CB1 activation exerts calming effect, induces sleep**
- **TRPV1 activation /agonism increases restful REM sleep**
- **PEA reduce Neuropathic pain & inflammatory pain**
  Neuropathic pain & inflammation both disturb slow wave sleep
- **PPAR alpha receptors are located in sleep-regulatory areas including thalamus**

Rothhaas, Rebecca, and Shinjae Chung. “Role of the preoptic area in sleep and thermoregulation.” Frontiers in Neuroscience 15 (2021).

Leys, Laura J., et al. “Disturbances in slow-wave sleep are induced by models of bilateral inflammation, neuropathic, and postoperative pain, but not osteoarthritic pain in rats.” PAIN® 154.7 (2013): 1092-1102.

Briskey, D., A. R. Mallard, and A. Rao. “Increased absorption of palmitoylethanolamide using a novel dispersion technology system (LipiSperse®).” J. Nutraceuticals Food Sci 5.3 (2020).

Rao, Amanda, et al. “Palmitoylethanolamide for Sleep Disturbance. A Double-blind, Randomised, Placebo-controlled Interventional Study.” (2021).

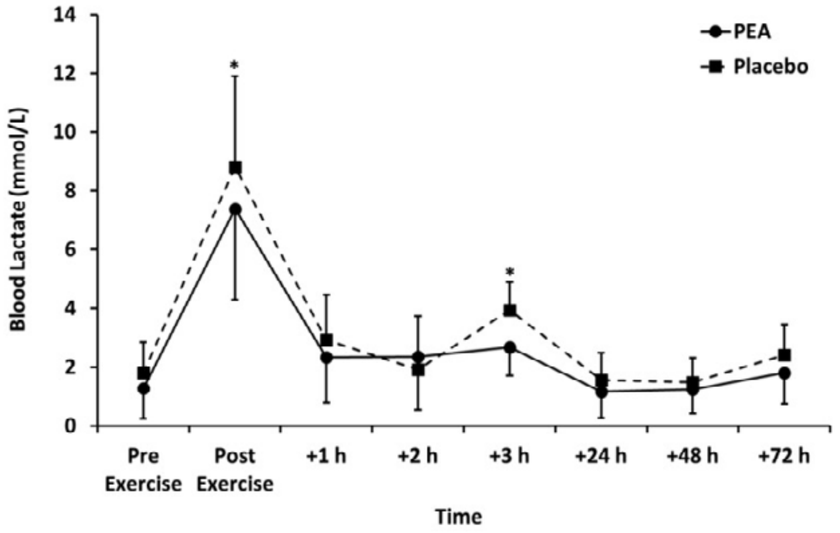
Recent studies show additional clinical possibilities

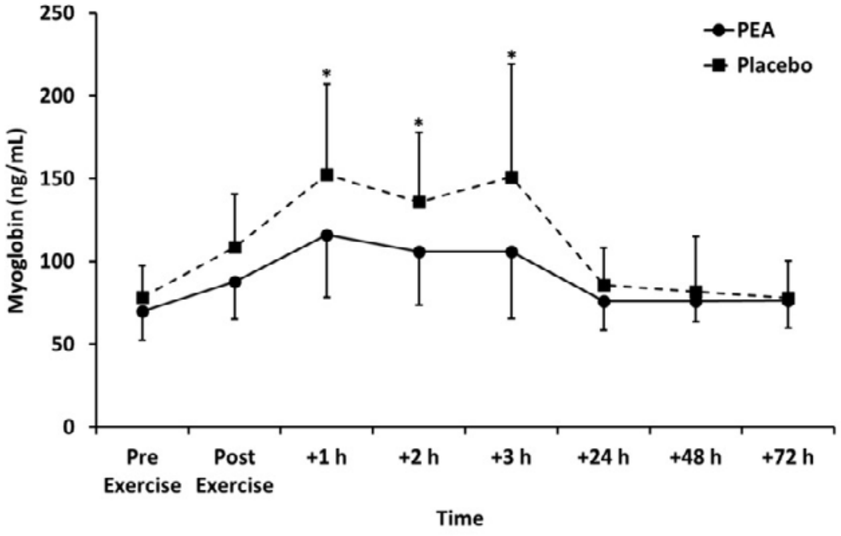
PEA Lipiseperse shows better recovery from sports performance

28 health male volunteers (18-35years old)
treatment regimen: PEA lipiSperse versus placebo

Outcome measures:

- Change in blood indicators of muscle damage / recovery, Creatine Kinase
- Muscle pain (VAS Pain score
- Muscle Soreness (DOMS)
- Change in blood indicators of muscle damage, Myoglobin, Lactate Dehydrogenase
- Exercise Recovery: the number of leg presses a participant can complete
- Lab markers CRP, IL-6, IL-10,TNF-alpha
- Pain Questionnaire
- Muscle swelling
- MSFI Multidimensional Symptoms Fatigue Inventory
- Safety markers like GI tolerance





Conclusions

- Athletes who consumed PEA lipiSperse® may be sublect to higher exercise intensities for longer time, allowing for an improved training response or performance
- A lower blood lactate concentration is correlated with increased aerobic energy metabolism
- Athletes can exercise at higher intensities for longer time
- Injured players recover better and faster
- Improved sleep quality among players with sleep disturbances

Mallard, Alistair, et al. "The Effect of Orally Dosed Levagen+™(palmitoylethanolamide) on Exercise Recovery in Healthy Males—A Double-Blind, Randomized, Placebo-Controlled Study." Nutrients 12.3 (2020): 596.

PEA as treatment for ASD: a randomized controlled trial

Methods:

- 62 children 4–12y with ≥ 12 on the Aberrant Behaviour Checklist-Community
- Randomised in two groups:
  - 31 risperidone + placebo
  - 31 risperidone + 600mg PEA 2x/d
- 10 week study
- Outcome? ABC-C score (irritability, lethargy, stereotype, hyperactivity and inappropriate speech)

Results:

PEA + risperidone: significant difference in improvement of symptoms on the ABC-irritability and hyperactivity subscales compared to risperidone + placebo

No differences in improvement on lethargy, stereotypic behaviour, and inappropriate speech, compared to risperidone + placebo

No report of serious side effects, no differences between the two groups

→ **PEA is effective as co-treatment to reduce ASD related symptoms**

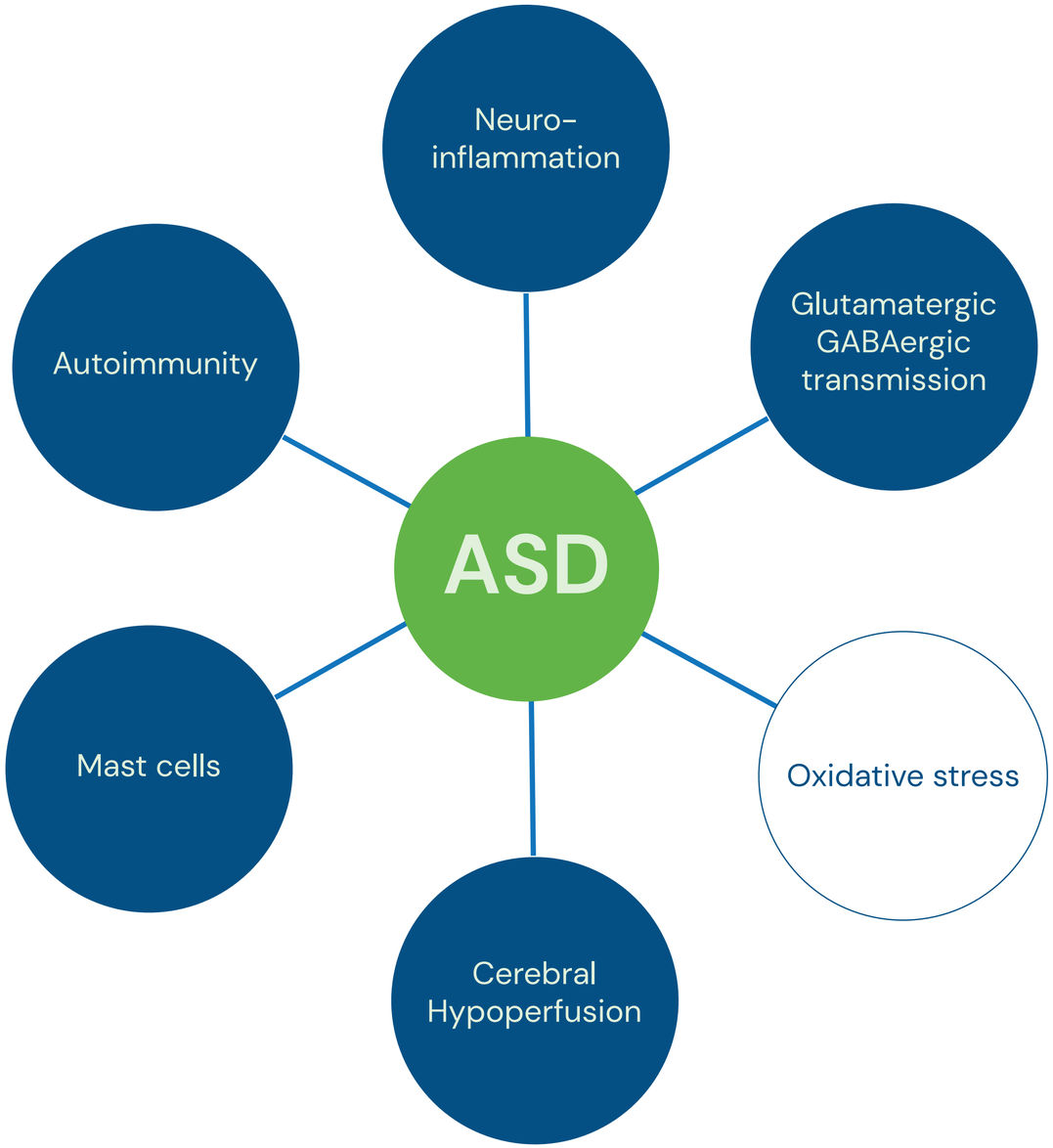
Khalaj M, Saghazadeh A, Shirazi E, Shalbafan MR, Alavi K, Shooshtari MH, et al. Palmitoylethanolamide as adjunctive therapy for autism: Efficacy and safety results from a randomized controlled trial. J Psychiatr Res. 2018;103:104–11.

PEA LipiSperse coating®
= N-Palmitoylethanolamide

indication	Reduces neuropathic pain. Downregulates mast cell activation and microglial activation.	
dosage	2 x 1 - 2 caps per day	
packaging	120 vegecaps per container	
composition (amount per 2 vegecaps)	PEA LipiSperse coating® (Certified grade)	600 mg

Please find our referenced version on the professional section of our website.
All information is exclusively aimed at and released to an audience of health care professionals.

PEA 5/6


Neuro-
inflammation
Autoimmunity
Glutamatergic
GABAergic
transmission
ASD
Mast cells
Oxidative stress
Cerebral
Hypoperfusion

Butyrate Coated

Short-chain fatty acids (SCFAs), the main metabolites produced in the colon by bacterial fermentation are speculated to play a key role in neuro-immunoendocrine regulation.

How is butyrate formed?

1. From host prebiotic
2. From exogenous prebiotics

Fuel to renew epithelial cells

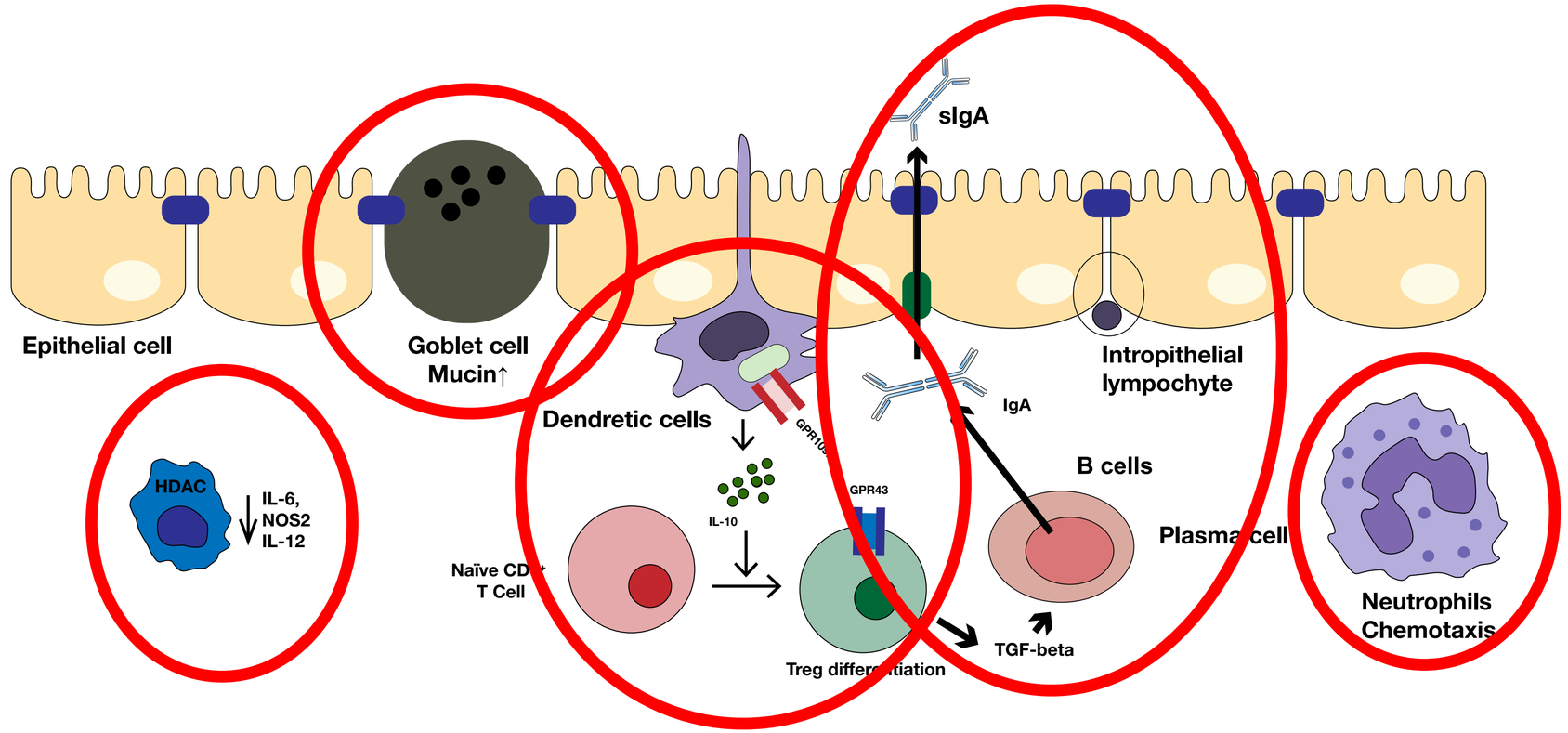
Impact on dendritic cells, more IL-10 & T regs

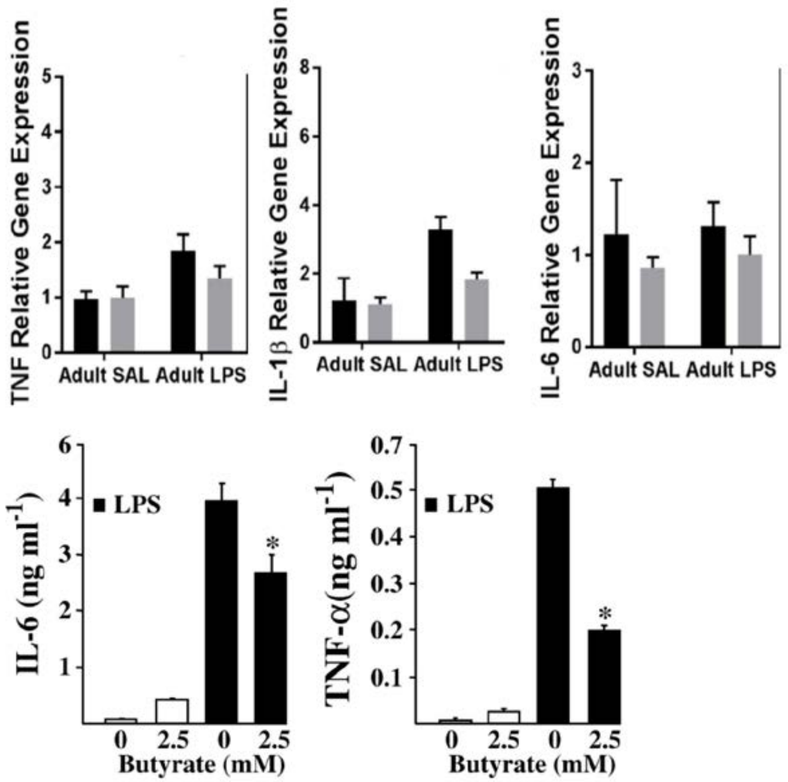
Goblet Cells release more mucins

Macrophages more tolerant towards commensal bacteria

Neutrophil chemotaxis

B cells synthesize more s IgA's





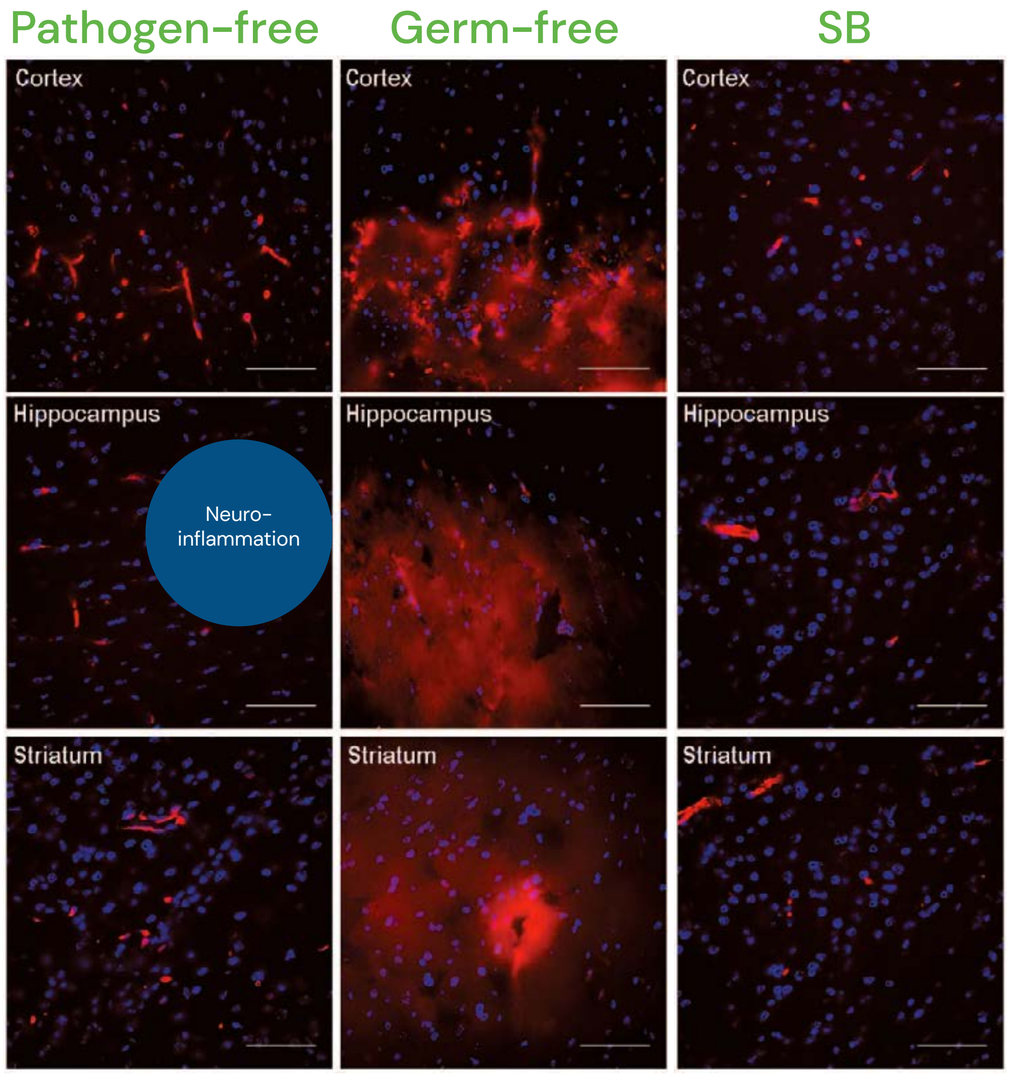
Butyrate and neuroinflammation

- Butyrate decreases gene expression of pro-inflammatory cytokines TNF, IL-1β and IL-6
  → Real-Time RT-PCR on microglia tissue,
  Similar results in hippocampal tissue
- Butyrate decreases gene expression of pro-inflammatory cytokines TNF and IL-6 in butyrate pre-treated microglia, 22h before LPS-injection
  Simultaneous administration of butyrate and LPS?
  Only IL-6 decreased
  → Butyrate pre-treatment induces adaptive changes in the microglial activation system, link with effect on histones

Matt SM, Allen JM, Lawson MA, Mailing LJ, Woods JA, Johnson RW. Butyrate and dietary soluble fiber improve neuroinflammation associated with aging in mice. Front Immunol. 2018 Aug 14;9(AUG).

Huuskonen J, Suuronen T, Nuutinen T, Kyrylenko S, Salminen A. Regulation of microglial inflammatory response by sodium butyrate and short-chain fatty acids. Br J Pharmacol. 2004 Mar;141(5):874–80.

Butyrate and BBB permeability



Germ-free mice: lack of normal gut bacteria

↓

Increased BBB permeability
(compared to pathogen-free mice)
Sodium butyrate: 1g/kg, oral administration, 3 days

↓

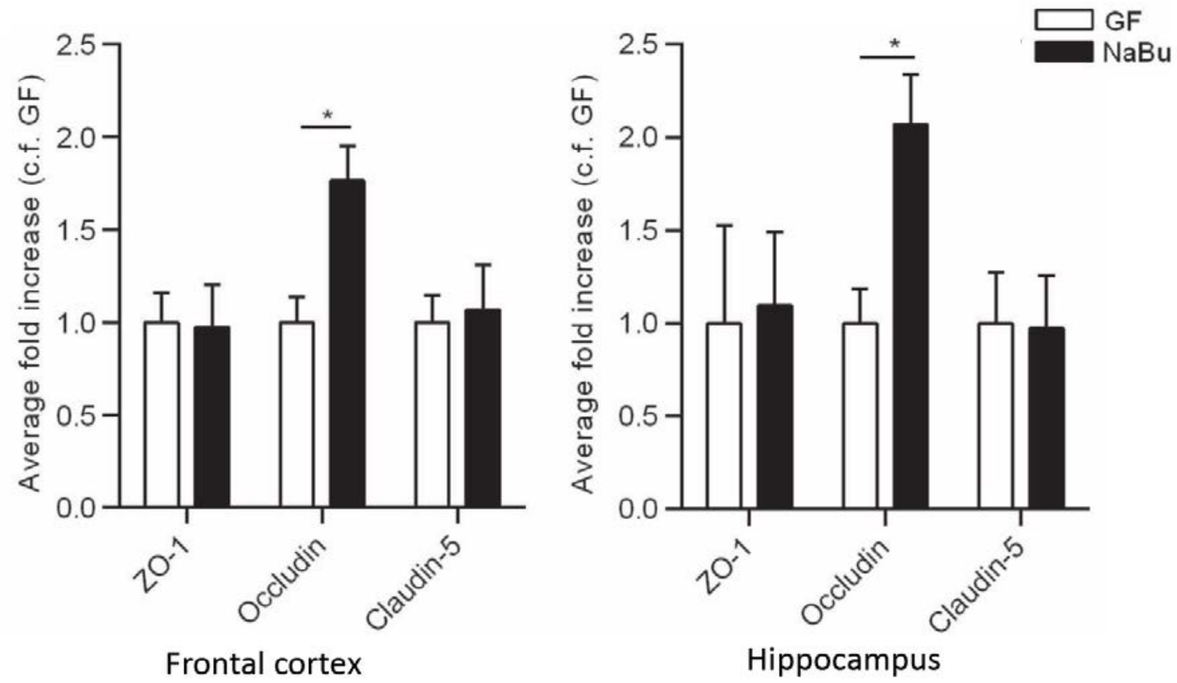
Restored BBB permeability

(equivalent to pathogen-free BBB)

Visualisation with Evans blue tracing

Neuro-inflammation

Braniste V, Al-Asmakh M, Kowal C, Anuar F, Abbaspour A, Tóth M, et al. The gut microbiota influences blood-brain barrier permeability in mice. Sci Transl Med. 2014;6(263).



Butyrate gavaged mice (72h): increases the expression of **occludin**, compared to water gavaged mice

No effect was found on claudin-5 expression

Occludin? Tight junction protein

Neuro-inflammation

Braniste V, Al-Asmakh M, Kowal C, Anuar F, Abbaspour A, Tóth M, et al. The gut microbiota influences blood-brain barrier permeability in mice. Sci Transl Med. 2014;6(263).

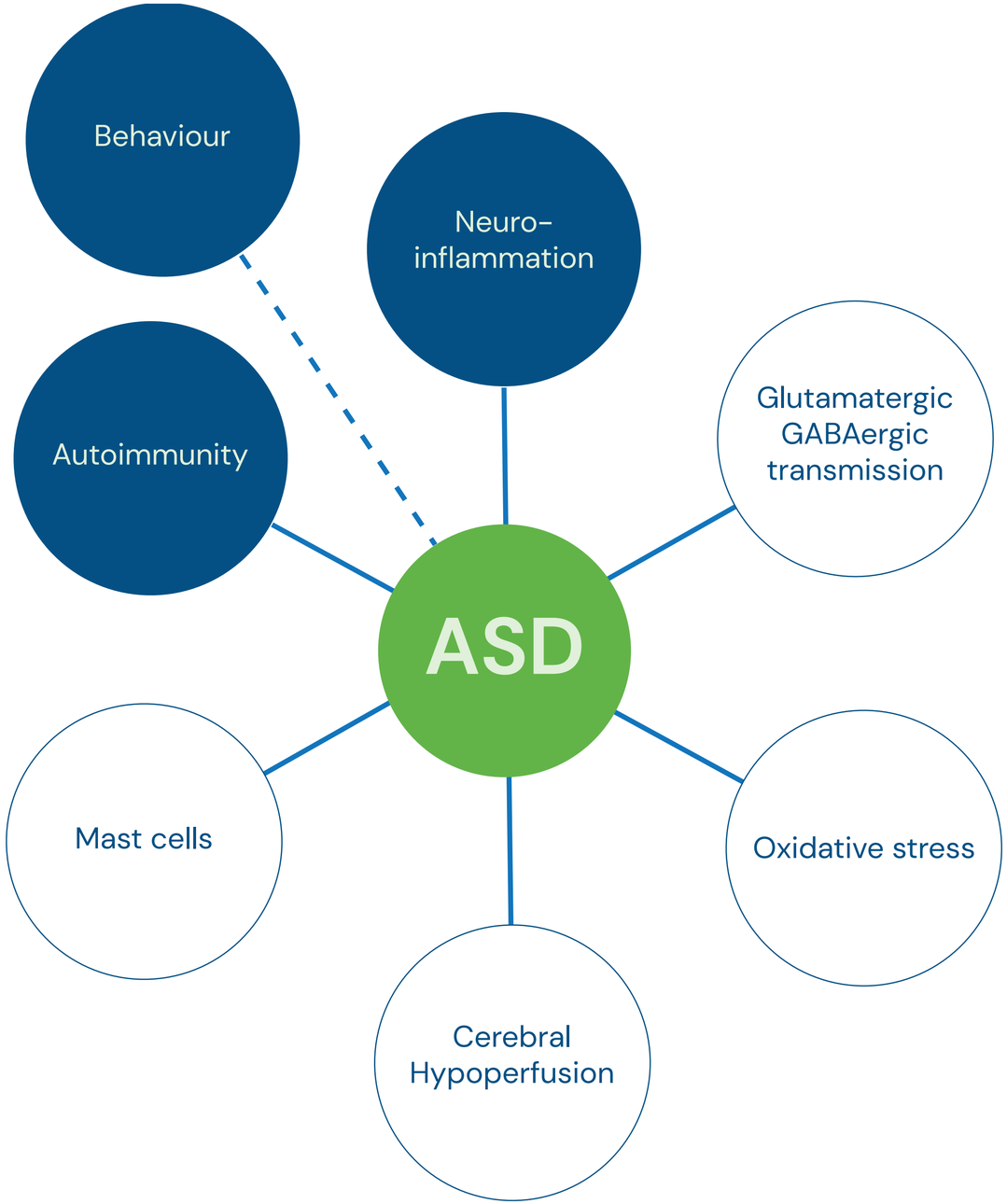
Different studies on mice showed improvement in ASD- related behaviour

Kratsman, Neta, Dmitriy Getselter, and Evan Elliott. “Sodium butyrate attenuates social behavior deficits and modifies the transcription of inhibitory/excitatory genes in the frontal cortex of an autism model.” Neuropharmacology 102 (2016): 136-145.

Gagliano, Humberto, et al. “High doses of the histone deacetylase inhibitor sodium butyrate trigger a stress-like response.” Neuropharmacology 79 (2014): 75-82.

Cho, Yongcheol, and Valeria Cavalli. “HDAC5 is a novel injury-regulated tubulin deacetylase controlling axon regeneration.” The EMBO journal 31.14 (2012): 3063-3078.

Butyrate Coated 3/6



Butyflam Coated

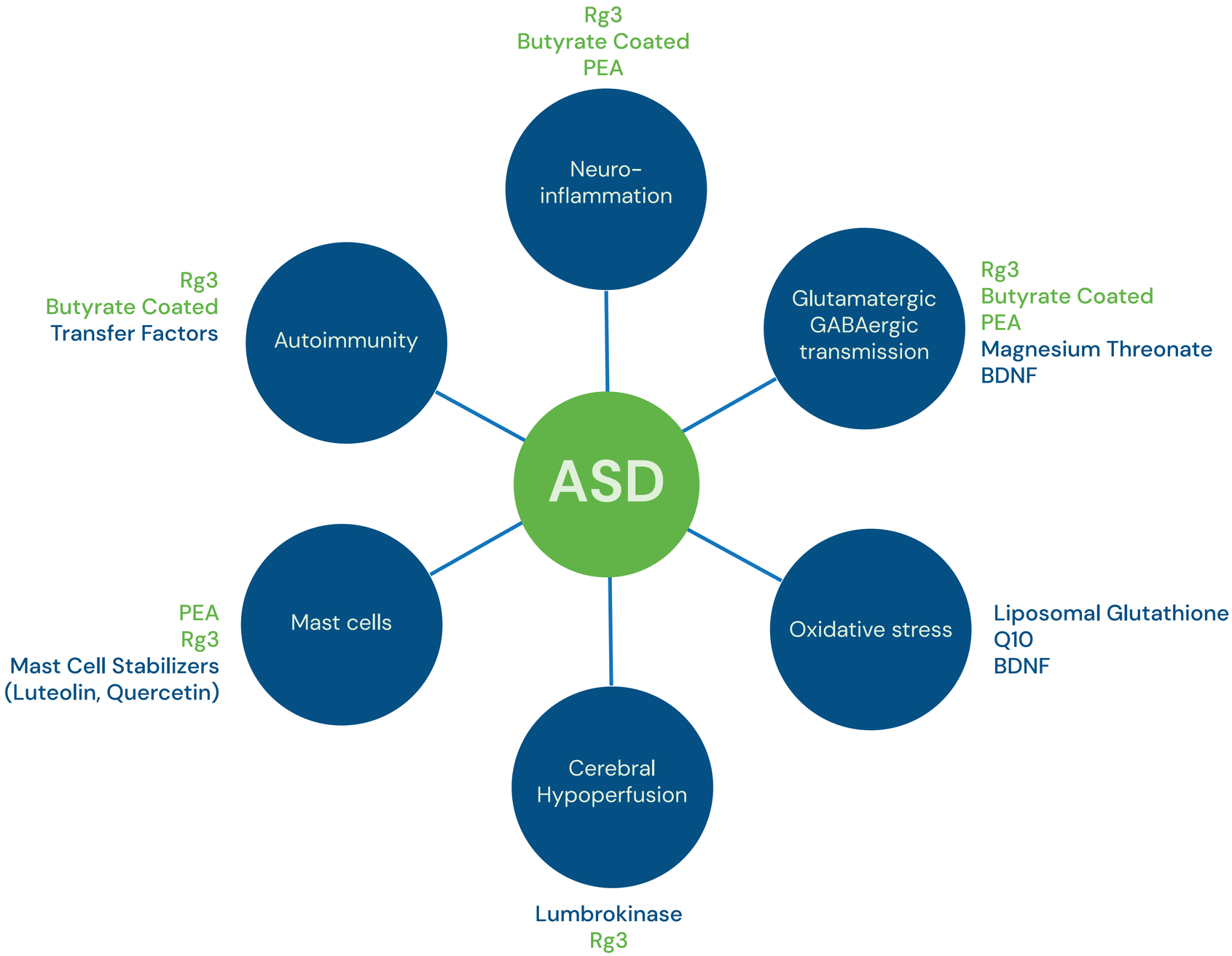
Butyrate is a short-chain fatty acid produced by the intestinal bacteria through fermentation of non-digestible fibers. Butyflam Coated delivers bio-available levels of butyrate in our intestines to guarantee immune tolerance and avoid excessive inflammation or auto-immune reactions.

indication	Neuroinflammation Immune modulating (T reg + IL-10 anti-inflammation) Remodeling intestinal barrier function
dosage	3 x 2 tablets per day
packaging	180 coated tablets per container
composition (amount per 6 tablets)	Butyrate - 3000 mg

Currently, only one medication (risperidone) is FDA-approved for the treatment of autism spectrum disorders (ASD)

Recent data suggest that ASD is at least partially the result of the defected interplay between the different biological systems we discussed today

Future pharmacological research should focus more on neuroinflammation and the affected interaction between microglia and astrocytes


Rg3
Butyrate Coated
PEA
Neuro-
inflammation
Rg3
Butyrate Coated
Transfer Factors
Autoimmunity
Glutamatergic
GABAergic
transmission
Rg3
Butyrate Coated
PEA
Magnesium Threonate
BDNF
ASD
PEA
Rg3
Mast Cell Stabilizers
(Luteolin, Quercetin)
Mast cells
Oxidative stress
Liposomal Glutathione
Q10
BDNF
Cerebral
Hypoperfusion
Lumbrokinase
Rg3

Medicin with a mission

At Nutrined we are well aware that the medical standards that we are all working so hard to obtain cannot be taken for granted. In other parts of the world basic healthcare, as well as other aspects of what we consider to be a healthy society, is far from self-evident. That is why we have decided to share our profits with a humanitarian project supporting the Boutyouss r egion in Morocco.

In 2016 Pol De Saedeleer and his friends, all with a strong passion for sports, joined a desert run near Zagora, a city in southern Morocco. They received a warm welcome from the locals and instantly fell in love with the region and its inhabitants. As they gained more insight in the struggles the local society was facing on a daily basis, the idea arose to take initiative to support the region. This is how the non-profit organisation Run To Start and the sports event Trans Zagora Trail were created. This trail, consisting of 200 km and 6 stages, takes place once a year in the Moroccon desert and it was the very first event to be organised to get funding for Run To Start.

Nutrined wants to join hands with Run To Start in supporting different projects for the Boutyouss region. One of the projects we feel strongly about is that of the Cherif Alauoui School in Zagora, a school that has a dedicated class for children with Down's Syndrome. The children need specialized transportation to get to the school, which is not always accessible for all due to financial reasons. Run To Start therefore provides funding to the school, so that all children can enjoy a good education. Another project s upports local farmers in substituting traditional watermelon culture with palm trees. As palm trees, in contrast to watermelons, do not require a lot of water to grow, they could help solve water scarcity in the region over t ime, while still providing lots of fruits in the form of dates.

Nutrined is proud to be able to provide funding for these valuable projects that will enhance the quality of life for so many people in Morocco. That is the spirit of Medicine with a Mission, promoting the idea that 'protecting our health' does not stop at country boundaries.