Immunomodulation in Autism Spectrum Disorders

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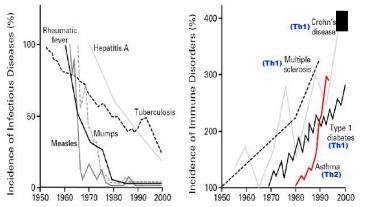
Autism One May 2011

This presentation

- Immune system refresher
- Hygiene Hypothesis aka "old friends"
- Immune abnormalities in Autism
  - Inflammation in brain and gut
  - Autoimmunity
  - Dysregulation
- Therapies to modulate (as opposed to suppress) the immune system

Chronic Disease Prevalence in the last 50 Years

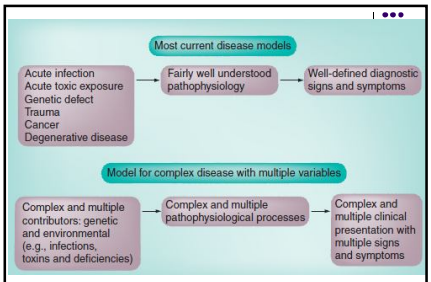
Decrease in infections is associated with increase immune disorders



Bach JF. N Engl J Med. 2002;347:911-920

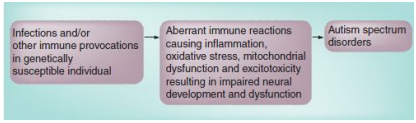
"We cannot solve the problems that we have created with the same thinking that created them."

-Albert Einstein



Bransfield RC, *Pediatric Health* 2009. 3(2): 125-140

Model for Infections/Immune Stimulation Contributing to Autism Spectrum Disorders

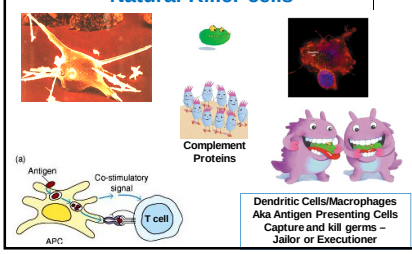


Bransfield RC, *Pediatric Health* 2009. 3(2): 125-140

The "Ideal" Immune System

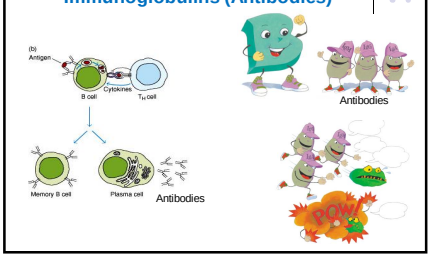
- Recognize all foreign organisms.
  - Bacteria, viruses, parasites (fungi,worms)
- Efficiently and rapidly destroy invaders.
- Prevent a second infection with the same microbe (have a memory).
- Never cause damage to self.

Innate Immunity: Phagocytes (Macrophages) and Natural Killer cells

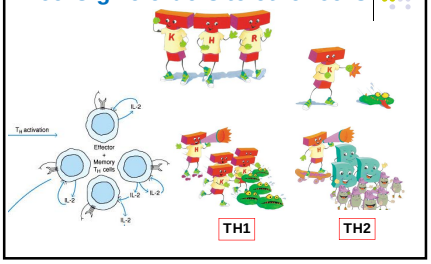


Dendritic Cells/Macrophages Aka Antigen Presenting Cells Capture and kill germs – Jailor or Executioner

Adaptive Immunity: B cells produce Immunoglobulins (Antibodies)



Adaptive Immunity: T cells give orders to other cells



Adaptive Immunity: Regulatory T cells keep things in balance

T regs tell B cells to stop making antibodies when the infection is over

T regs tell other T cells to stop "directing" and killing when the infection is over

All cell types work together in a healthy immune system !

Cytokines: Chemical messages that are the main communication system between cells of the immune system (and others, incl. nervous system).

Can be divided several ways:

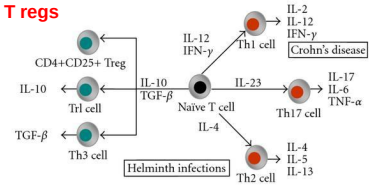
- **Th1** (adaptive/memory, cell mediated): IL-2, IFN-γ
- **Th2** (adaptive/memory, antibodies): IL-4, IL-5, IL-13
- **Innate**: TNF-α, IL-1, IL-6, IL-12
- **Pro-inflammatory**: TNF-α, IL-1, IL-6
- **Anti-inflammatory**: TGF-β, IL-10
- **Regulatory**: IL-10, IL-12,TGF-β

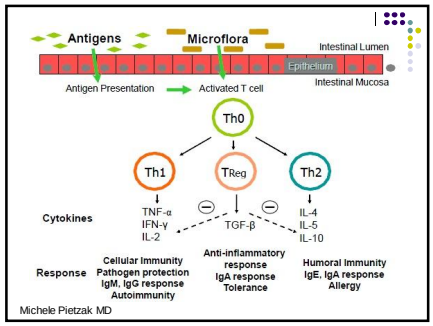
Multiple roles makes this confusing!!!!
Can do different things in different contexts.

RESEARCH TOOLS – not really available in a useful way

More than just Th1 and Th2…

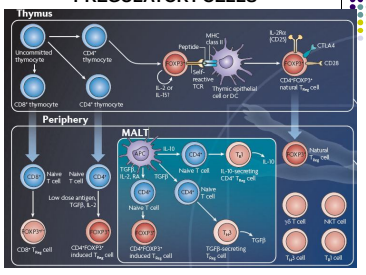
T regs



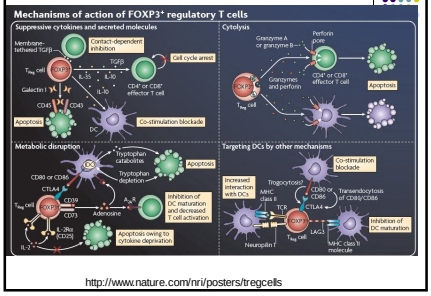


Michele Pietzak MD

T REGULATORY CELLS

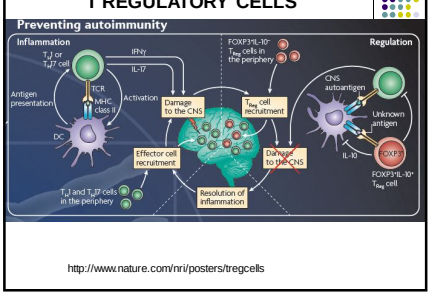


http://www.nature.com/nri/posters/tregcells

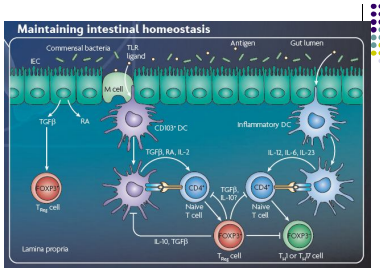


http://www.nature.com/nri/posters/tregcells

T REGULATORY CELLS



http://www.nature.com/nri/posters/tregcells



http://www.nature.com/nri/posters/tregcells

Things that can go wrong…

- **Immune deficiency/dysfunction: defective or ineffective response.**
- **Hypersensitivity: Over-reaction to innocuous foreign material, out of proportion to potential damage - Allergy.**
- **Autoimmunity: Inappropriate reaction towards self, loss of self-recognition.**
- **Inflammation: Too vigorous attack against invaders with "bystander" damage to normal tissue.**

Inflammation

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

Immune dysregulation and increased inflammation are frequent findings in autism

- **Over-active innate inflammatory response, especially increased pro-inflammatory cytokines, is a consistent finding.**
- **There is evidence of over-activity of the immune system in all parts of the immune system, with inflammation in the blood, in the brain, and in the GI tract of many of these children.**

Dysregulation and Inflammation!

- Nearly every study finds that some children have poor immune regulatory function so that immune responses do not turn "off" normally, staying "activated" or turned on and resulting in **inflammation.**
- Cytokines are often **"pro-inflammatory"**

T regs: TGF-Beta is decreased in autism

- **Decreased serum levels of transforming growth factor-β1 in patients with autism.** Okada K et al. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*. 31(1) 2007, Pages 187-190.

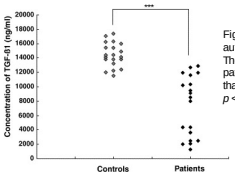


Fig. 1. The serum levels of TGF-β1 in autistic patients and normal controls. The serum levels of TGF-β1 in autistic patients ($n = 19$) were significantly lower than those of normal controls ($n = 21$). $p < 0.001$

Decreased IL-10 in Autism

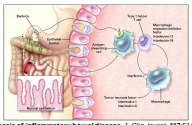
- Molloy, C., Morrow, A., Meinzen-Derr, J., Schleifer, K., Dienger, K., Manning-Courtney, P., Altaye, M., & Wills-Karp, M. (2006). **Elevated cytokine levels in children with autism spectrum disorder.** *Journal of Neuroimmunology*, 172, 198-205.
- **IL-10** levels were not elevated in individuals with **autism**, although both Th1 and Th2 cytokines were elevated.
- Unusual to see both the Th1 and Th2 arms of the adaptive immune response so active at the same time; even more unusual to see this increased activation without a proportional increase in the regulatory cytokine **IL-10**, which is involved in Th1 and Th2 system regulation.
- **Children with autism may not be able to down-regulate their Th1 and Th2 systems because of a dysfunction in the production or function of IL-10**

Hypotheses of Etiology of Inflammatory Bowel Disease

1. Abnormal (dysregulated) immune system, normal gut microbes

2. Normal immune system, abnormal microbes +/- abnormal barrier

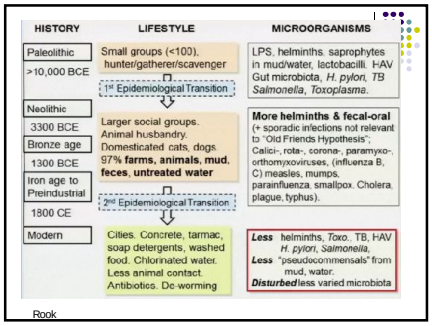
We conclude that IBD is characterized by **an abnormal mucosal immune response** but **that microbial factors and epithelial cell abnormalities can facilitate this response.**

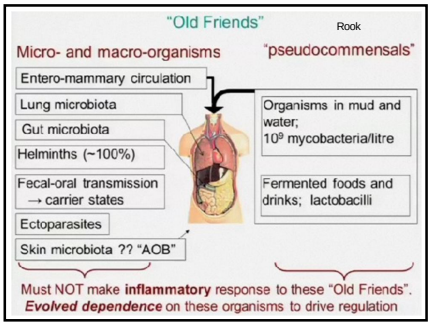


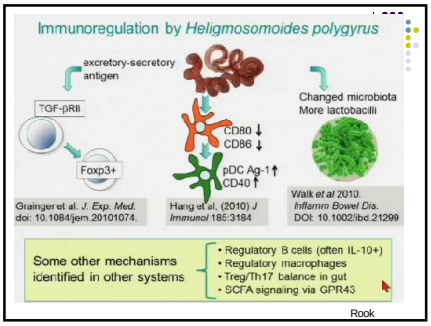
Strober W. **The fundamental basis of inflammatory bowel disease** J. Clin. Invest. **117**:514-521 (2007)

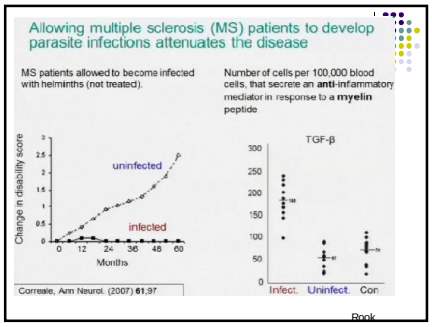
Old friends

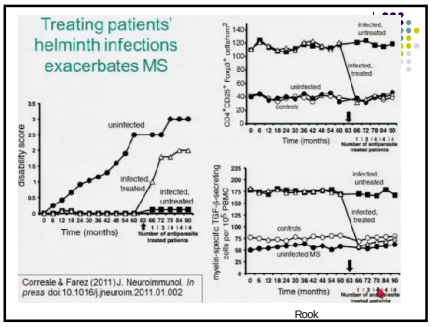
- Mammalian evolution has kept us in close contact with relatively harmless micro-organisms over a long period of time
- We recognize these "old friends" and they help to educate our immune system
- Decreased types of bacteria in our gut from antibiotics similar to effect of global warming to the planet

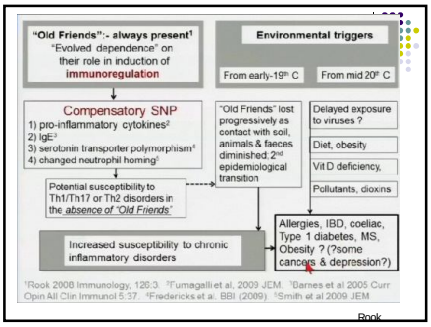

HISTORY
LIFESTYLE
MICROORGANISMS
Paleolithic
>10,000 BCE
Small groups (<100), hunter/gatherer/scavenger
LPS, helminths, saprophytes in mud/water, lactobacilli, HAV
Gut microbiota, H. pylori, TB
Salmonella, Toxoplasma.
1st Epidemiological Transition
Neolithic
3300 BCE
Bronze age
1300 BCE
Larger social groups.
Animal husbandry.
Domesticated cats, dogs.
97% farms, animals, mud, feces, untreated water
More helminths & fecal-oral
(+ sporadic infections not relevant to 'Old Friends Hypothesis';
Calici-, rota-, corona-, paramyxo-, orthomyxoviruses, (influenza B, C) measles, mumps, parainfluenza, smallpox, Cholera, plague, typhus).
Iron age to Preindustrial
1800 CE
2nd Epidemiological Transition
Modern
Cities. Concrete, tarmac, soap detergents, washed food. Chlorinated water. Less animal contact. Antibiotics. De-worming
Less helminths, Toxo, TB, HAV
H. pylori, Salmonella.
Less "pseudocommensals" from mud, water.
Disturbed less varied microbiota
Rook

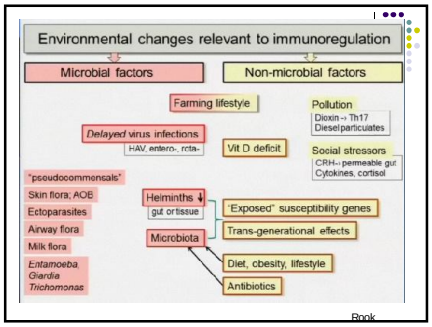

"Old Friends"
Rook
Micro- and macro-organisms
"pseudocommensals"
Entero-mammary circulation
Lung microbiota
Gut microbiota
Helminths (~100%)
Fecal-oral transmission → carrier states
Ectoparasites
Skin microbiota ?? "AOB"
Organisms in mud and water; 10^9 mycobacteria/litre
Fermented foods and drinks; lactobacilli
Must NOT make inflammatory response to these "Old Friends".
Evolved dependence on these organisms to drive regulation

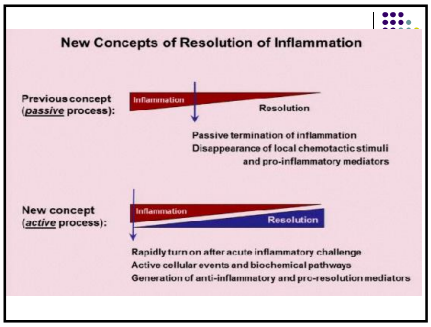

Immunoregulation by Heligmosomoides polygyrus
excretory-secretory antigen
TGF-βRII
Foxp3+
CD80 ↓
CD86 ↓
pDC Ag-1↑
CD40 ↑
Changed microbiota
More lactobacilli
Grainger et al. J. Exp. Med. doi: 10.1084/jem.20101074.
Hang et al. (2010) J Immunol 185:3184
Walk et al 2010. Inflamm Bowel Dis. DOI: 10.1002/ibd.21299
Some other mechanisms identified in other systems
• Regulatory B cells (often IL-10+)
• Regulatory macrophages
• Treg/Th17 balance in gut
• SCFA signaling via GPR43
Rook

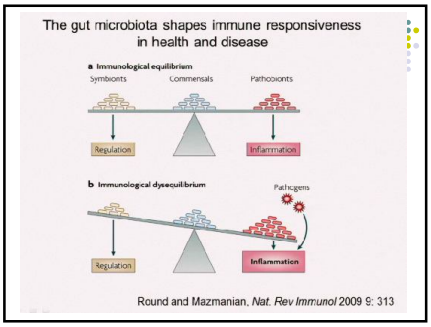

Allowing multiple sclerosis (MS) patients to develop parasite infections attenuates the disease
MS patients allowed to become infected with helminths (not treated).
Number of cells per 100,000 blood cells, that secrete an anti-inflammatory mediator in response to a myelin peptide
Change in disability score
uninfected
infected
Months
TGF-β
Infect.
Uninfect.
Con
Correale, Ann Neurol. (2007) 61:97
Rook


Treating patients' helminth infections exacerbates MS
disability score
uninfected
infected, treated
infected, untreated
Time (months)
uninfected controls
infected, untreated
infected, treated
controls
uninfected MS
Correale & Farez (2011) J. Neuroimmunol. In press doi:10.1016/j.jneuroim.2011.01.002
Rook


"Old Friends":- always present[1]
"Evolved dependence" on their role in induction of immunoregulation
Environmental triggers
From early-19th C
From mid 20th C
Compensatory SNP
1) pro-inflammatory cytokines[2]
2) IgE[3]
3) serotonin transporter polymorphism[4]
4) changed neutrophil homing[5]
"Old Friends" lost progressively as contact with soil, animals & faeces diminished 2nd epidemiological transition
Delayed exposure to viruses ?
Diet, obesity
Vit D deficiency,
Pollutants, dioxins
Potential susceptibility to Th1/Th17 or Th2 disorders in the absence of "Old Friends"
Increased susceptibility to chronic inflammatory disorders
Allergies, IBD, coeliac, Type 1 diabetes, MS, Obesity ? (?some cancers & depression?)
[1]Rook 2008 Immunology, 126:3. [2]Tumagalli et al. 2003 JEM. [3]Barnes et al 2005 Curr Opin All Clin Immunol 5:37. [4]Fredericks et al. BBI (2009). [5]Smith et al 2009 JEM
Rook


Environmental changes relevant to immunoregulation
Microbial factors
Non-microbial factors
Farming lifestyle
Pollution
Dioxin→ Th17
Diesel particulates
Delayed virus infections
HAV, entero-, rota-
Vit D deficit
Social stressors
CRH→ permeable gut
Cytokines, cortisol
"pseudocommensals"
Skin flora; AOB
Ectoparasites
Airway flora
Milk flora
Entamoeba, Giardia, Trichomonas
Helminths ↓
gut or tissue
Microbiota
"Exposed" susceptibility genes
Trans-generational effects
Diet, obesity, lifestyle
Antibiotics
Rook


New Concepts of Resolution of Inflammation
Previous concept (passive process):
Inflammation
Resolution
Passive termination of inflammation
Disappearance of local chemotactic stimuli and pro-inflammatory mediators
New concept (active process):
Inflammation
Resolution
Rapidly turn on after acute inflammatory challenge
Active cellular events and biochemical pathways
Generation of anti-inflammatory and pro-resolution mediators


The gut microbiota shapes immune responsiveness in health and disease
a Immunological equilibrium
Symbionts
Commensals
Pathobionts
Regulation
Inflammation
b Immunological dysequilibrium
Pathogens
Regulation
Inflammation
Round and Mazmanian, Nat. Rev Immunol 2009 9: 313

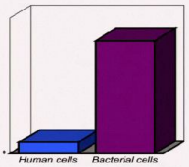
Role of Intestinal Microflora

- Occupy adhesion sites of other bacteria
- Fermentation of substrates
- Metabolism of proteins, bile acids
- Vitamin synthesis

- Modulate gut immune function
  - Barrier function (non-immune factors)
  - Immune stimulatory function
    - Innate immunity
    - Adaptive immunity

Pietzak

The intestinal commensal microbiome contains approximately 10^{14} bacteria of an estimated 1000 different species

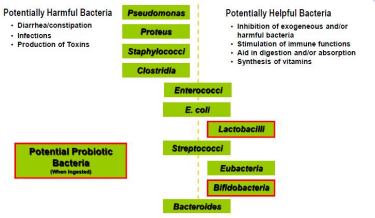


There is also an enteric viral microbiome

and, in the developing world, a parasite microbiome....

Cathryn Nagler

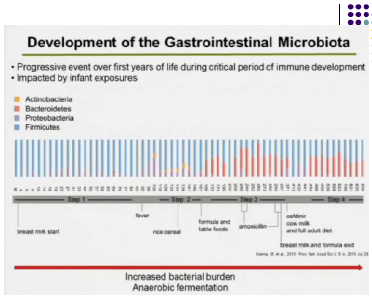
Intestinal Flora (A Balanced Ecosystem)



From: Gibson GR. J Nutrition 1995; 125:1401-1412

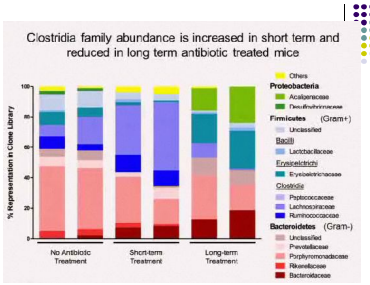
Pietzak

Development of the Gastrointestinal Microbiota



S Lynch

Clostridia family abundance is increased in short term and reduced in long term antibiotic treated mice



Cathryn Nagler

Dysregulated immune system with inflammation in children with ASD

- Jyonouchi H, et al. **Impact of innate immunity in a subset of children with autism spectrum disorders: a case control study** *Journal of Neuroinflammation* 2008, **5**:52 http://www.jneuroinflammation.com/content/5/1/52
- Ashwood, P., Wakefield, A.J.,2006. **Immune activation of peripheral blood and mucosal CD3+ lymphocyte cytokine profiles in children with autism and gastrointestinal symptoms.** *J. Neuroimmunol.* 173, 126–134.
- Molloy CA, et al. Elevated Cytokine Levels in Children with ASD. *J Neuroimmunol*. 172:198-205, March 2006 ["Children with ASD had **increased activation** of both Th1 and Th2 arms of the adaptive immune response, with a **Th2 predominance**, and **without the compensatory increase in the regulatory** cytokine IL-10."]
- Croonenberghs, J., Bosmans, E., Deboutte, D., Kenis, G., Maes, M., 2002. **Activation of the inflammatory response system in autism.** *Neuropsychobiology* 45, 1–6.
- Sweeten TL et al, **High Blood Monocyte Count and Neopterin Levels in Children with Autistic Disorder**. *Am J Psych.* 2003; 160:1691-1693.

Serum (Blood) Findings in ASD: Autoimmunity

- Many, many types of **autoantibodies** (against "self" tissues) have been found but the significance of the many types of anti-brain antibodies is not clear.

Singer HS, Morris CM, Williams PN, Yoon DY, Hong JJ, Zimmerman AW. **Antibrain antibodies in children with autism and their unaffected siblings.** J Neuroimmunol. 2006 Jul 12; [Epub ahead of print]

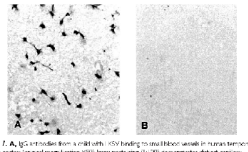
Autoantibodies in Autism Spectrum Disorders MIND Institute
Annals of the New York Academy of Sciences (2007) ;1107 (1) , 79–91

NEUROMUSCULAR DISEASE CENTER

Washington University, St. Louis, MO USA

Serum autoantibodies to brain in Landau-Kleffner variant, autism, and other neurologic disorders.
Journal of Pediatrics 1999 May;134(5):607-13. Connolly, et al

Antibodies Against Small blood Vessels In the brain



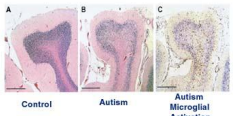
Courtesy of J Bradstreet

Dysregulated immune system with inflammation in children with ASD - BRAIN

- Chez, M.G., Dowling, T., Patel, P.B., Khanna, P., Kominsky, M. **Elevation of tumor necrosis factor-alpha in cerebrospinal fluid of autistic children.** *Pediatr. Neurol.* 2007; 36: 361–365.
- Zimmerman, A., Jyonouchi, H., Comi, A., Connors, S., Milstien, S., Varsou, A., Heyes, M., 2005. **Cerebrospinal fluid and serum markers of inflammation in autism.** *Pediatr.Neurol.* 35, 195-201.
- Li X, et al. **Elevated immune response in the brain of autistic patients**, *J. Neuroimmunol*. 2009 doi:10.1016/j.jneuroim.2008.12.002
- Vargas et al, **Neuroglial activation and neuroinflammation in the brain of patients with autism.** *Ann Neurol.* 2005 Jan;57(1):67-81.

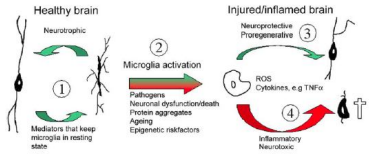
Brain findings: Vargas 2005

- We demonstrate an **active neuroinflammatory process** in the cerebral cortex, white matter, and notably in cerebellum of autistic patients. Immunocytochemical studies showed marked activation of microglia and astroglia.
- **Our findings indicate that innate neuroimmune reactions play a pathogenic role in an undefined proportion of autistic patients, suggesting that future therapies might involve modifying neuroglial responses in the brain.**
  - **Vargas et al, Neuroglial activation and neuroinflammation in the brain of patients with autism.** ***Ann Neurol.*** **2005 Jan;57(1):67-81.**



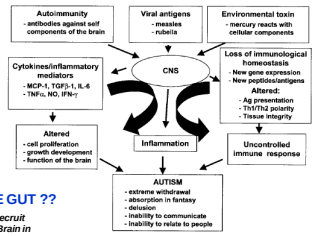
Microglial Balancing Act

F. Vilhardt / The International Journal of Biochemistry & Cell Biology 37 (2005) 17–21



Microglia and astrocytes need glutathione to protect neurons

Cohly HH and Panja A, **Immunological Findings in Autism.** *International Review of Neurobiology* (2005), 71:17-41.



ROLE OF THE GUT ??

Cerebral Microglia Recruit Monocytes into the Brain in Response to TNF Signaling During Peripheral Organ Inflammation *J Neuroscience 2009*

Fig. 1. Schematic presentation of immunopathogenesis of autism.

Goal: Decrease inflammatory stimulation

- Vaccines
  - Ask for IgG vaccine antibody titers to see if boosters are necessary or not, especially for live viral vaccines (MMR, varicella)
- Decrease Stress
  - Depresses immunity; causes Th1 -> Th2 shift
- Avoid/Remove Toxins
  - Cause autoimmunity, promotes immune dysregulation
- Decrease oxidative stress
  - Activates innate immunity

Improving Immunity

- **Diet**
  - Remove foods causing immune stimulation
  - Healthy, well balanced, Free of toxins

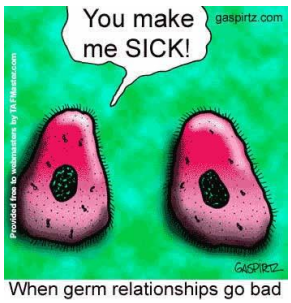
- **Supplements** to support metabolism
  - Vitamins
  - Minerals
  - Antioxidants
  - http://www.wfubmc.edu/Center-for-Integrative-Medicine/Patient-Resources/Dietary-Supplements.htm

Exercise and stress

- **Exercise has been shown to boost the immune response**
  - moderate exercise increases the immune response in all age groups
  - intensive exercise can stress the immune system
- **Lack of sleep and exhaustion decrease immune function**
- **Psychological stress has also been found to decrease immune function**
- **Metabolic stress – Oxidative Stress – also very detrimental to immune function and is pro-inflammatory**

Immunomodulatory Therapies

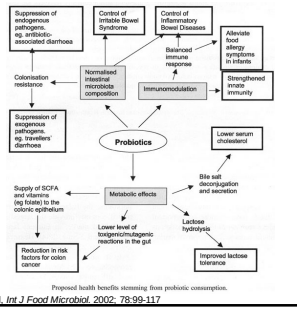
- **Probiotics**
  - **Probiotics** = dietary supplement containing live micro-organisms
  - **Early regulation of the immune system largely dependent on gut flora**
- **Omega 3 Fatty Acids**
  - **Natural anti-inflammatory agents**
- **Methyl B12**
  - **A crucial biochemical crossroads that helps in stabilizing membranes and making glutathione**
- **Glutathione**
  - **Helps to regulate T cells and regenerate gut epithelium**



When germ relationships go bad

Effects of Probiotics on the Immune System

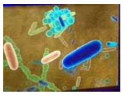
- Produce natural anti-microbials
- Block adhesion of toxins and pathogens
- Decrease gut permeability
- Modulate immune response
  - Enhanced natural killer cell activity
  - Increase mucosal and secretory IgA
  - Decrease pro-inflammatory cytokines
  - Increase anti-inflammatory cytokines and T regs
  - Barrier function



Saarela M et al, *Int J Food Microbiol*. 2002; 78:99-117

Common Probiotics

- Lactobacillus
  - can adhere to gut mucosa
  - L. acidophilus, L. rhamnosus GG, L. bulgaricus
- Bifidobacteria
  - predominant colonic flora of breast fed infant
  - B. bifidum, B. longum, B. breve, B. infantis, B. animalis
- Both are normal intestinal flora
- Both can be recovered in the stool after ingestion
  - -suggests colonization of gut



Pietzak

Probiotic Products for Infants and Children - Supplements

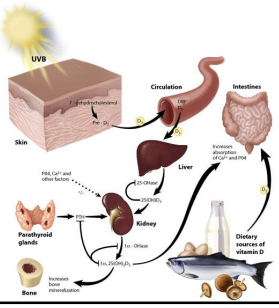
Manufacturer	Type of supplement	Probiotic
Baby's Only Essentials Probiotic from Nature's One	Powdered packet	*B. longum BB536* *B. breve M-16V* *B. infantis M-63*
Culturelle	Tablet or gelatin capsule	*L. rhamnosus GG*
VSL #3	Powder packet	*B. breve* *B. longum* *B. infantis* *L. acidophilus* *L. plantarum* *L. paracasei* *L. bulgaricus* *S. thermophilus*

Dose ranges between $10^9 - 10^{10}$ per serving

MANY GOOD BRANDS… few well studied!

Vitamin D

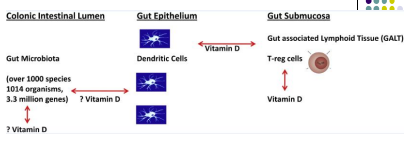
- Critical role in innate immunity and autoimmunity – really a hormone with receptors on many many cells
- Very frequently low in patients with autoimmune disease
- Low in people with darker skin or little sun exposure – made in skin when in sunlight
- Can measure 25 (OH) D level in the blood
  - Want levels 50 – 60 ng/ml range



Immune functions of Vit D

- **Nonclassic actions of Vitamin D.** Bikle D. *J Clin Endocrinol Metab* January 2009, 94(1): 26-34.
  - **Inhibits T cell proliferation**
  - **Increases IL-10 and TGF-beta (regulatory cytokines)**
  - **Increases T regs**
  - **Decreases innate inflammation**
- **Evidence that vitamin D3 reverses age-related inflammatory changes in the rat hippocampus.** Moore ME et al. *Biochemical Society Transactions* (2005) 33(4): 573- 577.
  - **… vitamin D3 acts as an anti-inflammatory agent and reverses the age-related increase in microglial activation in the brain.**

INTERACTION OF VITAMIN D and GUT MICROBES



Vit D may directly influence the number or diversity of the gut microbiota. It also influences dendritic cell antigen processing. It controls immune trafficking between dendritic cells and T regs. Finally, through IL-10 and TGF-beta, it modulates T reg function through all three effector arms of the adaptive immune system – Th1, Th2, and Th17.

Weiss, ST. Bacterial Components Plus Vitamin D: The ultimate solution to the asthma (autoimmune disease) epidemic? *J Allergy Clin Immunol* May 2011; 127(5) 1128-1130

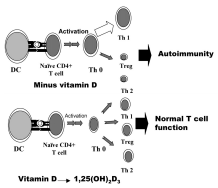


Figure 1. A model for the effects of vitamin D and 1,25-dihydroxy vitamin D_3 ($1,25[OH]_2D_3$) and T-cell development and function. The hypothesis is that vitamin D and $1,25(OH)_2D_3$ regulate T helper cell (Th1) development by inhibiting Th1 and inducing other CD4+ T cell populations, including regulatory T cells and Th2 cells. **In the absence of adequate vitamin D, the immune system favors the development of self-reactive T cells and autoimmunity.**

Cantorna and Mahon, Mounting Evidence for Vit D as an Environmental Factor Affecting Autoimmune Disease Prevalence. *Exp Biol Med* (2004) 229 (11): 1136.

How low are most people? NHANES

- 3454 random adults age > 21 yrs had 25 (OH)D levels checked:
  - 26% were < 15
  - 60% were 15-30
  - 14% were > 30
- 3136 random children age 1-21 yrs had 25 (OH)D levels checked:
  - 24% were < 15
  - 63% were 15-30
  - 13% were > 30

Sharif S et al, **Vitamin D levels and food and environmental allergies in the US: Results from the National Health and Nutrition Examination Survey 2005-2006** *J Allergy Clin Immunol* May 2011;127(5): 1195-1202

Vitamin D

- Safe to give children **2000 IU** per day and adults **4000-5000** IU daily of D3 (cholecalciferol) without checking a blood level.
- If measured value is low (< 30 ng/ml 25(OH)D), can give higher doses. Every 1,000 IU consumed raises the level a further 7-10 ng/ml.; don't go above 10,000 IU per day.
- Endocrinologists give adults with levels below 20 – 30 Ergocalciferol 50,000 IU once a week for 3 months, then once a month.
- Check levels, aim for **50-60**; keep < 90 ng/ml

Vitamin A

- **Research now recognizing the impact in the immune system**
  - **Decreases autoimmunity**
  - **Helps in regulation**
  - **Aides IgA function**
- **Vit A promotes the induction of Treg cells.**
- **Take recommended daily allowance in a multi-vitamin**

- **Retinoic acid-dependent regulation of immune responses by dendritic cells and macrophages.** Manicassamya, S and Pulendrana,B. *Seminars in Immunology* (2009) 21:22–27.
- **Regulation of FoxP3+ Regulatory T Cells and Th17 Cells by Retinoids.** Kim CH. *Clinical and Developmental Immunology* (2008)
- **Role of retinoic acid in the imprinting of gut-homing IgA-secreting cells**. Mora, J R and von Andrian U H. *Seminars in Immunology*. (2009) 21:28–35.

Antioxidants – Curcumin (Turmeric)

- **Antioxidant and anti-inflammatory properties of curcumin.** Adv Exp Med Biol. 2007;595:105-25.
  - The anti-inflammatory effect of curcumin is most likely mediated through its **ability to inhibit cyclooxygenase-2 (COX-2), lipoxygenase (LOX), and inducible nitric oxide synthase (iNOS),** all important enzymes that mediate inflammatory processes.
  - Cook with it or Supplements: Thorne – Meriva, Lee Silsby – Enhansa; start slow, work up to ~ 300 + mg.

Antioxidants – CoQ 10 and Quercetin

- **CoQ-10** is anti-inflammatory/anti-oxidant
  - Start with **50** mg a day, can go to 100 - 200 mg
- **Quercetin**
  - Natural antihistamine (for allergies)
  - Quercetin also has anti-inflammatory properties
  - Dose – start with **100** mg a day, can go to 200 mg

Omega-3 Fatty Acids

- **Omega-6 fatty acids** (in many processed foods) are **pro-inflammatory.**
- **Omega-3 fatty acids (fish oil, flax seed oil) are anti-inflammatory** - can have marked influence on both specific and nonspecific immune responses in modifying inflammatory precursors and replacing Omega-6 FAs in cell membranes.
- **1 - 2 grams a day** can be given safely. Start with a low dose and work up.
- See Handout for food content and supplement brands http://www.wfubmc.edu/Center-for-Integrative-Medicine/Patient-Resources/Dietary-Supplements.htm

Kankaanpaa P, Dietary fatty acids and allergy. *Annals of Med* **31(4): 282-7, 1999**
Grimm H, Regulatory potential of n-3 fatty acids in immunological and inflammatory process. *Brit J Nutrition* **87(sup 1): S59-67, 2002.**

Immunomodulatory Therapies

- **Probiotics**
- **Omega 3 Fatty Acids**
- **Methyl B12, vit D**
- **Glutathione**
- **Chelation** – Results of Jim Adam's trial of oral DMSA trial (3 days of 10 mg/kg given 3 times a day) – all 64 children normalized their Glutathione levels with this one "round" of oral DMSA without significant side effects. Children who went on to receive more "rounds" also lowered their high platelet counts, consistent with improvement in inflammation.
- **Anti-inflammatory medications**: Singulair, NSAIDs, etc

PANDAS: Bear with me

- **P**ediatric **A**utoimmune **N**europsychiatric **D**isorder **A**ssociated with **S**treptococcus.
- Onset of Tourette's Syndrome or Obsessive-Compulsive Disorder in children following a strep throat.
- Antibodies to strep cross-react with brain proteins (basal ganglia), causing symptoms.
- Treatable with immune modulation, antibiotics.

Swedo SE. *Molecular Psychiatry* 2002; 7 (sup 2):S24-5.

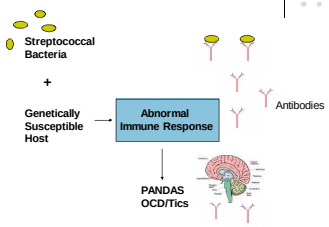
PANDAS

Criteria for PANDAS

I. Presence of OCD and/or Tic Disorder
II. Prepubertal onset
III. Episodic course of symptom severity
IV. Association with neurological abnormalities
V. Temporal relationship between symptom exacerbations and streptococcal infections

Waxing and waning of symptoms with parallel to titers critical to making absolute diagnosis

PANDAS MODEL



Molecular Psychiatry (2009), 1–15
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www.nature.com/mp

ORIGINAL ARTICLE

Passive transfer of streptococcus-induced antibodies reproduces behavioral disturbances in a mouse model of pediatric autoimmune neuropsychiatric disorders associated with streptococcal infection

K Yaddanapudi[1], M Horrig[1], R Serge, J De Miranda, A Baghban, G Villar and WI Lipkin

Center for Infection and Immunity and Department of Epidemiology, Mailman School of Public Health, Columbia University, New York, NY, USA

ELSEVIER
Journal of Neuroimmunology xx (2006) xx–xx
Journal of Neuroimmunology
www.elsevier.com/locate/jneuroim

Antibody-mediated neuronal cell signaling in behavior and movement disorders

Christine A. Kirvan [a], Susan E. Swedo [b], Lisa A. Snider [b], Madeleine W. Cunningham [a,b,1]

Mimicry and autoantibody-mediated neuronal cell signaling in Sydenham chorea Nature Medicine 2003: 9(7) 914

Christine A Kirvan[1], Susan E Swedo[2], Janet S Heuser[2] & Madeleine W Cunningham[1]

Streptococcus pyogenes–induced acute rheumatic fever (ARF) is one of the best examples of postinfectious autoimmunity due to molecular mimicry between host and pathogen. Sydenham chorea is the major neurological manifestation of ARF but its pathogenesis has remained elusive, with no candidate autoantigen or mechanism of pathogenesis described. Chorea monoclonal antibodies showed specificity for mammalian lysoganglioside and *N*-acetyl-(1-4)-α-glucosamine (GlcNAc), the dominant epitope of the group A streptococcal (GAS) carbohydrate. Chorea antibodies targeted the surface of human neuronal cells, with specific induction of calcium/calmodulin-dependent protein (CaM) kinase II activity by monoclonal antibody 24.3.1 and sera from active chorea. Convalescent sera and sera from other streptococcal diseases in the absence of chorea did not activate the kinase. The new evidence implicates antibody-mediated neuronal cell signaling in the immunopathogenesis of Sydenham chorea and will lead to a better understanding of other antibody-mediated neurological disorders.

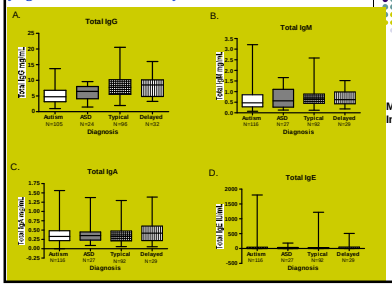
PANDAS Treatment

- **Antibiotic prophylaxis with azithromycin or penicillin for childhood-onset neuropsychiatric disorders.** Snider LA, Lougee L, Slattery M, Grant P, Swedo SE. *Biol Psychiatry*. 2005;57(7):788-92.
  - Pen V K 250 BID po
  - Zithromax 500 po q week
  - IM bicillin
- Nearly impossible to get insurance to pay for IVIG
  - 1.5 – 2 gms/kg
- Plasmaphoresis very invasive, also rarely covered

Intravenous Immune Globulin

- Low dose (400 – 600 mg/kg every 4 weeks) for those with low IgG levels and recurrent infections (true immunodeficiency)
- Higher doses used for autoimmune disease
- IVIG will only work in the short term, not long term, unless one is able to shut down the persistently activated auto-reactive T cells that are driving the production of cytokines and antibodies.
- Like steroids (anti-inflammatory but with major side effects) ***in my opinion IVIG is a "last ditch" therapy and never a "front line" one***

Reduced levels of IgG and IgM are indicative of an underlying defect in the immune system of children with autism.



M.I.N.D. Institute

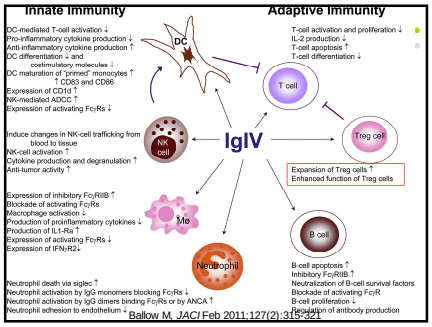
Brief Report: Dysregulated Immune System in Children with Autism: Beneficial Effects of Intravenous Immune Globulin on Autistic Characteristics[1]

Gupta S et al, **J Autism Dev Disord. 1996 Aug;26(4):439-52.**

DISCUSSION

In this preliminary study a marked abnormality of immune parameters was observed in children with autism when compared to age-matched controls. This included abnormalities of various lymphocyte subsets and serum levels of various immunoglobulin classes and subclasses. Furthermore, intravenous immunoglobulin treatment resulted in improved autistic features.

Improvement in children with autism treated with intravenous gamma globulin Marvin Boris MD ; Allan Goldblatt PA-C ; Stephen M. Edelson PhD. Journal of Nutritional & Environmental Medicine, December 2005 15: 169 - 176



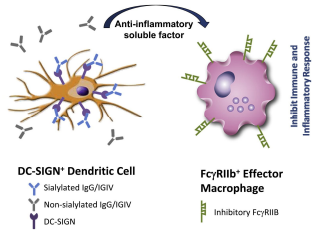
Ballow M, *JACI* Feb 2011;127(2):315-321

Identification of a receptor required for the anti-inflammatory activity of IVIG Anthonya RM, et al www.pnas.orgcgidoi10.1073pnas.0810163105

- The anti-inflammatory activity of intravenous Ig (IVIG) results from a minor population of the pooled IgG molecules that contains terminal 2,6-sialic acid linkages on their Fc-linked glycans. These anti-inflammatory properties can be recapitulated with a fully recombinant preparation of appropriately sialylated IgG Fc fragments.
- We now demonstrate that these sialylated Fcs require a specific C-type lectin, SIGN-R1, (specific ICAM-3 grabbing nonintegrin- related 1) expressed on macrophages in the splenic marginal zone. A human orthologue of SIGN-R1, DC-SIGN, displays a similar binding specificity to SIGN-R1.
- **These studies thus identify an antibody receptor specific for sialylated Fc, and present the initial step that is triggered by IVIG to suppress inflammation.**

Antibody receptor specific for sialylated Fc

- This switching between sialylated IgG and asialylated IgG suggests a mechanism by which the immune response can distinguish between IgG antibodies in the steady state and those generated in response to a specific antigenic challenge, thereby protecting the host against coincidental activation of inflammatory pathways in the absence of a pathogenic challenge.



Ballow M, *JACI* Feb 2011;127(2):315-321

Role of steroids/IVIG in LKS and intractable seizures

- Landau-Kleffner syndrome: consistent response to repeated intravenous gamma-globulin doses: a case report. Fayad MN. Epilepsia. 38(4):489-94, 1997.
- Successful use of intravenous immunoglobulins in Landau-Kleffner syndrome. Lagae LG. Pediatric Neurology. 18(2):165-8, 1998.
- Successful use of intravenous immunoglobulin as initial monotherapy in Landau-Kleffner syndrome. Mikati MA. Saab R. Epilepsia. 41(7):880-6, 2000.
- Treatment in typical and atypical rolandic epilepsy. Rating D. Epileptic Disorders. 2 Suppl 1:S69-72, 2000. (in favor of steroids)

Role of steroids/IVIG in LKS and intractable seizures

- Efficacy of intravenous immunoglobulin in Landau-Kleffner syndrome. Mikati MA. Pediatric Neurology. 26(4):298-300, 2002.
- The use of immunoglobulins in the treatment of human epilepsy. Villani F. Neurological Sciences. 23 Suppl 1:S33-7, 2002 .
- Management of Landau-Kleffner syndrome. Mikati MA. Paediatric Drugs. 7(6):377-89, 2005. (good review, waffles about IVIG)

Steroids?

Miroslav Kovacevic MD http://www.webpediatrics.com/ivig.html

Steroid burst (Prednisone 1 mg/kg/day, max. dose 50 mg, as a single AM dose for FIVE consecutive days) response does parallels the expected (and achieved) IVIG benefits. He considers it positive only if there is a significant and objective improvement in patient's core symptoms.

Response to IVIG not as good in ASD children and less response in older children

Michael Chez MD Abstract Child Neurology Society 1998

Tried "pulse" high dose oral steroids in children with LKS/seizures as 5 mg/kg/day on Saturdays and Sundays, gradually weaned off over a year to 2 years while on valproate (Depakote). EEG improvement seen after treatment with valproate preparations showed additional responses to pulse dose steroids.

COLON COMRADES!

Worms: Trichuris Suis Ova

- Based on work with Crohn's disease – like "super" probiotics. Elevations of anti-inflammatory and immunosuppressive cytokines (such as IL-10) occur during long-term helminth infections.
- Pig whipworms so can not colonize humans.
- www.ovamed.org/english/home/home.html
- Dose studied is 2500 ova every other week; mix in liquid and drink. May be able to use less once response is established.
- www.biomonde.co.th to order. Expensive! ~ $900/mo.
- www.autismtso.com

Trichuris suis therapy in Crohn`s disease Summers RW et al. *Gut* 2005; 54:87-90.

Summers, Elliott, Urban, et al

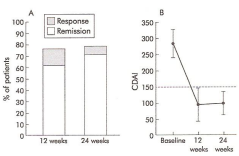
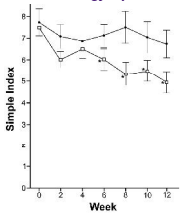


Figure 1 (A) Percentage of patients achieving remission or response at week 12 or 24 after initiating ova therapy. (B) Mean change in Crohn's disease activity index (CDAI, mean (SD)) for respondents to ova therapy. CDAI <150 is remission. p<0.0001, week 12 or week 24 compared with baseline (time 0).

Open Label

Trichuris suis Therapy for active ulcerative colitis, a randomized controlled trial. Summers, RW et al

***Gastroenterology* April 2005**



Ulcerative Colitis Symptom Index for Placebo (solid circles) and Ova-treated Patients (open squares)

CNS effects

- Sewell D, Qing Z, Reinke E, Elliott D, Weinstock J, Sandor M, Fabry Z. **Immunomodulation of experimental autoimmune encephalomyelitis by helminth ova immunization**. *International Immunology* 15:59-69, 2003.
- La Flamme AC, Ruddenklau K, Backstrom BT. **Schistosomiasis decreases central nervous system inflammation and alters the progression of experimental autoimmune encephalomyelitis**. *Infection & Immunity* 71:4996-5004, 2003.

Mechanism

- Doetze, A et al. **Antigen-specific cellular hyporesponsiveness in a chronic human helminth infection is mediated by T(h)3/T(r)1-type cytokines IL-10 and transforming growth factor-beta but not by a T(h)1 to T(h)2 shift.**
  International Immunology, 12:623, 2000 .

Another possibility?

- Earth Dragon from Allergy Research Group: *Lumbricus rubellus* aka earthworm powder with Green Tea extract and some other TCM compounds - $38.00 for 150 capsules; http://www.allergyresearchgroup.com/Earth-Dragon-150-Vegetarian-Caps-p-72.html
- Dose not clear, but company recommends 3 caps twice a day for adults.

Therapies under investigation or used in other neuroinflammatory disorders

Actos – Boris and Goldblatt – down regulates microglial activation and decreases inflammatory cytokines.

Low Dose Naltrexone – Jaqueline McCandless – decreases inflammation, increases NK cell activity and causes shift towards Th2 ?

Spironolactone - Jeff Bradstreet

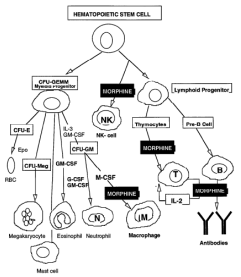
Minocycline – NIH trial - down-regulates microglia.

NeuroProtek – Theo Theorides

HBOT – clearly helpful in acute inflammation; evidence that it is helpful in chronic as well.

Low Dose Naltrexone (LDN)

- http://www.lowdosenaltrexone.org/
- **Dr. Jaquelyn McCandless has been the champion of this therapy. She uses 3mg transdermally every *night* in ASD children; made by compounding pharmacies .**
- Typically see improvement in sociability and irritability within 3 months; if parents report some improvement then continue for one year. It is relatively inexpensive and easy to apply.
- Mechanism of action not really clear – opioids are generally anti-inflammatory, so an anti-opioid (opioid antagonist) like naltrexone might have made things worse, but the thinking is that by using a low dose to down regulate the system at night, there is a "rebound effect" when the antagonist wears off, resulting in an increase in the opioids themselves.



Roy S, Loh HH. *Effects of opioids on the immune system.* Neurochem Res 1996;21:1375-1386

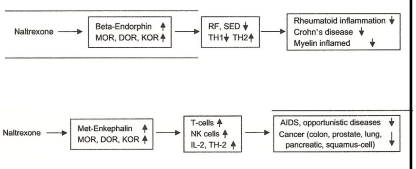
Multiple Sites of Action of Morphine

Therapy with the Opioid Antagonist Naltrexone Promotes Mucosal Healing in Active Crohn's Disease: A Randomized Placebo-Controlled Trial.

Dig Dis Sci. 2011 Mar 8. PMID: 21380937

- **BACKGROUND:** Endogenous opioid peptides have been shown to play a role in the development and/or perpetuation of inflammation. We hypothesize that the endogenous opioid system is involved in inflammatory bowel disease, and antagonism of the opioid-opioid receptor will lead to reversal of inflammation.
- **METHODS: 40 adults** with active Crohn's disease, patients randomized to daily 4.5-mg oral naltrexone or placebo for 12 weeks. Providers and patients were blinded.
- **RESULTS: Eighty-eight percent** of those treated with naltrexone had at least a 70-point decline in Crohn's Disease Activity Index score (CDAI) vs **40%** of placebo-treated patients ($p = 0.009$). After 12 weeks, 78% of subjects treated with naltrexone exhibited an endoscopic response as indicated by a 5-point decline in the Crohn's disease endoscopy index severity score (CDEIS) from baseline compared to 28% response in placebo-treated controls ($p = 0.008$), and 33% achieved remission with a CDEIS score <6, whereas only 8% of those on placebo showed the same change. Fatigue was the only side effect reported that was significantly greater in subjects receiving placebo.
- **CONCLUSIONS: Naltrexone improves clinical and inflammatory activity of subjects with moderate to severe Crohn's disease compared to placebo-treated controls.**

Proposed Immunologic actions of LDN



Low-dose naltrexone for disease prevention and quality of life
Panksepp J and Brown N, *Medical Hypotheses* 72 (2009) 333–337

Medical Hypotheses (2007) 68, 970–987
ELSEVIER
medical hypotheses
http://intl.elsevierhealth.com/journals/mehy

Spironolactone might be a desirable immunologic and hormonal intervention in autism spectrum disorders

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Table 2. Summary of the proposed effects of spironolactone on autism findings

Clinical finding	Autism finding	Effect of spironolactone
Interferon gamma	↑ [19]	↓ [55]
TNF-α	↑ [24,25]	↓ [54,55]
MCP-1	↑ [34]	↓ [54,56]
Inflammation	↑ [27,34]	↓ [53,55]
Oxidative stress	↑ [57]	↓ [62]
Testosterone effects	↑* [5–7]	↓ [59]

* Elevated in a subset of autistic individuals.

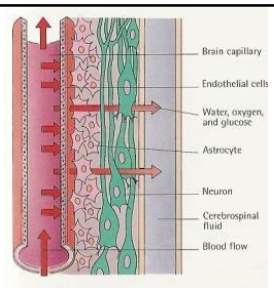
Originally used as a diuretic but at low doses is anti-inflammatory and partially blocks Testosterone receptors so particularly useful in adolescent boys

Minocycline

- Worked for Fragile X in the recent trial - suppressed a protein called MMP-9, which is overproduced in fragile X brains
- Really no effect on autism in small NIH trial
  - open-label preliminary trial of 6 months of **minocycline** therapy (1.4 mg/kg) in 10 children with regressive ASD (mean age 7.58yrs; range 3-12 yrs).
  - No significant clinical effects were seen. However, changes in the pre-/post-treatment profiles of the proform of BDNF in CSF and blood, HGF in CSF and IL8 in serum, suggest that **minocycline** may have effects in the CNS by modulating the production of neurotropic growth factors.

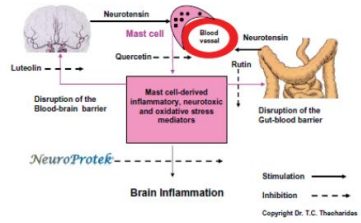
Systemic Mast Cell Activation Disorders

- Heterogeneous group of pathological conditions characterized by
- Systemic and severe MC mediator related symptoms (including anaphylaxis)
  - Severity of symptoms are unrelated with mast cell burden
  - With or without a known trigger
  - With or without specific IgE antibodies against the suspected trigger(s)
  - With or without increased baseline serum tryptase levels
  - Need for intensive and continuous anti-mediator therapy (usually except in cases triggered by insects)



BLOOD BRAIN BARRIER

How might NeuroProtek® be helpful? The active ingredients were selected to provide the maximum anti-oxidant, anti-inflammatory, mast cell inhibitory and neuroprotective effects.



Transfer Factors

- Small RNAs which label infected host cells, serving as a "target" for cell-mediated destruction.
- Very specific for pathogens – like antibodies (Ab to strep only will recognize strep; Transfer factor to Hepatitis C will only recognize Hepatitis C)
- Present in colostrum

Summary: Immune dysregulation and increased inflammation are frequent in autism

- **There is evidence of over-activity of the immune system indicating DYSREGULATION**
  - **The innate immune system is involved**
  - **Adaptive immune system appears to be dysregulated as well**
  - **Inflammation in the blood, in the brain, and in the GI tract of many of these children**
  - **Evidence that T regulatory piece is not functioning properly, so boosting T regs may help**