Immunomodulation in Autism Spectrum Disorders

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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

“We cannot solve the problems that we have created with the same thinking that created them.”
- Albert Einstein

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

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

**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

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

**T REGULATORY CELLS**

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

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Things that can go wrong...

- **Immune deficiency/dysfunction:** defective or ineffective response.
- **Hypersensitvity:** 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**

- Fig. 1. 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**

- 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.

**Old friends**

- Mammalian evolution has kept us in close contact with relatively harmless microorganisms 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
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

The intestinal commensal microflora contains approximately $10^{11}$ bacteria of an estimated 1000 different species.

Intestinal Flora (A Balanced Ecosystem)

Development of the Gastrointestinal Microbiota

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

Dysregulated immune system with inflammation in children with ASD

  “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.”

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.
- Antibodies Against Small Blood Vessels in the brain


NEUROMUSCULAR DISEASE CENTER
Washington University, St. Louis, MO USA

Serum autoantibodies to brain in Landau-Kleffner variant, autism, and other neurologic disorders.

Antibodies Against Small Blood Vessels in the brain

Dysregulated immune system with inflammation in children with ASD - BRAIN

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 astrocytes.

- 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.


Microglial Balancing Act

Microglia and astrocytes need glutathione to protect neurons

ROLE OF THE GUT ??

Cerebral Microglia Recruit Monocytes into the Brain in Response to TNF Signaling


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 microorganisms
  - Early regulation of the immune system largely dependent on gut flora
- Omega 3 Fatty Acids
  - Natural anti-inflammatory agents
  - Methyl B12
    - A crucial biochemical crossroad that helps in stabilizing membranes and making glutathione
- Glutathione
  - Helps to regulate T cells and regenerate gut epithelium

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

You make me SICK!

When germ relationships go bad
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

  - Inhibits T cell proliferation
  - Increases IL-10 and TGF-beta (regulatory cytokines)
  - Increases T regs
  - Decreases innate inflammation

  - ...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.


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

**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
  - Acts on T lymphocytes
- V3A promote the induction of Treg cells.
- Take recommended daily allowance in a multi-vitamin:
  - Antioxidants: Thorne

**Antioxidants – Curcumin (Turmeric)**
  - 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 Silisy – Enhansa; start slow, work up to 300 – 500 mg.

**Omega-3 Fatty Acids**
- Omega-3 fatty acids (in many processed foods) are anti-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 processes 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.thorne.com/CenterForIntegrativeMedicine/Patients/Resources/Primary-Supplements.html](http://www.thorne.com/CenterForIntegrativeMedicine/Patients/Resources/Primary-Supplements.html)

**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: Singular, NSADs, etc.

**PANDAS: Bear with me**
- Pediatric Autoimmune Neuropsychiatric Disorder Associated with Streptococcus.
- 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-S.

**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

**Antioxidants – CoQ10 and Quercetin**
- CoQ10 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
Antibiotic prophylaxis with azithromycin or penicillin for childhood-onset neuropsychiatric disorders.


- 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
- 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.


In this preliminary study a marked abnormality of immune parameters was observed in children with autism who were treated with IVIG. Immune parameters characterized by increased levels of IgM and IgA and decreased levels of IgG and IgE were observed. Furthermore, long-term immunological 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 antibodies

- The anti-inflammatory activity of intravenous, Ig (IVIG) results from a minor population of the pooled IgG molecules that contain 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 Fc's require a specific C-type lectin, SIGN-R1, (specific ICAM-3 grabbing nonintegrin-related) 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.
Role of steroids/IVIG in LKS and intractable seizures


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 MD dose for PM: continuous daily dose) response does parallels the expected (and achieved) IVIG benefits. He concludes however 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 improvements seen after treatment with valproate preparations showed additional responses to pulse dose steroids.

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

Gastroenterology April 2005

CNS effects


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

Mechanism

- Doetze, A et al. Antigen-specific cellular hyporesponsiveness in a chronic human helminth infection is mediated by T(h)3/T(1)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: Lumbicus 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.

Low Dose Naltrexone (LDN)

- Dr. Jaqueline 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.

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?
- Spiroinolactone – Jeff Bradstreet
- Minocycline – NIH trial - down-regulates microglia.
- NeuroProtek – Theo Theorides
- HBO – clearly helpful in acute inflammation; evidence that it is helpful in chronic as well.

Proposed Immunologic actions of LDN

Low-dose naltrexone for disease prevention and quality of life

Low-dose naltrexone for disease prevention and quality of life

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.
**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

**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 mast cells)

**Blood Brain Barrier**

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