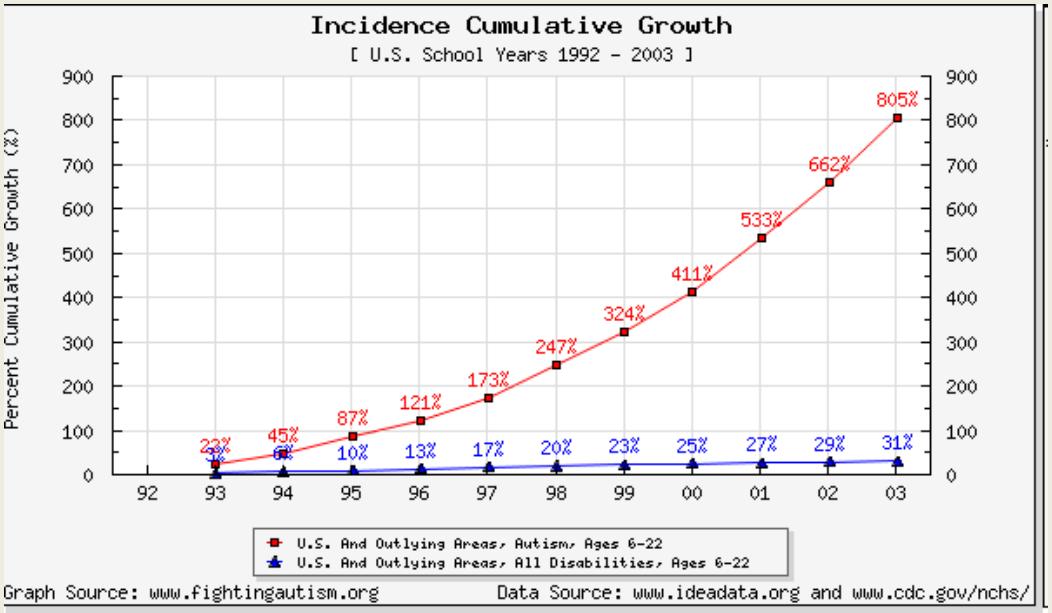
# INTEGRATIVE APPROACH FOR TESTING PATIENTS WITH AUTISM SPECTRUM DISORDERS

## The Importance of Assessing Inflammation, Infections and Gastrointestinal Disorders in Autism Spectrum Disorders (ASD) Patients

*Tanja Mijatovic, PhD*
*R.E.D. Labs CSO & Lab Manager*

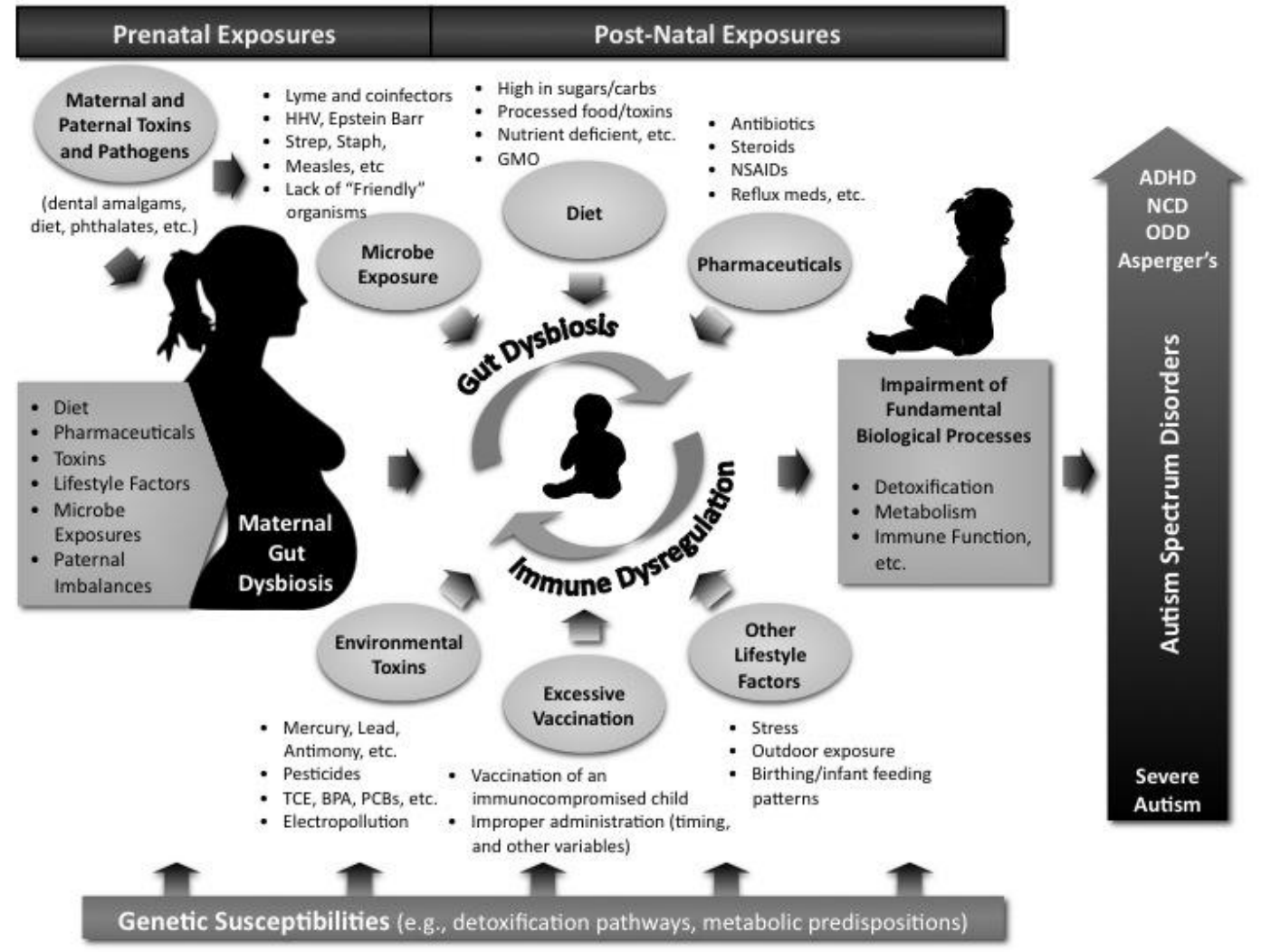
# Autism diagnosis




**1.7%**
is the average percentage identified with ASD

**1 in 59**
8-year-old children were identified with ASD by ADDM in 2014

# Immune dysfunction in autism



# Inflammation and ASD – well documented issue

Spotlight

## Inflammation and Autism: From Maternal Gut to Fetal Brain

Ivan Osokine[1] and Adrian Erlebacher[1,*]

Trends in Molecular Medicine, December 2017, Vol. 23, No. 12

AIMS Molecular Science

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http://www.aimspress.com/journal/Molecular

*Communication*

**Biomedical approach in autism spectrum disorders—the importance of assessing inflammation**

**Tatjana Mijatovic[1,*], Dario Siniscalco[2,3,4], Krishnamurthy Subramanian[5], Eugene Bosmans[1], Vincent C. Lombardi[5,6] and Kenny L. De Meirleir[5,7]**

Prata *et al. Journal of Neuroinflammation* (2017) 14:179
DOI 10.1186/s12974-017-0938-y

Journal of Neuroinflammation

REVIEW Open Access

## Bridging Autism Spectrum Disorders and Schizophrenia through inflammation and biomarkers - pre-clinical and clinical investigations

Joana Prata[1,2†], Susana G. Santos[2,3*†], Maria Inês Almeida[2,3], Rui Coelho[1,2] and Mário A. Barbosa[2,3,4]

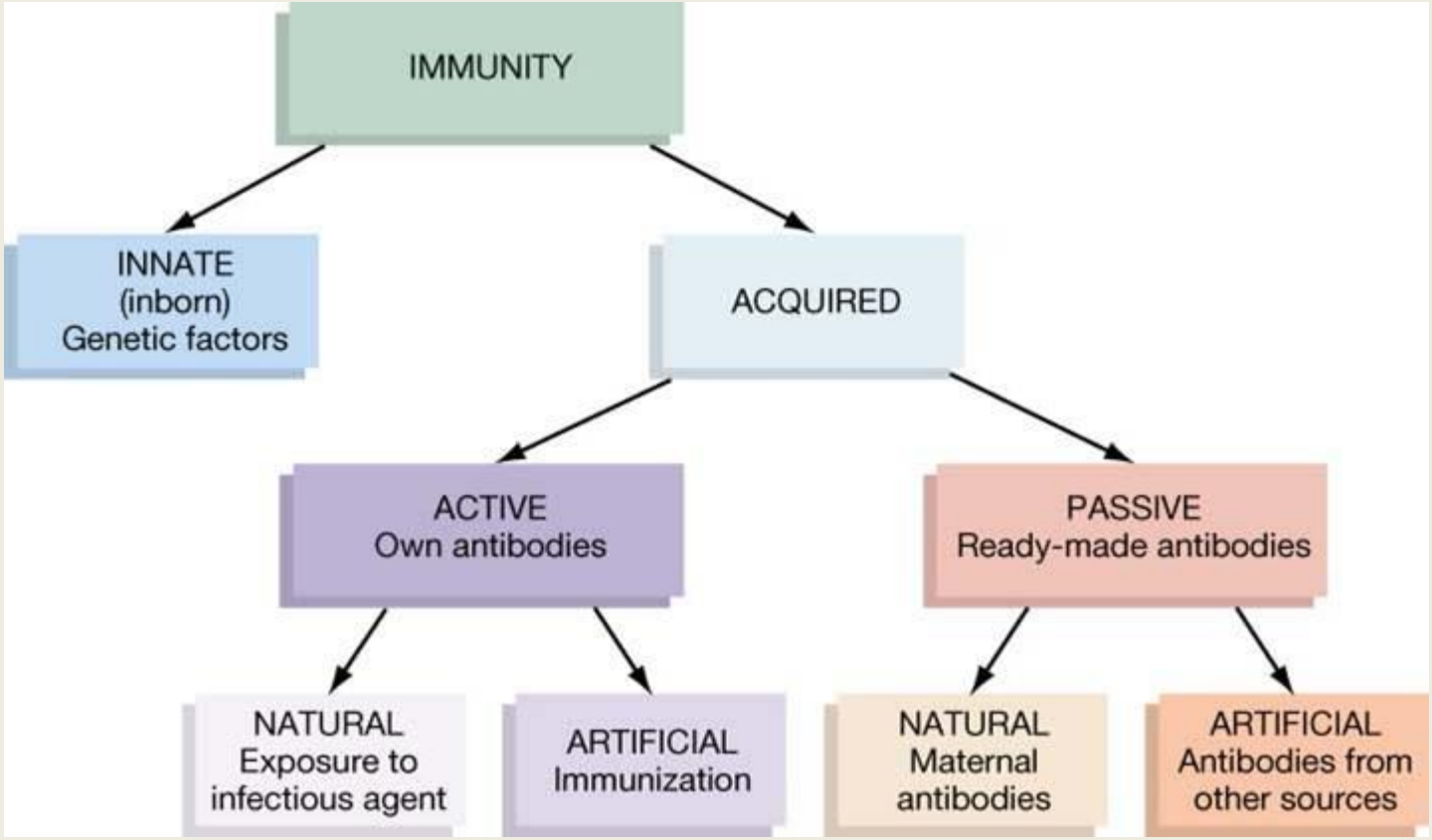
**And many many more**

# Immune dysfunction in autism: a pathway to treatment

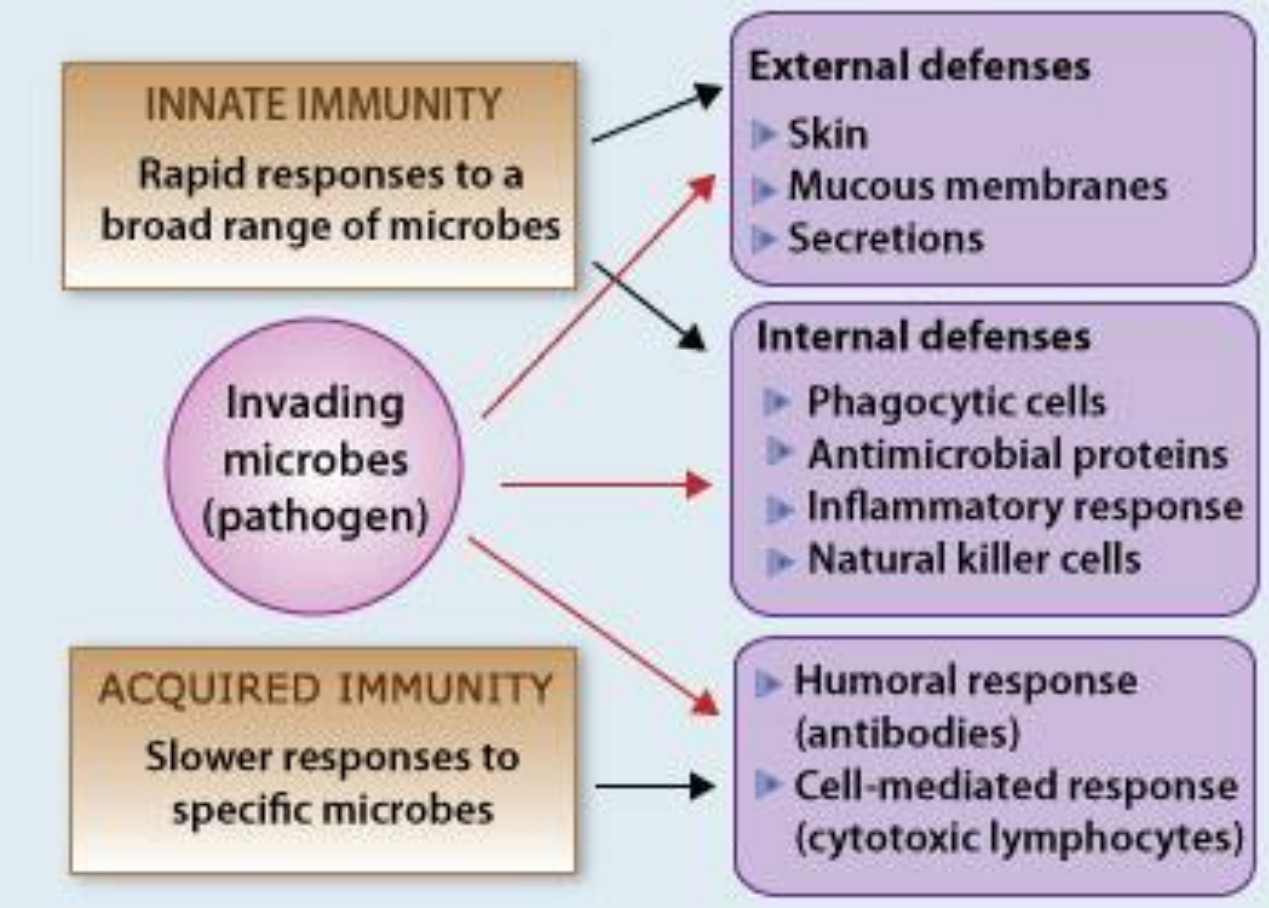
*"Autism is a complex and clinically heterogeneous disorder with a spectrum of symptoms. Published findings have identified widespread changes in the immune systems of children with autism, at both systemic and cellular levels. Together, these reports suggest that* ***autism may in fact be a systemic disorder with connections to abnormal immune responses****. Such immune system dysfunction may represent novel targets for treatment." (Careaga et al. Neurotherapeutics 2010)*

- The autism diagnosis is suffering by the lack of a specific biomarker
- Several biochemical pathways are associated with ASDs
- Many testing panels are currently available for biomedical evaluation of multifactorial afflictions
- Autism spectrum disorders are even more complex than most of multifactorial syndromes, needing thus a very specialized and personalized approach.

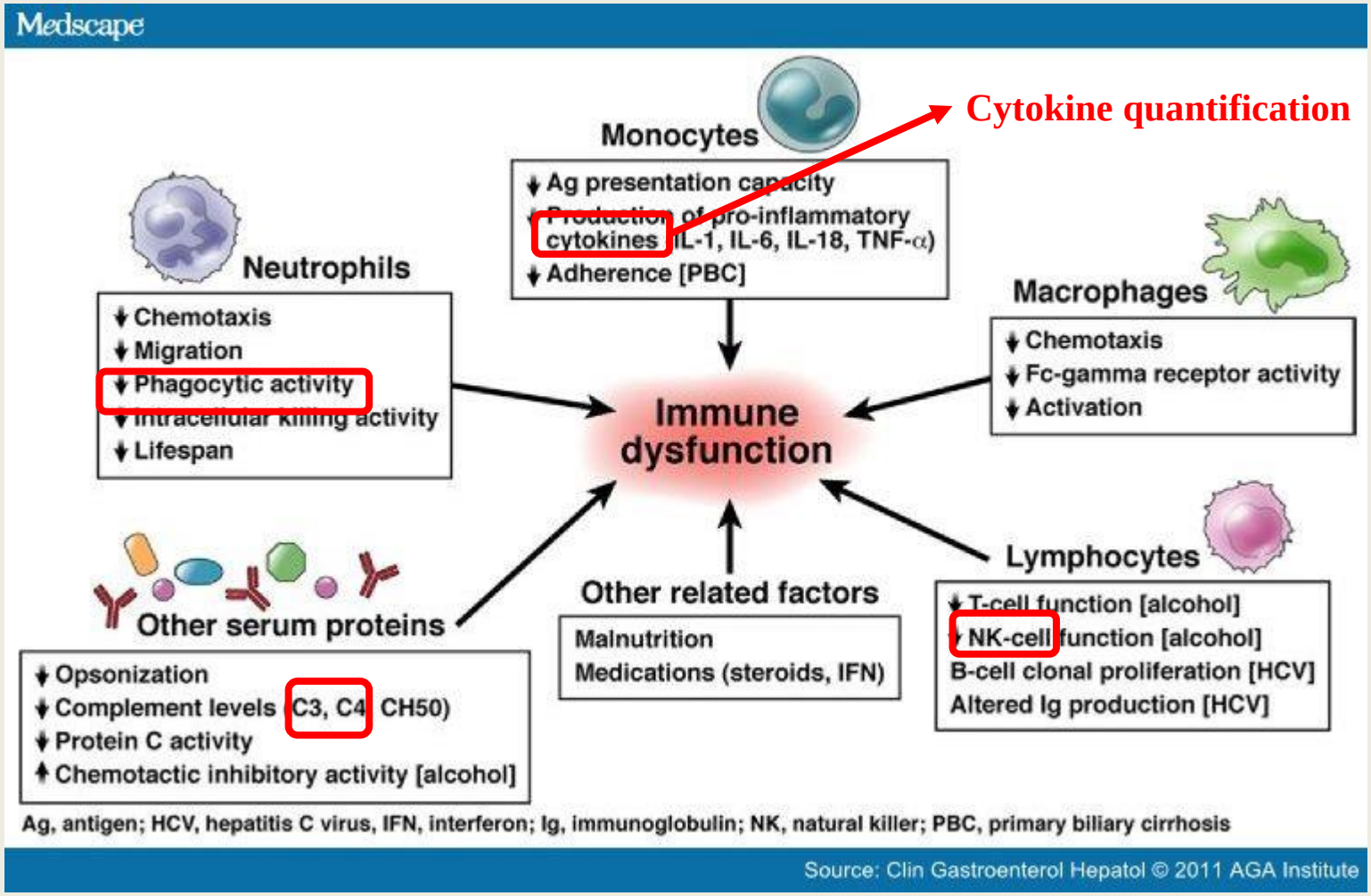
# Immunity


IMMUNITY
INNATE
(inborn)
Genetic factors
ACQUIRED
ACTIVE
Own antibodies
PASSIVE
Ready-made antibodies
NATURAL
Exposure to
infectious agent
ARTIFICIAL
Immunization
NATURAL
Maternal
antibodies
ARTIFICIAL
Antibodies from
other sources

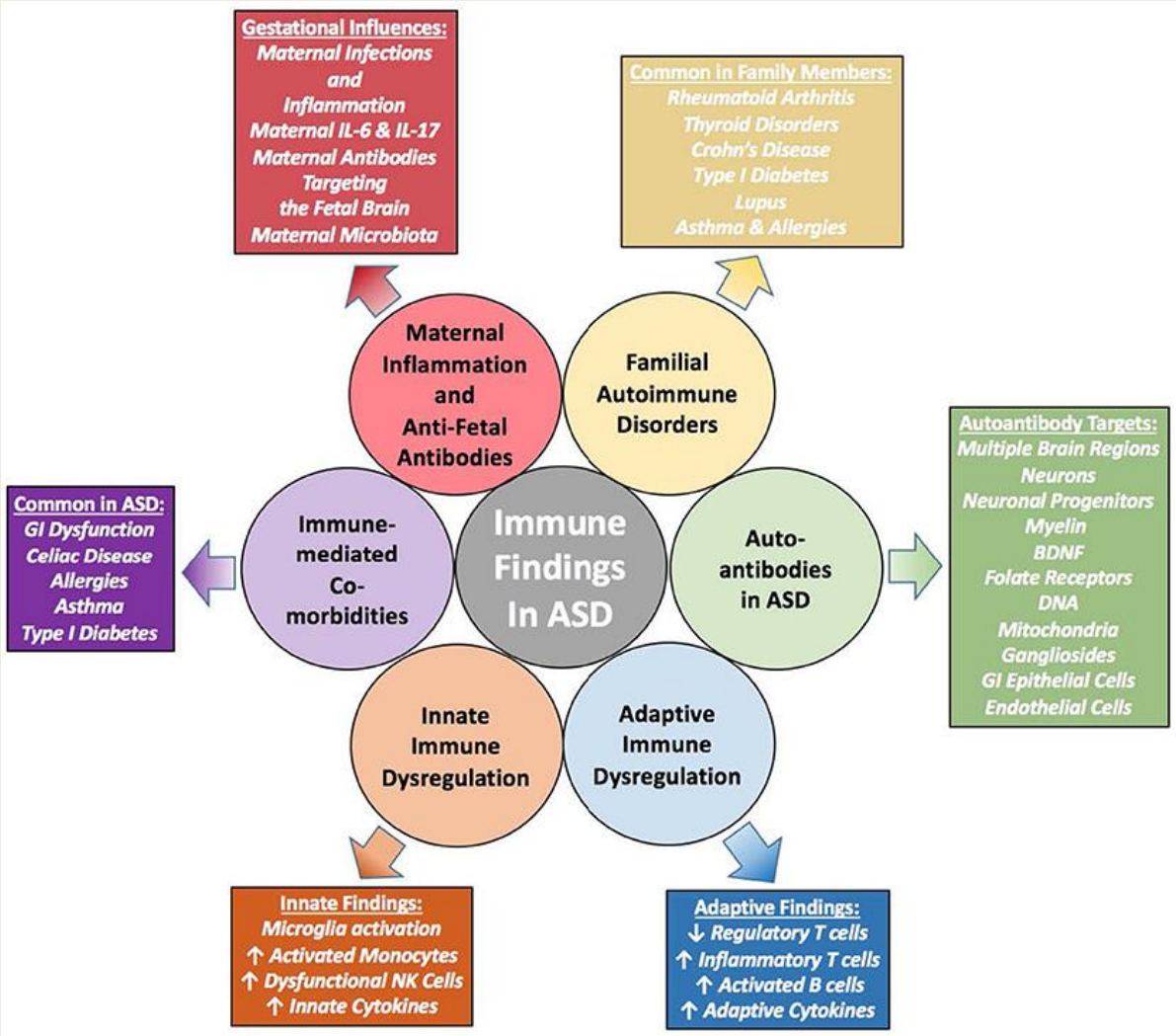
# Innate and acquired immunity



# Immune dysfunction



# Immune findings in ASD




*Hughues et al. Front. Cell. Neurosci., 13 November 2018*

# A majority of children with autism exhibited low NK cell activity

## Absolute count of CD56+ and CD57+ Natural killer cells

- Vojdani et al (J Neuroimmunol. 2008) reported on low natural killer cell cytotoxic activity in autism. In their study, involving 1027 blood samples from autistic children obtained from ten clinics and compared the results to 113 healthy controls, 45% of the children with autism exhibited low NK cell activity.
- CD57+/CD3- cells are a subset of NK cells. Their exact function, and what differentiates them from CD56+ NK cells, is not well understood. The absolute number of CD57+/CD3- cells is low in patients suffering from chronic Lyme disease (a disease that follows an infection by a bacteria called Borrelia). Patients with very low CD57 have significantly more co-infections and persistent immunologic defects than patients with higher counts. In patients that respond to antibiotic therapy, the number of cells come back to normal, hence this is a useful marker to follow the effect of a therapy.
- Our study (Siniscalco et al, InVivo 2016) showed that on 107 autistic patients enrolled, 73 (68.2%) of them showed CD57 below 100 cells/µl of whole blood (mean±SE 49.12±3.12), and among them 47 (64.4%) patients showed CD57 below the lower limit of the normal range (the normal range is considered 60-360 cells/µl of whole blood).

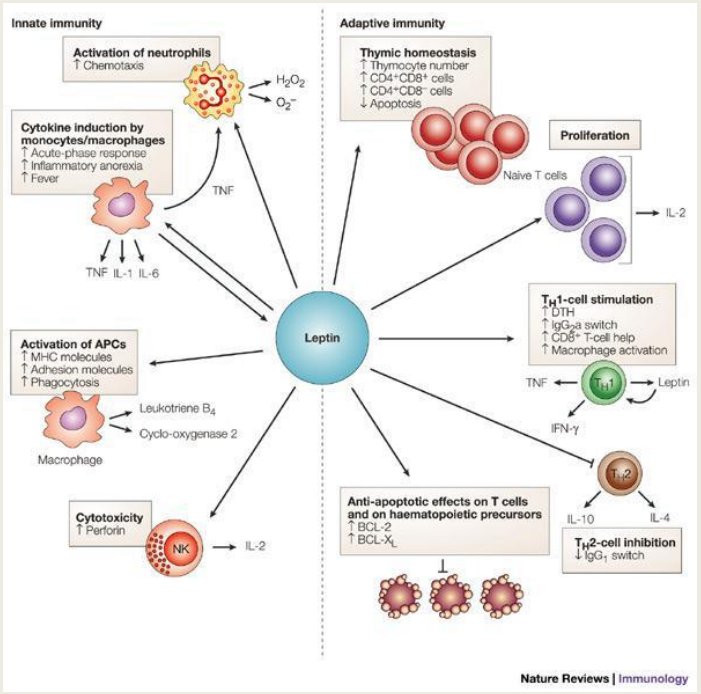
# *Perforin mRNA expression : a mean to evaluate NK cell activation*

Since NK cells play a central role in the defense against bacteria and viruses, decreased NK activity can lead to the development of opportunistic infections.

NK cells exert their cytotoxic effect by releasing perforin, a protein that will destroy the cytoplasmic membrane of target cells and finally kill them.

**Increased Perforin indicates that an infection is in place, while low perforin would indicate on ongoing persistent viral infection.**

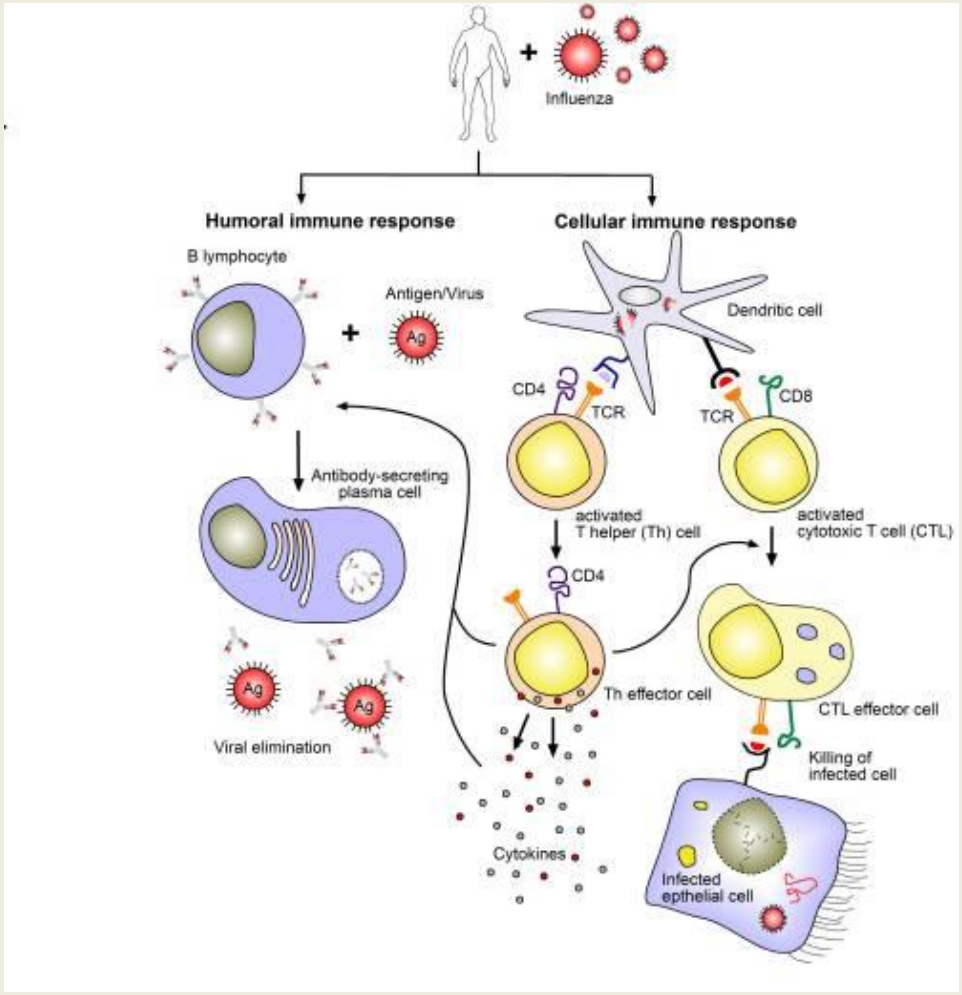
# Inflammation




**The response of our natural immunity to stress is called inflammation**

- Neutrophils and macrophages congregate at the site of injury/infection.
- They phagocyte invaders and release toxic substances such as oxygen radicals.
- Macrophages release **pro-inflammatory cytokines** IL-6 and TNFalpha to organize further inflammatory response.
- Mast cells and eosinophils will be involved in parasitic defense and allergy.
- Natural killer cells eliminante non-self cells by releasing toxic substances.

# Immune response




- **Cellular immune response Th1**

This is how we eliminate intracellular pathogens and non-self cells.

- **Humoral immune response Th2**

This is our ability to produce antibodies to neutralize pathogens.

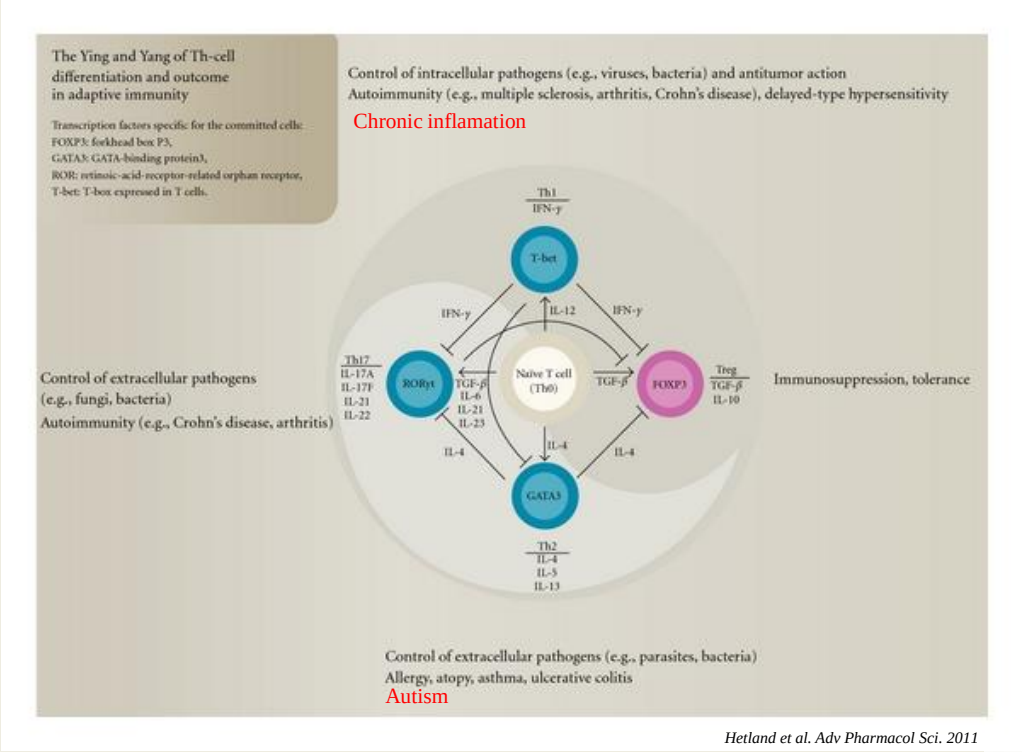
***Th1 and Th2 should always be in balance!***

# Specific immunity

- **Th1 and Th2 produce and release cytokines that trigger a domino effect leading to an immune reaction:**
- **Cytokines** released by **Th1** are: IL-2, IL-12, INFɣ, INFα and INFβ
- **Cytokines** released by **Th2** are: IL-4, IL-5, IL-10
- **Th1 cytokines suppress Th2 cytokines and vice versa**
- If the pathogen is defeated, the immune system returns to a balance between Th1 and Th2
- Unfortunately, some conditions involve chronic activation or suppression of one of the two categories.

# Th1/Th2 Balance

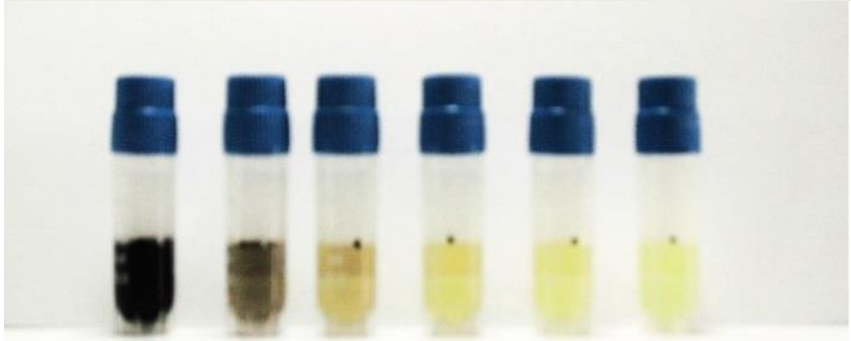
## Th1/Th2 balance




*Hetland et al. Adv Pharmacol Sci. 2011*

## urine-based Th1/Th2 balance test

- may detect disturbances of this delicate equilibrium in time in order to restore balance whenever required and before irreversible conditions are developing
- allows patients to follow-up on Th1/Th2 balance during therapy (anti-oxydants, probiotics, nutraceuticals).



**Far Th2** **Th2** **Balanced** **Th1** **Far Th1**

# Cytokine imbalances in autism spectrum disorders

-Ashwood et al. (Brain Behav Immun. 2011) reported on **significant increases in plasma levels of a number of cytokines**, including IL-1β, IL-6, IL-8 and IL-12p40 in the ASD group compared with controls.

-Suzuki et al. (PLoS One 2011) reported that the plasma concentrations of IL-1β, IL-1RA, IL-5, IL-8, IL-12(p70), IL-13, IL-17 and GRO-α were significantly higher in subjects with ASD compared with the corresponding values of matched controls.

-Okada et al. (Prog Neuropsychopharmacol Biol Psychiatry 2007) and Ashwood et al. (J Neuroimmunol. 2008 ) reported on **decreased serum levels of transforming growth factorbeta1 (TGFb1) in patients with autism**, with lower TGFb1 levels associated with lower adaptive behaviors and worse behavioral symptoms, suggesting that

-Al-Ayadhi LY1, Mostafa GA - J Neuroinflammation. 2012 9:158. reported that Children with autism had **significantly higher serum IL-17A** levels than healthy controls (P <0.001), with increased serum levels of IL-17A found in 48.9% of the autism group.

- Patients with severe autism had significantly higher serum IL-17A levels than those with mild to moderate autism (P=0.01), and raised serum IL-17A levels were significantly more common in children with severe autism (67.9%) than in those with mild to moderate autism (17.6%), P=0.001.
- Serum IL-17A levels were raised in the group with autism, and the levels correlated significantly with the severity of autism
- Further research is warranted to determine whether the increase of serum IL-17A levels plasma has a pathogenic role in autism, and whether anti- IL-17A therapy could be useful.

# The Importance of Assessing Inflammation-Related Markers

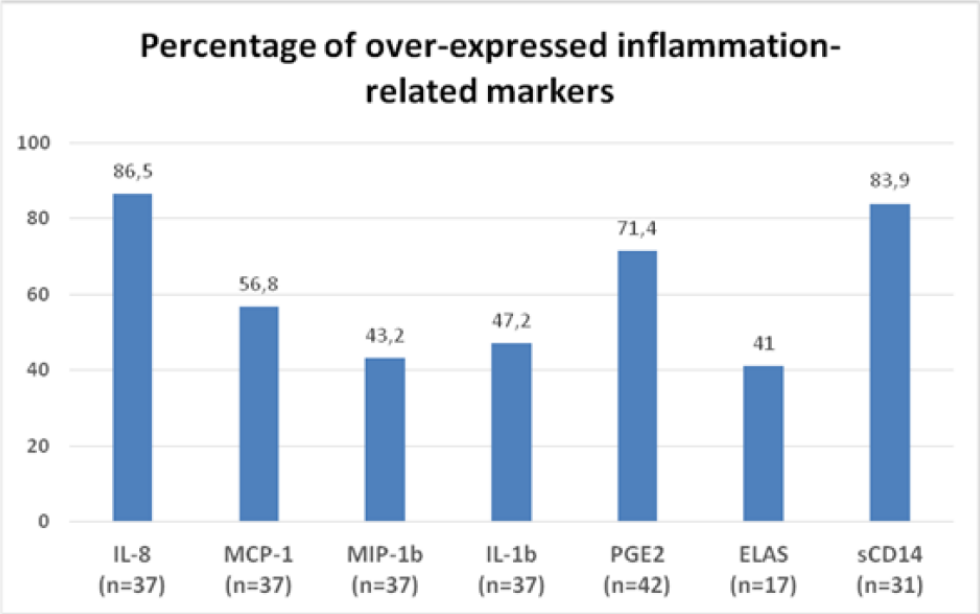
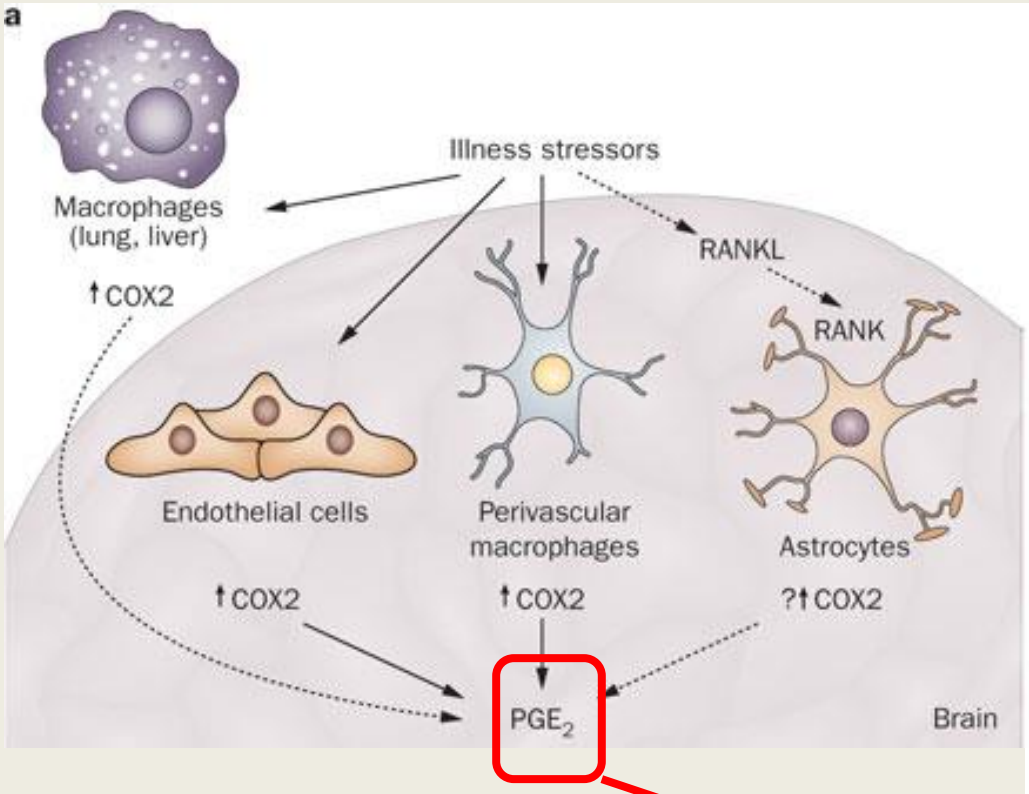


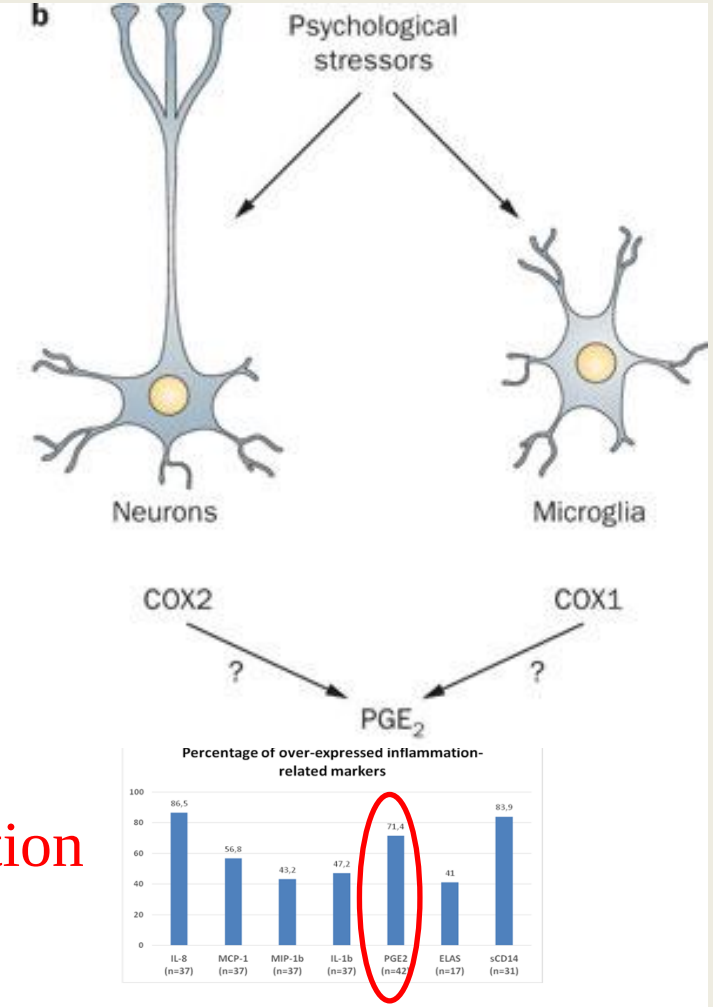

**Figure 1.** Percentage of over-expressed inflammation-related markers. The values of the graph represent the percentage of ASD patients with abnormal laboratory results for serum interleukin 8 (IL-8), MCP-1, MIP-1β, IL-1β, PGE2 and sCD14 and mRNA levels for elastase (ELAS). The numbers in brackets indicate the total number of patient records used to derive each respective value.

*Mijatovic T. et al., 2018, AIMS Molecular Science 5:173-182.*

# $PGE_2$ production during illness and psychological stress




quantification

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Furuyashiki, T. & Narumiya, S. (2010) Stress responses: the contribution of prostaglandin $E_2$ and its receptors
*Nat. Rev. Endocrinol.* doi:10.1038/nrendo.2010.194

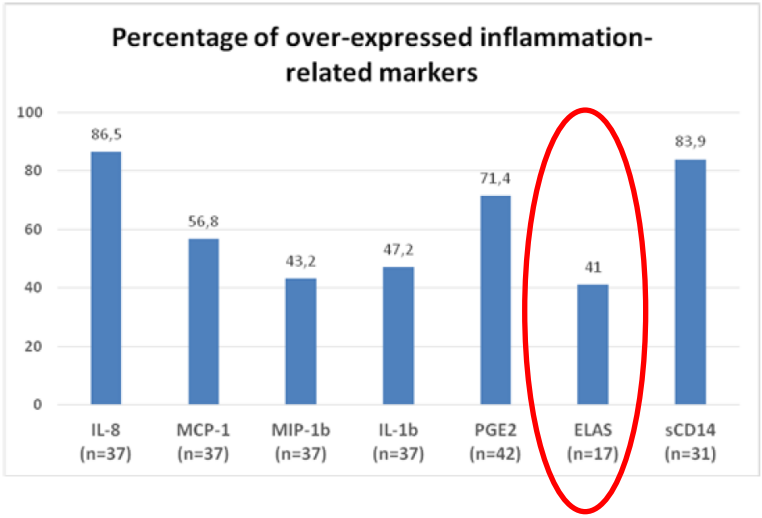
# PGE2 has been found as significantly higher in autistic patients

## Quantification of serum levels of Prostaglandine E2

- Prostaglandin E2 (PGE2) has been found as significantly higher in autistic patients, recording an increase of 91.15% (El-Ansary & Al-Ayadhi, Lipids Health Dis. 2012).
- PGE2 is a compound derived from membrane phospholipids and is a key mediator of immunopathology in chronic infections and cancer
- PGE2 selectively suppresses effector functions of macrophages and neutrophils and the Th1-, CTL-, and NK cell-mediated type 1 immunity, but it promotes Th2, Th17, and regulatory T cell responses.
- PGE2 modulates chemokine production, inhibiting the attraction of proinflammatory cells while enhancing local accumulation of regulatory T cells cells and myeloid-derived suppressor cells.

# *Elastase mRNA expression : a marker of inflammation*

Elastase is an inflammatory protease expressed in immune cells (monocytes, neutrophils) that contributes to immune defense by inactivating foreign bacteria but at the same time it causes damage to connective tissue, breaks down cytokines, immunoglobulins and immune cells receptors. An excess, chronic production of elastase is therefore detrimental detrimental (destroyed gamma-globulins ➔ decreased immunity; broken elastin ➔ diminished tissue elasticity, problems with neck) .

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# Environmental exposure and oxidative stress

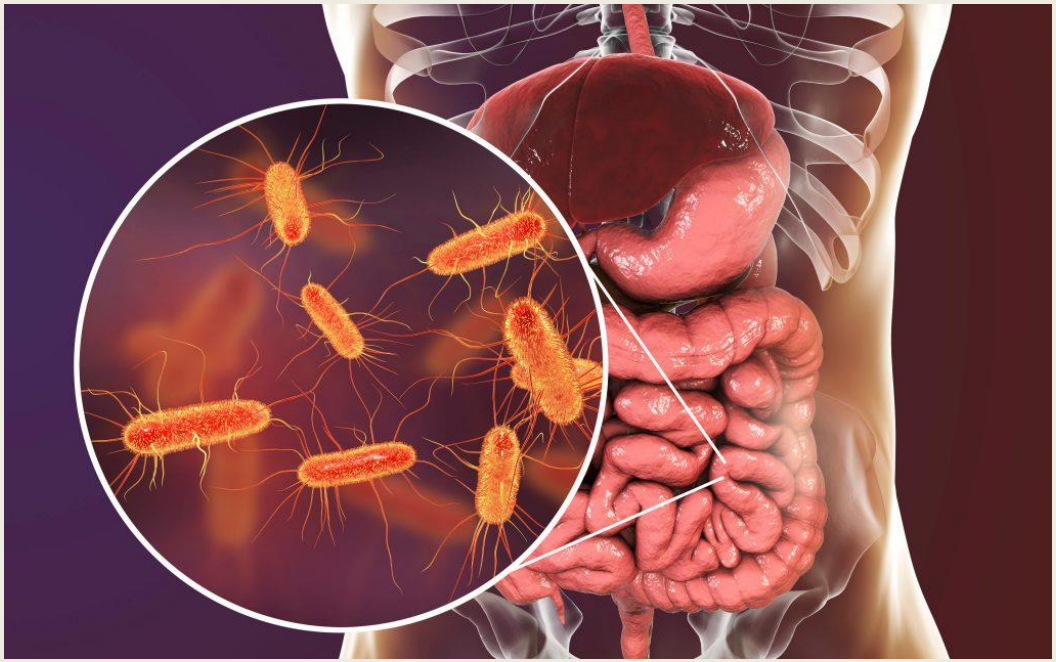
- Oxidative stress is implicated in a large number of diseases, including neurodegenerative diseases and autoimmune diseases
- Indicators of oxidative stress have been detected in muscles and blood
- Oxidative damage to cellular membranes can alter the permeability of the blood-brain barrier, which could lead to some of the cognitive symptoms observed in patients.
- Increased oxidative stress could have several origins: chronic inflammation (activated neutrophils release pro-oxidative molecules), excess nitric oxide production (NO reacts with free radicals to produce peroxynitrite, a potent oxidant), or exposure to environmental toxins (exposure to certain chemicals leads to the depletion of essential antioxidants such as glutathione and selenium; heavy metals can also directly inhibit antioxidant enzymes like superoxide dismutase or glutathione reductase).
- Oxidative stress markers are useful to evaluate the need for antioxidant therapy.

# SUMMARY - Testing for immune and metabolic dysfunctions

**The extent of the global immune and / or metabolic dysfunctions are evaluated by testing:**

- cytokine expression
- elastase and perforin mRNA expression
- oxidative stress, heavy metals, molds
- macrophage phagocytic activity
- alpha-N-acetylgalactosaminidase activity (Nagalase testing)
- sCD14 expression
- C3a & C4a expression
- CD57 cell subset absolute count
- prostaglandine E2 (PGE2) synthesis and hsCRP
- VEGF synthesis
- ammonia accumulation
- kynurenic and quinolinic acid accumulation, and many more

# Testing for Intestinal dysfunction in autism



Although ASD primarily impacts the brain, over recent years, links with other systems have become clear — in particular, gastrointestinal (GI) issues seem to occur more often in individuals with ASD than in the rest of the population.

The GI issues that come with ASD might be due to two factors: firstly, inappropriate immune activation, causing inflammation of the tract; and, secondly, differences in the types of gut bacteria that are present.

# Gut-Brain Axis in Autism

Autism-Open Access

Siniscalco, Autism-Open Access 2014, 4:3
http://dx.doi.org/10.4172/2165-7890.1000e124

Editorial Open Access

## Gut Bacteria–Brain Axis in Autism

Dario Siniscalco*[1,2,3]

Starting from an early idea of Nobel laureate Luc Montagnier that metabolites from gut bacteria end up in the plasma and could trigger damage to the brain [3], we can now coin the term "BBB" in autism as bacteria-brain-behavior influence that we see in autistic children. It is noteworthy to consider that nowadays almost all the autistic patients suffer from gastrointestinal symptoms and show an altered intestinal barrier, such as an impaired gut barrier function [4,5]. This impaired gut barrier permits the passage of dietary-derived non-self antigens and has a dramatic consequence on the immune system responses of the autistic child [6].

# Intestinal dysfunctions in autism

- Gut flora and gastrointestinal status in children with autism correlate with autism severity
  - **Adams et al. (BMC Gastroenterology 2011) reported that gastrointestinal symptoms were strongly correlated with the severity of autism. From four types of beneficial bacteria that were investigated, the children with autism had much lower levels of Bifidobacterium (-45%), slightly lower levels of Enterococcus (-16%), and much higher levels of Lactobacillus (+100%).**
  - **Finegold et al. (Anaerobe 2010) reported that in the control children's stools, Firmicutes accounted for 63.6% of the total flora but only 38-39% of the flora of autistic children's stools. Bacteroidetes accounted for 30% of the stool flora in controls and for 51% in the flora of stools of autistic children. Actinobacteria made up 1.8% of stool flora of control children and between 0.4 and 0.7% of the flora of autistic children. Proteobacteria made up 0.5% of the flora of control children and between 2.3 and 3.1% of the flora of autistic children. In summary, the fecal flora of autistic children was statistically significantly different from the fecal flora of healthy children.**

➢ ***R.E.D. Laboratories are offering specialty test to deeply analyze gut microbiota: MSA assay***

# METAGENOMIC STOOL ASSAY (MSA)

## Intestinal microbiota analysis : from culture to high-throughput sequencing:

- *Until recently research into microbiota composition relied almost exclusively on culture ; 40 to 80% of gut bacteria cannot be cultured*
- *Identification of colonies can be difficult*
- *Bacteria must be alive: studies of anaerobes very difficult, major loss during collection and processing of samples*
- *Culture approach may address only a small fraction of all bacterial species (10%?)*
- *E.coli once thought to be a dominant species, is a minor member…*

## R.E.D. Labs scientists have developed and validated a new procedure to analyze bacterial populations in a stool sample : MSA assay

- ***New molecular technique involving sequencing of specific regions of bacterial DNA (metagenomics)***
- Can be performed on dead organisms (exposure to oxygen, freezing are not a problem)
- Identification of each bacteria by comparing sequence with public databases: extremely precise, not subjective
- High-throughput technology allows identification of tens or even hundreds of thousends organisms in a single sample
- Bacterial DNA was extracted from stool samples, PCR amplification was performed on 16S rRNA gene regions, and PCR amplicons were sequenced Bacteria were classified by phylum, family and genus.

# ASD child MSA

**Prevotella**: strong hydrogen sulfide ($H_2S$) producers. In excess, $H_2S$ acts as a mitochondrial poison and a potent neurotoxin. It can directly inhibit enzymes involved in the cellular production of energy. $H_2S$ also interferes with oxygen transport by blocking hemoglobin in the red blood cells. Finally, $H_2S$ is lowering gut pH preventing the growth of many beneficial bacteria,

| | | | JAN 2019 | Ref. | Oct 2018 | JAN 2018 |
|---|---|---|---|---|---|---|
| PHYLUM | FAMILY | GENUS | % of total | | % of total | % of total |
| Firmicutes (gram +) | *Lachnospiraceae* | Anaerostipes | 0.01 | <1 | 0.01 | 0.13 |
| | | Coprococcus | 4.06 | <10 | 1.97 | 1.72 |
| | | Dorea | 4.24 | <15 | 3.92 | 4.55 |
| | | Moryella | 0.01 | <1 | 0 | 0.03 |
| | | Roseburia | 3.81 | <50 | 3.7 | 3.31 |
| | | Sporobacterium | 0 | <1 | 0 | 0 |
| | | Syntrophococcus | 0 | <1 | 0 | 0 |
| | *Ruminococcaceae* | Acetanaerobacterium | 0 | <5 | 0 | 0 |
| | | Acetivibrio | 0 | <1 | 0 | 0 |
| | | Ethanoligenens | 0 | <1 | 0 | 0 |
| | | Faecalibacterium | 22.95 | <25 | 18.55 | 9.08 |
| | | Papillibacter | 0 | <1 | 0 | 0 |
| | | Ruminococcus | 0.52 | >2 | 0.24 | 0.01 |
| | | Sporobacter | 0 | <1 | 0 | 0 |
| | | Subdoligranulum | 2.65 | <25 | 0.08 | 0.02 |
| | *Clostridiaceae* | Butyricicoccus | 5.71 | <5 | 2.59 | 2.67 |
| | | Clostridium Sensu Stric. | 0.05 | <5 | 0.04 | 3.78 |
| | | Lactonifactor | 0.01 | 0 | 0.02 | 0 |
| | *Eubacteriaceae* | Anaerofustis | 0 | <0,5 | 0 | 0 |
| | | Eubacterium | 0 | 0 | 0.03 | 0 |
| | *Blautia* | Blautia | 20 | <50 | 17.15 | 3.68 |
| | *Howardella* | Howardella | 0 | <1 | 0 | 0 |
| | *Lactobacillaceae* | Lactobacillus | 0.08 | <1 | 0.01 | 0.42 |
| | *Enterococcaceae* | Enterococcus | 0 | 0 | 0 | 0 |
| | *Streptococcaceae* | Lactococcus | 0.01 | <1 | 0.02 | 0.03 |
| | | Streptococcus | 1.12 | <5 | 4.75 | 18.35 |
| | *Leuconostoc* | Leuconostoc | 0 | <0,3 | 0.01 | 0.19 |
| | *Erysipelotrichaceae* | Catenibacterium | 0 | <0,3 | 0 | 0 |
| | | Coprobacillus | 0.07 | <1 | 0.05 | 0.23 |
| | | Holdemania | 0.02 | <1 | 0.01 | 0.01 |
| | | Turicibacter | 0 | >0,5 | 0 | 0 |
| | *Veillonellaceae* | Dialister | 0 | 0-1 | 0 | 0 |
| | | Megamonas | 0 | 0 | 0 | 0 |
| | | Megasphera | 0 | 0 | 0 | 0 |
| | *Oscillospiraceae* | Oscillibacter | 2.47 | <4 | 1.05 | 0.17 |
| | *Staphylococcus* | Staphylococcus | 0 | <0,05 | 0.05 | 0.01 |
| Bacteroidetes (gram -) | *Bacteroidaceae* | Bacteroides | 7.3 | <10 | 8.53 | 4.42 |
| | *Rikenellaceae* | Alistipes | 1.19 | <3 | 0.65 | 0.11 |
| | *Porphyromonadaceae* | Barnesiella | 1.3 | <2 | 0.95 | 0.11 |
| | | Odoribacter | 0.13 | <0,5 | 0.15 | 0.01 |
| | | Parabacteroides | 0.66 | <3 | 1.77 | 0.23 |
| | *Prevotellaceae* | Prevotella | 15.61 | <5 | 26.07 | 42.02 |
| | | Xylanibacter | 0 | <1 | 0 | 0.03 |
| Actinobacteria (gram+) | *Bifidobacteriaceae* | Bifidobacterium | 0 | >5 | 0.01 | 0 |
| | *Actinomycineae* | Actinomyces | 0.37 | <1 | 1.29 | 0.62 |
| | *Micrococcineae* | Rothia | 0.27 | <0,2 | 1.92 | 1.37 |
| | *Coriobacterineae* | Asaccharobacter | 0 | >0,1 | 0 | 0 |
| | | Collinsella | 0.1 | <25 | 0.14 | 0.35 |
| | | Olsenella | 0 | 0 | 0 | 0 |
| | | Slackia | 0.39 | <1 | 0.16 | 0.15 |
| Proteobacteria (gram-) | *Enterobacteriaceae* | Escherichia/Shigella | 0 | <0,5 | 0 | 0.02 |
| | | Klebsiella | 0 | <0,5 | 0 | 0 |
| | *Sutterellaceae* | Sutterella | 0 | <1 | 0 | 0 |
| | *Desulfovibrionaceae* | Lawsonia | 0 | <0,5 | 0 | 0 |

# ASD child MSA

**Bacteroides**: dysbiotic bacterias found in most anaerobic infections with an associated mortality of more than 19 %. Species of the genus Bacteroides have the most antibiotic resistance mechanisms and the highest resistance rates of all anaerobic pathogens.

| PHYLUM | FAMILY | GENUS | % of total | Ref. |
|---|---|---|---|---|
| Firmicutes (gram +) | *Lachnospiraceae* | Anaerostipes | 0.08 | <1 |
| | | Coprococcus | 0.11 | <10 |
| | | Dorea | 1.73 | <15 |
| | | Moryella | 0.05 | <1 |
| | | Roseburia | 11.49 | <50 |
| | | Sporobacterium | 0 | <1 |
| | | Syntrophococcus | 0 | <1 |
| | *Ruminococcaceae* | Acetanaerobacterium | 0 | <5 |
| | | Acetivibrio | 0.01 | <1 |
| | | Ethanoligenens | 0 | <1 |
| | | Faecalibacterium | 18.71 | <25 |
| | | Papillibacter | 0 | <1 |
| | | Ruminococcus | 0 | >2 |
| | | Sporobacter | 0 | <1 |
| | | Subdoligranulum | 0.02 | <25 |
| | *Clostridiaceae* | Butyricicoccus | 0.05 | <5 |
| | | Clostridium Sensu Stric. | 0.24 | <5 |
| | | Lactonifactor | 0 | 0 |
| | *Eubacteriaceae* | Anaerofustis | 0 | <0,5 |
| | | Eubacterium | 0 | 0 |
| | *Blautia* | Blautia | 1.2 | <50 |
| | *Howardella* | Howardella | 0 | <1 |
| | *Lactobacillaceae* | Lactobacillus | 0.01 | <1 |
| | *Enterococcaceae* | Enterococcus | 0.01 | 0 |
| | *Streptococcaceae* | Lactococcus | 0.01 | <1 |
| | | Streptococcus | 1.3 | <5 |
| | *Leuconostoc* | Leuconostoc | 0 | <0,3 |
| | *Erysipelotrichaceae* | Catenibacterium | 0 | <0,3 |
| | | Coprobacillus | 0 | <1 |
| | | Holdemania | 0 | <1 |
| | | Turicibacter | 0.02 | >0,5 |
| | *Veillonellaceae* | Dialister | 0.01 | 0-1 |
| | | Megamonas | 0 | 0 |
| | | Megasphera | 0 | 0 |
| | *Oscillospiraceae* | Oscillibacter | 0.24 | <4 |
| | *Staphylococcus* | Staphylococcus | 0 | <0,05 |
| Bacteroidetes (gram-) | *Bacteroidaceae* | Bacteroides | 12.57 | <10 |
| | *Rikenellaceae* | Alistipes | 0.01 | <3 |
| | *Porphyromonadaceae* | Barnesiella | 0.01 | <2 |
| | | Odoribacter | 0 | <0,5 |
| | | Parabacteroides | 0 | <3 |
| | *Prevotellaceae* | Prevotella | 0.02 | <5 |
| | | Xylanibacter | 0 | <1 |
| Actinobacteria (gram+) | *Bifidobacteriaceae* | Bifidobacterium | 41.94 | >5 |
| | *Actinomycineae* | Actinomyces | 1.21 | <1 |
| | *Micrococcineae* | Rothia | 0.32 | <0,2 |
| | *Coriobacterineae* | Asaccharobacter | 0 | >0,1 |
| | | Collinsella | 4.65 | <25 |
| | | Olsenella | 0.03 | 0 |
| | | Slackia | 0 | <1 |
| Proteobacteria (gram-) | *Enterobacteriaceae* | Escherichia/Shigella | 0 | <0,5 |
| | | Klebsiella | 0 | <0,5 |
| | *Sutterellaceae* | Sutterella | 1.19 | <1 |
| | *Desulfovibrionaceae* | Lawsonia | 0 | <0,5 |

| | Value | Ref. |
|---|---|---|
| Total Lachnospiraceae | 13.46 | >5 |
| Total Ruminococcaceae | 18.74 | >5 |
| Total Clostridiaceae | 0.29 | <5 |
| Enterococcus | 0.01 | 0 |
| Streptococcus | 1.3 | <5 |
| Ruminococcus | 0 | >2 |
| Lactonifactor | 0 | 0 |
| Turicibacter | 0.02 | >0,5 |
| Bacteroides | 12.57 | <10 |
| Prevotella | 0.02 | <5 |
| Bifidobacterium | 41.94 | >5 |
| Asaccharobacter | 0 | >0,1 |

| | |
|---|---|
| Firmicutes | 35.29 % |
| Bacteroidetes | 12.61 % |
| Actinobacteria | 48.15 % |
| Proteobacteria | 1.19 % |
| Other | 2.76 % |

Range of Firmicutes % in European population: 50-85%

| Firmicutes/Bacteroidetes ratio | |
|---|---|
| High | |
| Average | |
| Low | 2.80 |

Low ratio may be associated with gut inflammation

| Gram+ / Gram- ratio | |
|---|---|
| High | |
| Average | |
| Low | 6.05 |

| | |
|---|---|
| Diversity Index | 3.13 |

Low <4, Average 4-5, High >5

Dysbiosis associated with low diversity

Electronically validated on: 16/01/2019

by E. Bosmans

Requesting physician: Dr. Anne VAN DE WINCKEL

Observations:

# ASD child MSA

**1st visit April 2017:**

High both Bacteroides & Prevotella

**2nd visit December 2017:**

Still high Bacteroides but normalized Prevotella

**3rd visit December 2018:**

Normalized both Bacteroides and Prevotella

| PHYLUM | FAMILY | GENUS | % of total | Ref. | % of total | % of total |
|---|---|---|---|---|---|---|
| Firmicutes (gram +) | *Lachnospiraceae* | Anaerostipes | 0.05 | <1 | 0.03 | 0,01 |
| | | Coprococcus | 19.75 | <10 | 13.52 | 8,83 |
| | | Dorea | 3.96 | <15 | 9.5 | 5,38 |
| | | Moryella | 0 | <1 | 0 | 0 |
| | | Roseburia | 8.02 | <50 | 0.97 | 13,26 |
| | | Sporobacterium | 0 | <1 | 0 | 0 |
| | | Syntrophococcus | 0.01 | <1 | 0 | 0 |
| | *Ruminococcaceae* | Acetanaerobacterium | 0 | <5 | 0 | 0 |
| | | Acetivibrio | 0 | <1 | 0 | 0 |
| | | Ethanoligenens | 0 | <1 | 0.01 | 0,01 |
| | | Faecalibacterium | 2.96 | <25 | 18.87 | 25,32 |
| | | Papillibacter | 0 | <1 | 0 | 0 |
| | | Ruminococcus | 0.01 | >2 | 8.06 | 0,19 |
| | | Sporobacter | 0.01 | <1 | 0 | 0 |
| | | Subdoligranulum | 0.01 | <25 | 0.01 | 0,02 |
| | *Clostridiaceae* | Butyricicoccus | 1.07 | <5 | 5.16 | 3,68 |
| | | Clostridium Sensu Stric. | 0.64 | <5 | 0.04 | 0 |
| | | Lactonifactor | 0 | 0 | 0.07 | 0 |
| | *Eubacteriaceae* | Anaerofustis | 0.05 | <0,5 | 0.01 | 0 |
| | | Eubacterium | 0.05 | 0 | 0 | 0,03 |
| | *Blautia* | Blautia | 6.69 | <50 | 5.88 | 19,01 |
| | *Howardella* | Howardella | 0.07 | <1 | 0 | 0 |
| | *Lactobacillaceae* | Lactobacillus | 0.1 | <1 | 0.01 | 0 |
| | *Enterococcaceae* | Enterococcus | 0 | 0 | 0.15 | 0 |
| | *Streptococcaceae* | Lactococcus | 0 | <1 | 0.01 | 0,01 |
| | | Streptococcus | 2.27 | <5 | 1.44 | 4,42 |
| | *Leuconostoc* | Leuconostoc | 0.01 | <0,3 | 0.39 | 0 |
| | *Erysipelotrichaceae* | Catenibacterium | 0 | <0,3 | 0 | 0 |
| | | Coprobacillus | 0.02 | <1 | 0.01 | 0 |
| | | Holdemania | 0 | <1 | 0 | 0 |
| | | Turicibacter | 0.07 | >0,5 | 0 | 0 |
| | *Veillonellaceae* | Dialister | 2.69 | 0-1 | 0 | 0 |
| | | Megamonas | 0 | 0 | 0 | 0 |
| | | Megasphera | 0 | 0 | 0 | 0 |
| | *Oscillospiraceae* | Oscillibacter | 0.98 | <4 | 3.65 | 0,53 |
| | *Staphylococcus* | Staphylococcus | 0 | <0,05 | 0 | 0,01 |
| Bacteroidetes (gram-) | *Bacteroidaceae* | Bacteroides | 13.8 | <10 | 20.21 | 6,88 |
| | *Rikenellaceae* | Alistipes | 1.66 | <3 | 0.55 | 0,23 |
| | *Porphyromonadaceae* | Barnesiella | 0.25 | <2 | 0.27 | 0,04 |
| | | Odoribacter | 0.1 | <0,5 | 0.17 | 0,02 |
| | | Parabacteroides | 2.49 | <3 | 0.67 | 0,3 |
| | *Prevotellaceae* | Prevotella | 21.71 | <5 | 3.85 | 3,77 |
| | | Xylanibacter | 0 | <1 | 0 | 0 |
| Actinobacteria (gram+) | *Bifidobacteriaceae* | Bifidobacterium | 2.21 | >5 | 0.11 | 0 |
| | *Actinomycineae* | Actinomyces | 0.16 | <1 | 0.08 | 0,23 |
| | *Micrococcineae* | Rothia | 0.01 | <0,2 | 0.05 | 1,14 |
| | *Coriobacterineae* | Asaccharobacter | 0 | >0,1 | 0 | 0 |
| | | Collinsella | 2.01 | <25 | 0.57 | 1,57 |
| | | Olsenella | 0 | 0 | 0 | 0 |
| | | Slackia | 0.58 | <1 | 0.28 | 0,19 |
| Proteobacteria (gram-) | *Enterobacteriaceae* | Escherichia/Shigella | 0.05 | <0,5 | 0 | 0 |
| | | Klebsiella | 0 | <0,5 | 0 | 0 |
| | *Sutterellaceae* | Sutterella | 0.81 | <1 | 0 | 0 |
| | *Desulfovibrionaceae* | Lawsonia | 0 | <0,5 | 0 | 0 |

ASD adult 28y

**Streptococcus:** well known in ASD. Strep antibodies might interact with the part of the brain known as the basal ganglia. This is believed to cause the sudden onset of tics or obsessive compulsive behaviors.

| PHYLUM | FAMILY | GENUS | % of total | Ref. |
|---|---|---|---|---|
| Firmicutes (gram +) | *Lachnospiraceae* | Anaerostipes | 0 | <1 |
| | | Coprococcus | 0,02 | <10 |
| | | Dorea | 1,61 | <15 |
| | | Moryella | 0 | <1 |
| | | Roseburia | 0,23 | <50 |
| | | Sporobacterium | 0 | <1 |
| | | Syntrophococcus | 0 | <1 |
| | *Ruminococcaceae* | Acetanaerobacterium | 0 | <5 |
| | | Acetivibrio | 0 | <1 |
| | | Ethanoligenens | 0 | <1 |
| | | Faecalibacterium | 0,27 | <25 |
| | | Papillibacter | 0 | <1 |
| | | Ruminococcus | 0,02 | >2 |
| | | Sporobacter | 0 | <1 |
| | | Subdoligranulum | 0,07 | <25 |
| | *Clostridiaceae* | Butyricicoccus | 0,05 | <5 |
| | | Clostridium Sensu Stric. | 0,01 | <5 |
| | | Lactonifactor | 0,02 | 0 |
| | *Eubacteriaceae* | Anaerofustis | 0 | <0,5 |
| | | Eubacterium | 0 | 0 |
| | *Blautia* | Blautia | 0,6 | <50 |
| | *Howardella* | Howardella | 0 | <1 |
| | *Lactobacillaceae* | Lactobacillus | 4,47 | <1 |
| | *Enterococcaceae* | Enterococcus | 8,54 | 0 |
| | *Streptococcaceae* | Lactococcus | 0,02 | <1 |
| | | Streptococcus | 40,61 | <5 |
| | *Leuconostoc* | Leuconostoc | 0,01 | <0,3 |
| | *Erysipelotrichaceae* | Catenibacterium | 0 | <0,3 |
| | | Coprobacillus | 0 | <1 |
| | | Holdemania | 0,04 | <1 |
| | | Turicibacter | 0 | >0,5 |
| | *Veillonellaceae* | Dialister | 0,35 | 0-1 |
| | | Megamonas | 0 | 0 |
| | | Megasphera | 0 | 0 |
| | *Oscillospiraceae* | Oscillibacter | 0,3 | <4 |
| | *Staphylococcus* | Staphylococcus | 0 | <0,05 |
| Bacteroidetes (gram-) | *Bacteroidaceae* | Bacteroides | 3,91 | <10 |
| | *Rikenellaceae* | Alistipes | 0,05 | <3 |
| | *Porphyromonadaceae* | Barnesiella | 0 | <2 |
| | | Odoribacter | 0,02 | <0,5 |
| | | Parabacteroides | 0,18 | <3 |
| | *Prevotellaceae* | Prevotella | 0,01 | <5 |
| | | Xylanibacter | 0 | <1 |
| Actinobacteria (gram+) | *Bifidobacteriaceae* | Bifidobacterium | 35,39 | >5 |
| | *Actinomycineae* | Actinomyces | 0,09 | <1 |
| | *Micrococcineae* | Rothia | 0,24 | <0,2 |
| | *Coriobacterineae* | Asaccharobacter | 0 | >0,1 |
| | | Collinsella | 0 | <25 |
| | | Olsenella | 0 | 0 |
| | | Slackia | 0 | <1 |
| Proteobacteria (gram-) | *Enterobacteriaceae* | Escherichia/Shigella | 1,03 | <0,5 |
| | | Klebsiella | 0,02 | <0,5 |
| | *Sutterellaceae* | Sutterella | 0 | <1 |
| | *Desulfovibrionaceae* | Lawsonia | 0 | <0,5 |

| | Value | Ref. |
|---|---|---|
| Total Lachnospiraceae | 1,86 | >5 |
| Total Ruminococcaceae | 0,36 | >5 |
| Total Clostridiaceae | 0,08 | <5 |
| Enterococcus | 8,54 | 0 |
| Streptococcus | 40,61 | <5 |
| Ruminococcus | 0,02 | >2 |
| Lactonifactor | 0,02 | 0 |
| Turicibacter | 0 | >0,5 |
| Bacteroides | 3,91 | <10 |
| Prevotella | 0,01 | <5 |
| Bifidobacterium | 35,39 | >5 |
| Asaccharobacter | 0 | >0,1 |

| | | |
|---|---|---|
| Firmicutes | 57,24 | % |
| Bacteroidetes | 4,17 | % |
| Actinobacteria | 35,72 | % |
| Proteobacteria | 1,05 | % |
| Other | 1,82 | % |

Range of Firmicutes % in European population: 50-85%

| Firmicutes/Bacteroidetes ratio | |
|---|---|
| High | |
| Average | 13,73 |
| Low | |

Low ratio may be associated with gut inflammation

| Gram+ / Gram- ratio | |
|---|---|
| High | |
| Average | 17,81 |
| Low | |

| Diversity Index | 2,79 |
|---|---|

Low <4, Average 4-5, High >5

Dysbiosis associated with low diversity

Electronically validated on: 24/09/2018

by E. Bosmans

Requesting physician: Himmunitas

Observations:

# Leaky gut in ASD children

**Involvement of Dietary Bioactive Proteins and Peptides in Autism Spectrum Disorders**

Dario Siniscalco[1,2,3,*] and Nicola Antonucci[4]

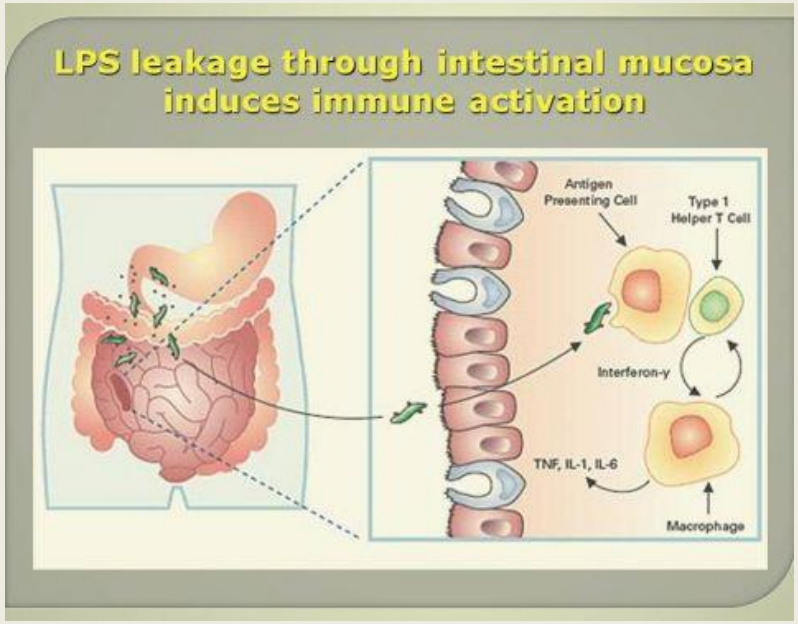
*Current Protein and Peptide Science*, 2013, *14*,

permeability [34]. ASD pathogenesis could be affected by this altered permeability. The impaired gut barrier function is the basis of the proposed leaky-gut hypothesis: ASD children show an elevated tight junctions-mediated intestinal permeability that allows the passage and absorption of dietary-derived incompletely digested peptides in the intestinal lamina propria. The intestinal barrier defects predispose autistic children to sensitization to environmental antigens. These

# Leaky gut

***ZONULIN ELISA test in stool samples***

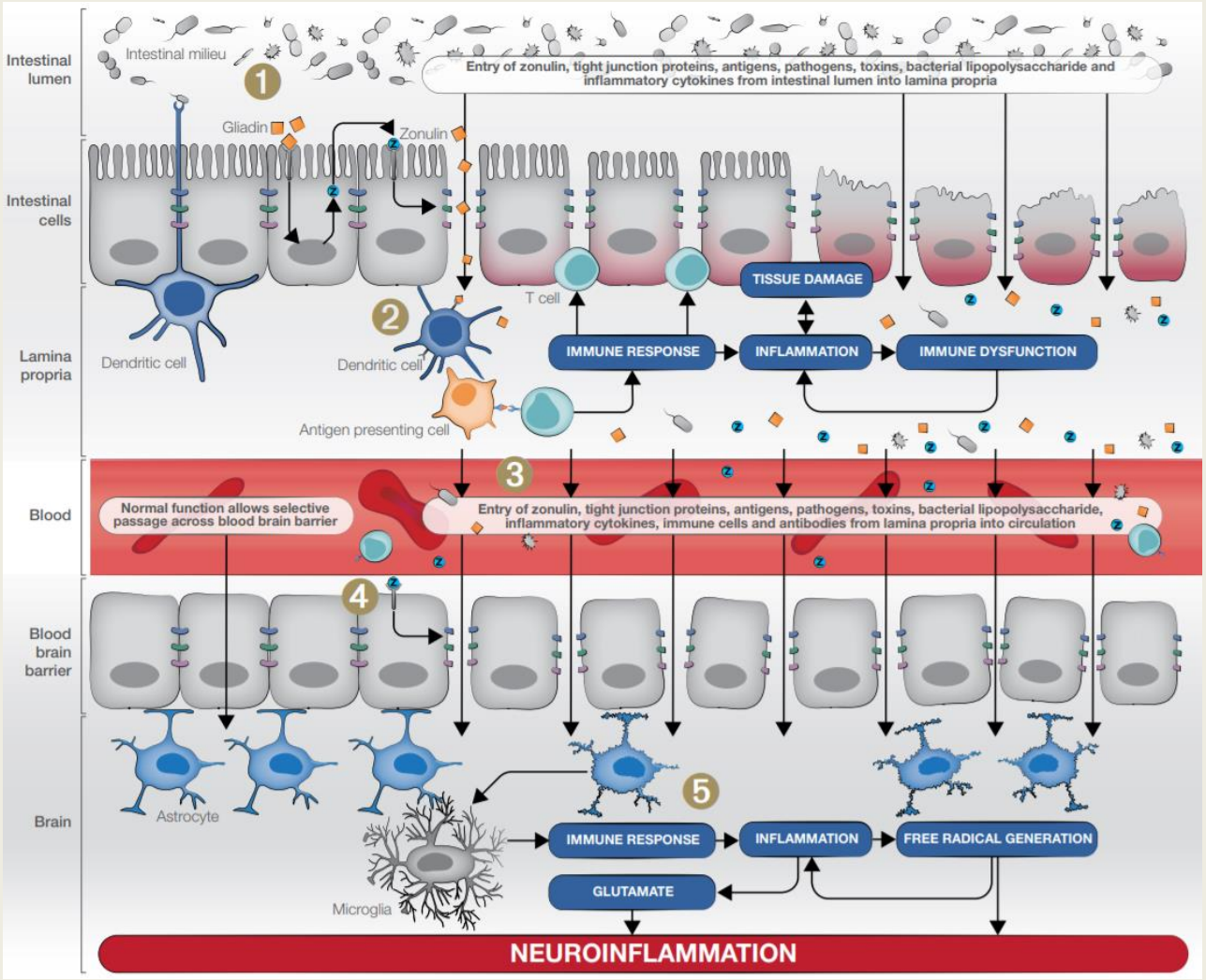
Zonulin is the "doorway" to leaky gut. Zonulin opens up the spaces between the cells of the intestinal lining. When leaky gut is present, the spaces between the cells open up too much allowing larger protein molecules and bacteria to get into the bloodstream where an immunologic reaction can take place. As the zonulin level rises, the seal between the intestinal cells diminishes. Zonulin is the only physiological modulator of intercellular tight junctions described so far that is involved in trafficking of macromolecules and, therefore, in tolerance/immune response balance.



# Consequences of the leaky gut - Chronic activation (inflammation) of the immune system

- Lipopolysaccharide (LPS) - bacterial compound that can easily make its way to the blood.
- Present in the bloodstream LPS will induce a strong pro-inflammatory response in monocytes and macrophages, involving recognition by a receptor (Toll-like receptor-4) and the subsequent secretion of cytokines such as IL-1, IL-6, TNF-alpha.
- LPS also induces the NK-kB-mediated production of nitric oxide. Because NO is increased, NK function is inhibited and opportunistic infections such as mycoplasma infections are often observed.
- Herpesviruses, which tend to reactivate in a context of immune activation, will also be frequently detected.

# Consequences of the leaky gut




https://www.fxmedicine.com.au

# sCD14 – an interesting marker

- CD14 is expressed in monocytes/macrophages and plays a critical role in the recognition of bacterial cell wall components (LPS).
- The extracellular part of CD14 can be cleaved and released in the plasma, where it will inactivate circulating LPS.
- Serum soluble CD14 levels are significantly elevated in patients with inflammatory bowel disease, Crohn's disease, but also in patients suffering from Brucellosis or Lyme disease.

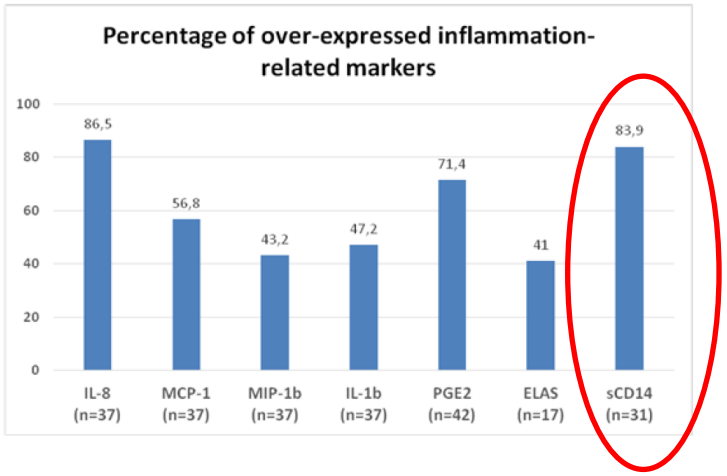



**Figure 1.** Percentage of over-expressed inflammation-related markers. The values of the graph represent the percentage of ASD patients with abnormal laboratory results for serum interleukin 8 (IL-8), MCP-1, MIP-1β, IL-1β, PGE2 and sCD14 and mRNA levels for elastase (ELAS). The numbers in brackets indicate the total number of patient records used to derive each respective value.

# Toxic metabolites from bacteria

- ***D-lactate in serum***
  - a product of bacterial metabolism, it is neither produced nor metabolized by mammalian cells. Typically, elevated D-lactate levels are due to bacterial infection or short bowel syndrome in humans. Due to slow metabolism and excretion, high D-lactate can cause acidosis and encephalopathy.

- ***Ammonia in serum***
  - Ammonia is derived from bacterial enzymatic action on ingested amino acids. It is absorbed from the gastrointestinal tract and delivered through the portal vein to the liver, which converts most of it into urea. Abnormally high levels of ammonia can result from colic or "enteric hyperammonemia" (combination of increased bacterial production and increased gut permeability) that occurs despite normal hepatic function. Hyperammonemia is a metabolic condition characterized by elevated levels of ammonia in the blood. Increased entry of ammonia to the brain is a primary cause of neurologic disorders, metabolic disorders and some toxic encephalopathies.

# Intestinal inflammation in autism

- Autism and gastrointestinal inflammation
  - **Several reports have revealed a high prevalence of gastrointestinal symptoms, inflammation, and dysfunction in children with autism (reviewed by Horvath and Perman, Curr Gastroenterol Rep. 2002).**
  - **Mild to moderate degrees of inflammation were found in both the upper and lower intestinal tract. In children with ASD, the presence of GI dysfunction is often associated with increased irritability, tantrums, aggressive behavior, and sleep disturbances (reviewed by Critchfield et al., Gastroenterol Res Pract. 2011).**

# Stool-based assays for Intestinal inflammation

***- sIgA ELISA test in stool samples***

- sIgA key function is to bind to invading micro organisms and toxins and entrap them in the mucus layer or within the epithelial cells, so inhibiting microbial motility, agglutinating the organisms and neutralizing their exotoxins and then assist in their harmless elimination from the body in the fecal flow.

- The concentration of sIgA gives us information about the intestinal immune defense:

***A lack of sIgA*** *indicates a* ***diminished activity of the intestinal immune system***

***An increased*** *level of sIgA shows* ***intestinal inflammation****.*

***- beta-Defensin-2 ELISA test in stool samples***

- Defensins exert a variable degree of antimicrobial activity against bacteria, fungi, and some enveloped viruses. The expression of ß-defensins is induced by the pro-inflammatory cytokines and also through microorganisms (e.g. E. coli, H. pylori or P. aeruginosa) and by probiotic microorganisms. A ß-defensin-2 deficiency can, for example, be observed in the intestinal mucous of patients with Crohn's disease. The defense system of the mucous membrane is therefore restricted and allows an increased invasion of bacteria, which could possibly lead to a typical infection in Crohn's disease patients. Recent results imply that ß-defensin-2 is overexpressed in active intestinal inflammation, especially in ulcerative colitis.

***- EDN / EPX ELISA test in stool samples***

- The accumulation of EDN in the intestine is associated with inflammation and tissue damage. Fecal EDN is considered the best of the cytotoxic granule proteins for assessment of gut inflammation. Elevated levels of fecal EDN are linked to multiple inflammatory conditions, like food allergy/sensitivity, pathogenic infections (C. difficile and H. Pylori), IBS, Eosinophilic Gastrointestinal Disorders.

***- Inflammation markers in stool samples***

- ***Hemoglobin*** : discharged with the feces in gastrointestinal bleeding diseases
- ***Transferrin***: a blood-derived component ; a good marker for gastrointestinal bleeding
- ***Calprotectin***: a neutrophil cytosolic protein with antimicrobial properties, which is present at increased concentration in stool during bowel inflammation
- ***Lactoferrin***: a primary component of the acute inflammatory response released from fecal leukocytes; may serve as a marker of inflammation in the intestine

# The Importance of Assessing Intestinal Dysfunctions

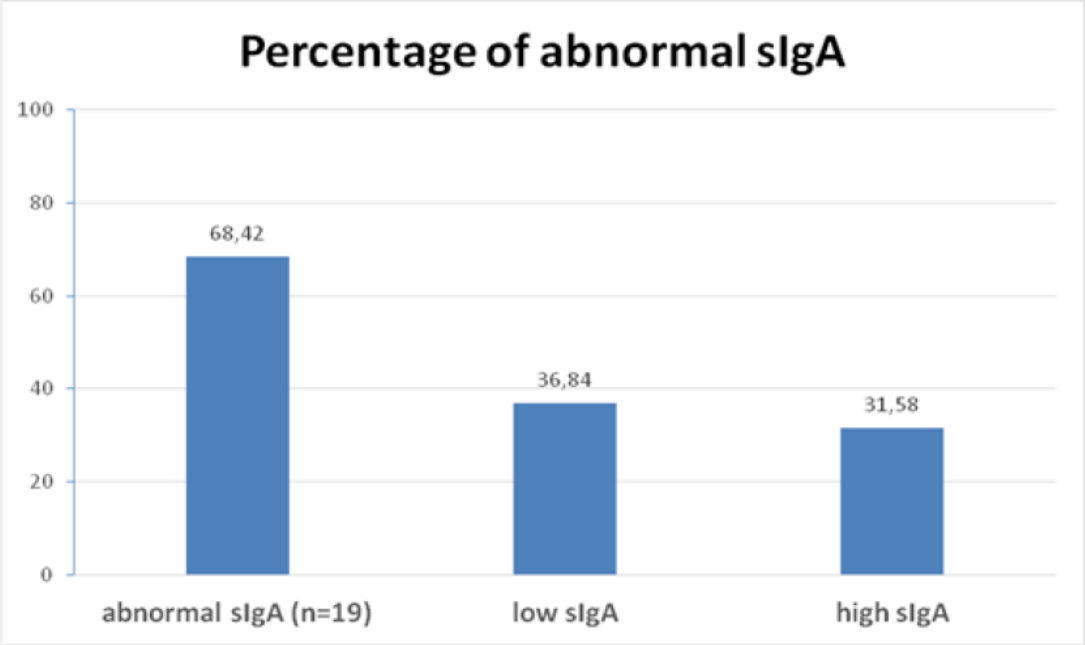



**Figure 2.** Percentage of abnormal sIgA results. The values of the graph represent the percentage of ASD patients with abnormal sIgA as measured in the stool. The numbers in brackets indicate the total number of patient records used to derive each respective value. The abnormal results may represent values that are either higher or lower than the established healthy range.

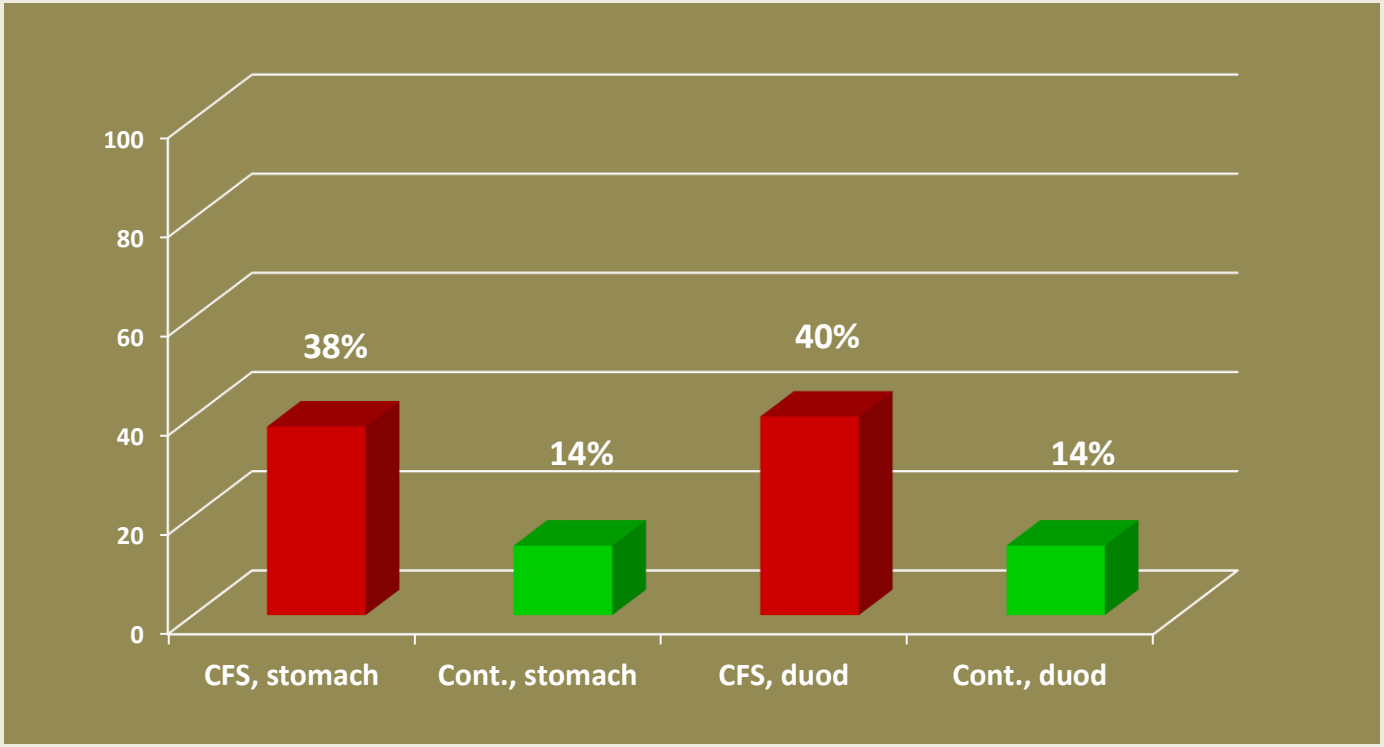
*Mijatovic T. et al., 2018, AIMS Molecular Science 5:173-182.*

# Assays for Intestinal Infections

***- INFECTIONS - assessment in stool samples***

*Ag-based testing for Clostridium, Yersinia, Enterovirus, Parasites, etc*

***- INFECTIONS – PCR-based viral and bacterial assessment in intestinal biopsies***




*Frémont et al., In vivo 2009*

# Importance of testing for gastrointestinal disorders

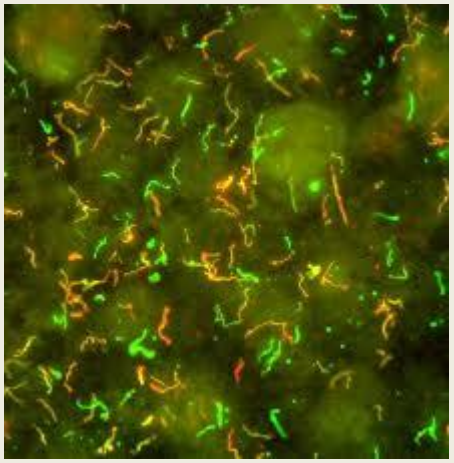
- GI symptoms may overlap with ASD core symptoms through different mechanisms.
- Shared pathogenetic factors and pathophysiological mechanisms possibly linking ASD and GI disturbances, as shown by most recent studies, include among others
  - intestinal inflammation with or without autoimmunity,
  - intestinal permeability,
  - food allergies,
  - dysbiosis.
- Dysregulation of the gut microbiome has also been shown to be involved in modulating GI functions with the ability to affect intestinal permeability, mucosal immune function, and intestinal motility and sensitivity.
- Immune dysregulation, GI inflammation, dysbiotic microbiome and dietary metabolites may contribute to brain dysfunction and neuroinflammation. Unexplained worsening of nonverbal behaviors (agitation, anxiety, aggression, self-injury, sleep deprivation) should alert professionals about this possibility.

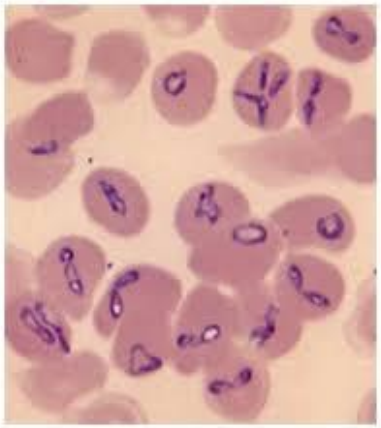
# SUMMARY - Testing for gastrointestinal disorders

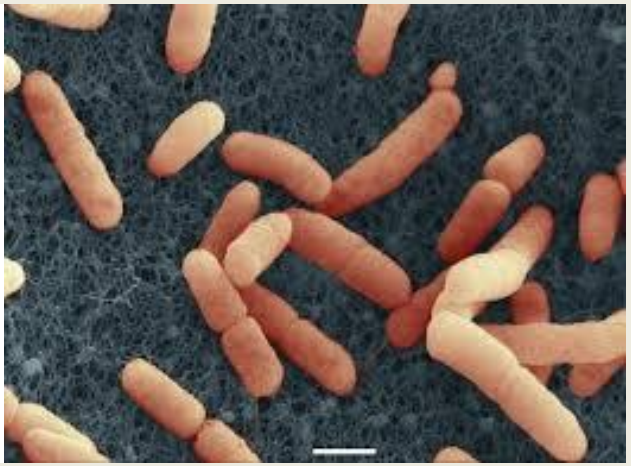
- **Useful assays to investigate intestinal dysfunctions:**
  - **BLOOD-BASED Tests: sCD14, Ammonia concentration, Lactase deficiency assay, D-lactate**
  - **BIOPSY-BASED Tests: PCR-based detection of viral and bacterial infections**
  - **STOOL-BASED Tests:**
    - **Intestinal Inflammation: sIgA, Beta-2 Defensin, EPX / EDN, Inflammation markers in stool samples**
      - ! Gut inflammation contributes to increased bacterial translocation.
    - **Intestinal Infections : immunochromatography antigenic testing for intestinal infections**
    - **Leaky gut: ZONULIN ELISA test in stool samples**
    - **Dysbiosis: MSA assay (metagenomic stool test)**

      *Important insights:*
      - High Prevotella : gut acidification; $H_2S$ production ➔ mitochondrial collapse,
      - Low Bifidobacterium: Bifido cannot stand low pH
      - High Clostridium IV ➔ production of toxic metabolites
      - Enterococcus – facultative anaerobes, if inflammation ➔ overgrowth

# Testing for Infections /chronic infections







# Tick-borne infections and autism

- An association between Lyme disease (LYD) and other tick-borne infections (TBI) during fetal development and in infancy with autism, autism spectrum disorders (ASD) and autistic symptoms has been noted by numerous clinicians and parents.
- Treatment of TBI during pregnancy can prevent the development of ASD associated with TBI (Bransfield et al., Med. Hypotheses 2008),
- Bransfield et al. wrote in 2008 a review paper (Med. Hypotheses 2008) to collate information from conference presentations on this issue with other sources that further address this association. They indicated that the preliminary data suggests ***Borreliosis may be a contributor in 20– 30% of ASD, and pathogenic Mycoplasma may be a contributor in 58%.***
- Autism spectrum disorder **results from multiple etiologies with both genetic and environmental contributions, including at least 23 different infections**, seven of which are chronic infections (Babesia, Bartonella, B. burgdorferi, Ehrlichia, Human Herpesvirus-6, Chlamydia pneumoniae and Mycoplasma), and the immune reactions associated with these infections (Bransfield, Pediatr Health 2009).

# Lyme Disease Testing

- **Importance to enlarge borreliosis-related testing targets (i.e. not testing only for B. burgdorferi sl)**

  *The overall high expansion of undiagnosed Lyme disease cases worldwide might be linked to the screening choice focusing only on B. Burgdorferi sl and only rarely testing for B. miyamotoi while the later one seems to be much more prevalent. Searching for actual bacterial presence using phage - based testing might pacify the debate and controversies on testing choices and late/chronic stage patients.*

- **Importance of novel testing approaches**

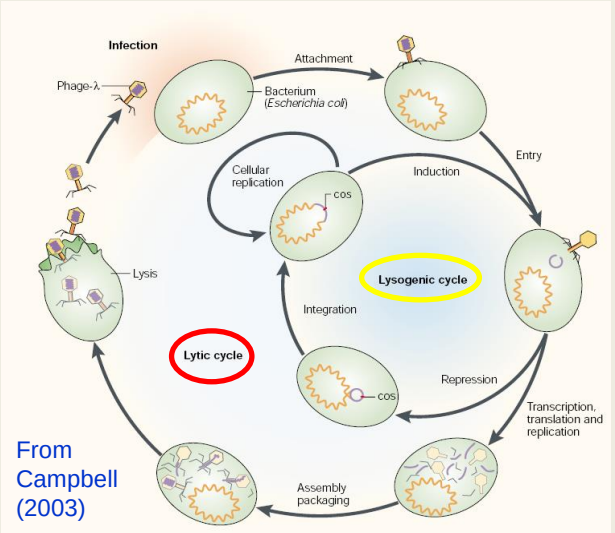
  *The overall high failure rate of tick-borne infection (TBI)-related testing underscores the necessity for novel approaches, i.e. not relying on serology and two-tier testing.*

# Novel Testing Approaches – Phage-based Test

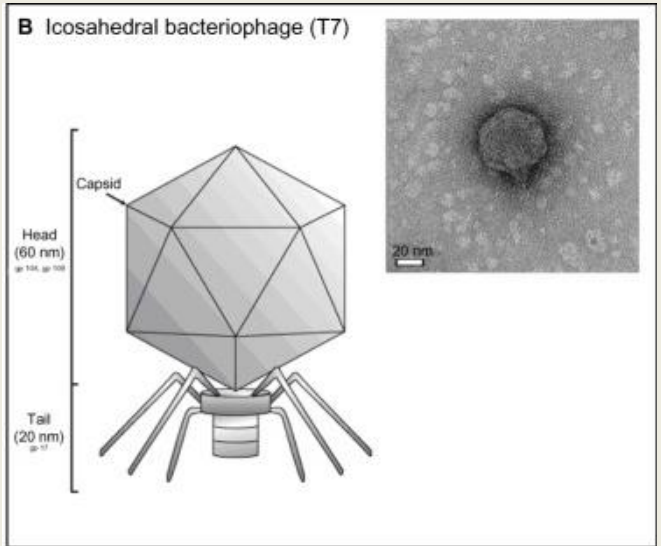
- The importance of novel testing approaches

  Phelix Phage Borrelia detection method (Patent WO2018083491A1) consists of targeting the presence of outnumbered prophages part of the bacteria lysogenic cycle. Bacteriophages are present only on active bacterial infections; hence a phage-based test is a direct proof of an active infection.

- The lysogenic cycle: The phage infects a bacterium and inserts its DNA into the bacterial chromosome, allowing the phage DNA (now called a prophage) to be copied and passed on along with the cell's own DNA.
- The lytic cycle: The phage infects a bacterium, hijacks the bacterium to make lots of phages, and then kills the cell by making it explode (lyse).




From Campbell (2003)



# Phelix Phage Borrelia Test

- Bacteriophages could become a diagnostic tool based on the principle that if there are phages it is because there are living bacteria.
- Phelix Charity together with Leicester University microbiology department have recently developed a Borrelia Phage-based PCR test searching for 3 major Borrelia groups:

  Borrelia burgdorferi sl (including B. burgdorferi ss, B. afzelii, B. garinii, B. spielmanii, etc)

  Borrelia miyamotoi and

  Relapsing fever group (B. recurrentis, B. hermsii, etc).
- This method is efficiently used to assess both human samples and ticks.
- Highly sensitive and specific.
- Do not generate positive signal against other bacterial strains.
- False positive are ruled out by sequencing.

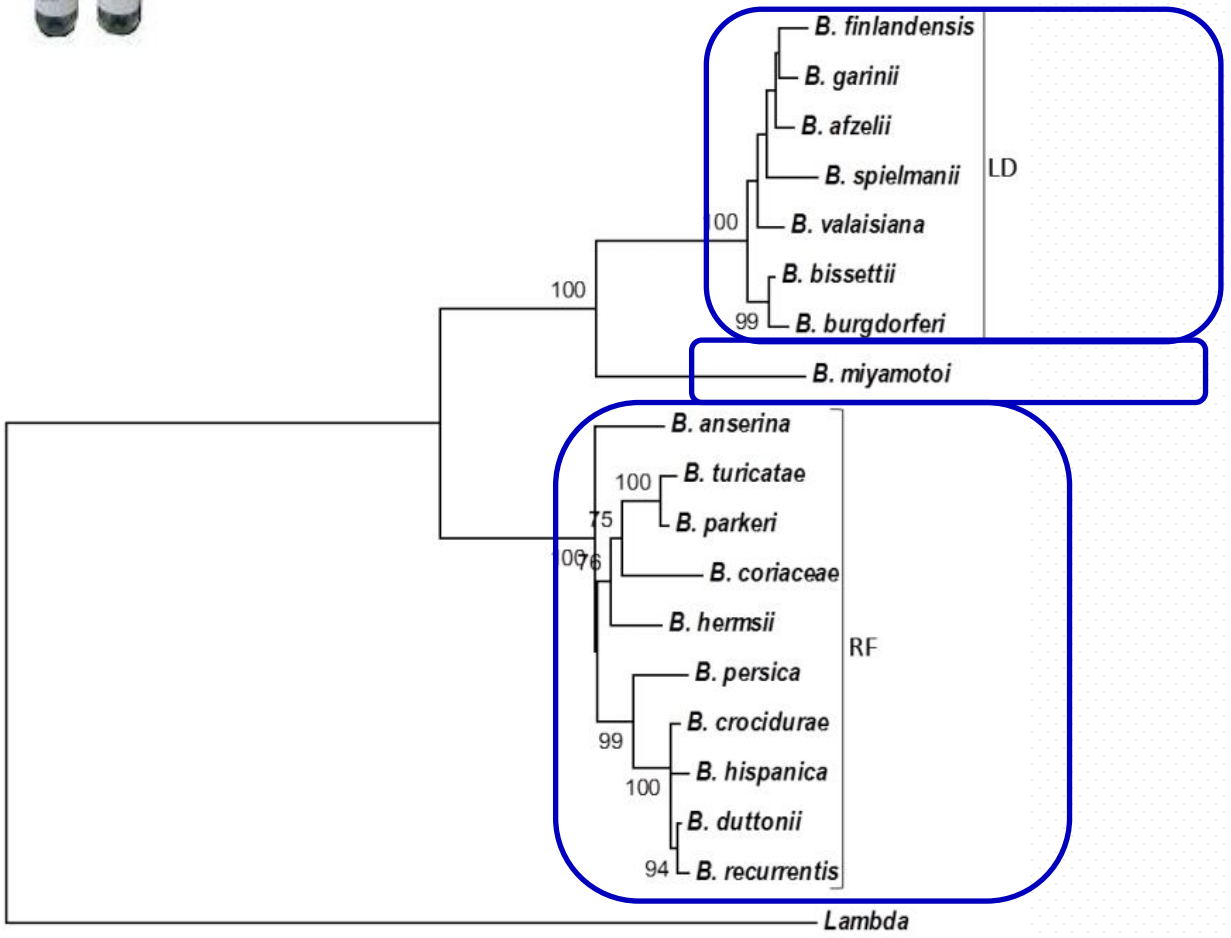
# Phelix Phage Borrelia Test – How it works

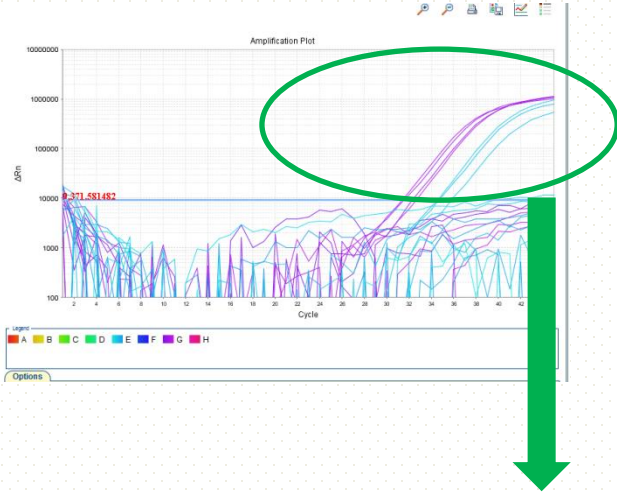
Manual DNA Phenol / Chloroform extraction

QCs

- Extraction control PCR *quality of extracted DNA*
- IAC control PCR *absence of PCR inhibitors*


3 different real-time PCRs, each sample in 4 replicates per target




Confirmatory sequencing for positive-like samples

# Immune Dysfunction in TBD

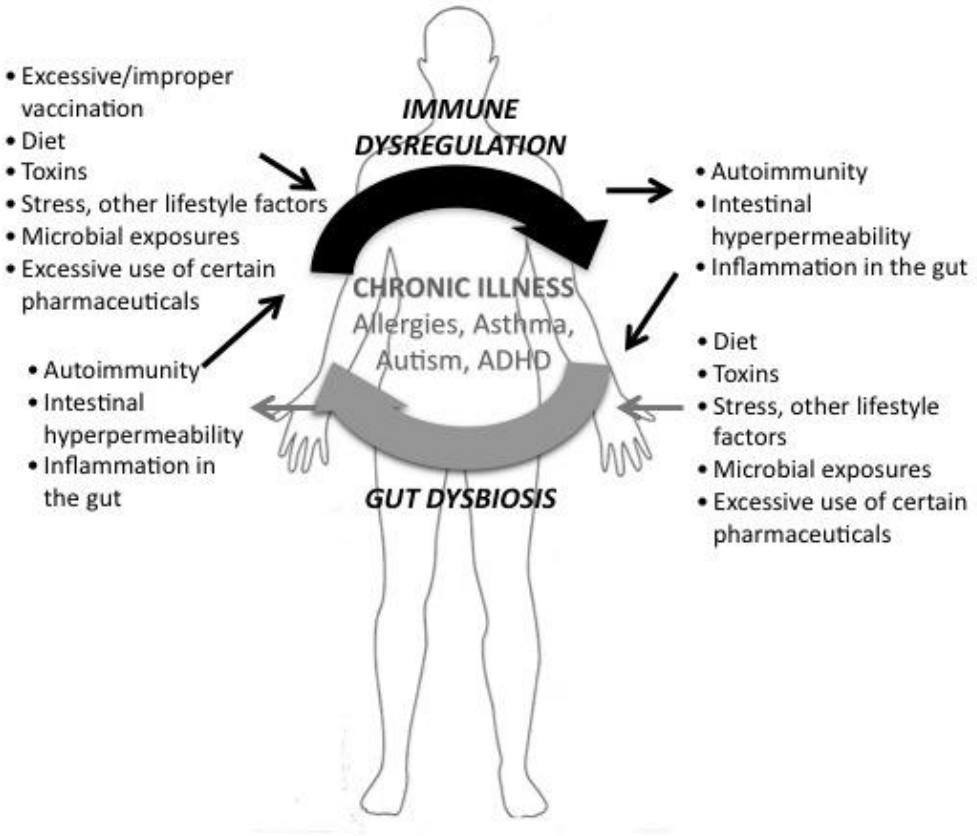
- A hallmark of chronic Lyme disease is an **underlying immune dysfunction** that not only limits the ability to accurately diagnose Lyme, leaving many such patients misdiagnosed, but also serves as a major reason for lack of treatment response to antibiotics with an inability to eradicate the chronic infection.
- The Borrelia bacteria (cause of Lyme disease) transforms from an acute to a chronic infection by transforming the body to a TH2 "extracellular" dominant response and then converting from a free swimming spirochete form in the blood into an intracellular form (L-form) to escape the elevated TH2 immunity. The suppressed and down-regulated TH1 intracellular immune response becomes an ineffective immune response by the body and an effective evasion strategy, which is the hallmark of transformation to late-stage Lyme dissemination.
- Appropriate immune modulating therapies that are able to restore normal functioning immunity may be the biggest necessary leap forward in the development of an effective treatment protocol for this multi-system illness.

# Mycoplasma ssp., Human HV6 coinfections, Intracellular pathogens in ASD

- Nicolson et al. (J Neurosci Res. 2007) examined the blood of 48 patients from central and southern California diagnosed with autistic spectrum disorders (ASD) and found **that a large subset of ASD patients shows evidence of bacterial and/or viral infections.**
  - **a large subset (28/48 or 58.3%) of patients showed evidence of Mycoplasma spp. infections** compared with two of 45 (4.7%) age-matched control subjects.
  - the prevalence human herpes virus-6 (HHV-6, 14/48 or 29.2%) coinfections in ASD patients versus 4/48 or 8.3% in Control subjects.

- As reported by Blinstock (Med. Hypotheses 2001), several autism-spectrum subgroups derive from intra-monocyte pathogens such as measles virus, cytomegalovirus, human herpesvirus 6, and Yersinia enterocolitica. In some such children, one or more of these pathogens persists as a chronic-active, seemingly subclinical infection etiologically significant to the child's autistic traits. Within these subgroups, immune impairments and atypical infections may be treatable.

# A VICIOUS CIRCLE



# Conclusions

- More and more evidence points towards a combination of factors (genetic, infectious, environmental, etc.) being important in the development of chronic immune dysfunctions, the cardinal finding in autistic patients.
- In many countries ASD are still considered as psychiatric despite clear biomedical evidence.
- For better management of this affliction suffering from lack of medical recognition, need to consider SPECIALTY TESTS
- Based on the recent publications and extensive collaborations with MDs specialized in management of autism-related disorders, we developed dedicated testing offer focusing on:
  - ***global immune dysfunctions***
  - ***persistent and/or chronic infections, especially tick-borne infections***
  - ***intestinal dysfunctions and intestinal inflammation***
- **Wider network of new collaborations needed for further advancement in autism biomarkers deciphering and validation**

# R.E.D. Laboratories

## *WHO WE ARE*

- R.E.D. Laboratories is Belgian company developing tests for chronic immune diseases and intestinal dysfunctions
- We are involved in several international groups aiming to advance knowledge in biological markers of autism.
- At R.E.D. Laboratories, we are continuously developing new tests according to the specific needs from health care providers.
- All generated benefits are used for research and development of new assays.

# R.E.D. Laboratories

## *OUR PHILOSOPHY*

- We focus on setting up the tests that are not (or rarely) available elsewhere.
- Personalization of testing panel leads to more efficient and rapid management of patients with complex clinical picture.
- Assay development programs at R.E.D. Laboratories focus on disorders that contribute to the onset and pathogenesis of diseases such as chronic fatigue syndrome, **autism**, multiple sclerosis, chronic infections or autoimmune diseases.

# Questions and Contacts

- **Material available on the website (www.redlabs.com)**
- Check regularly our **website** (**www.redlabs.com**) for the updates
- **Questions and contact:**
  - **General queries, logistics : E-mail to info@redlabs.be**
  - **Scientific questions : E-mail to tmijatovic@redlabs.be**